FUNDAMENTALS OF FERMENTATION TECHNOLOGY

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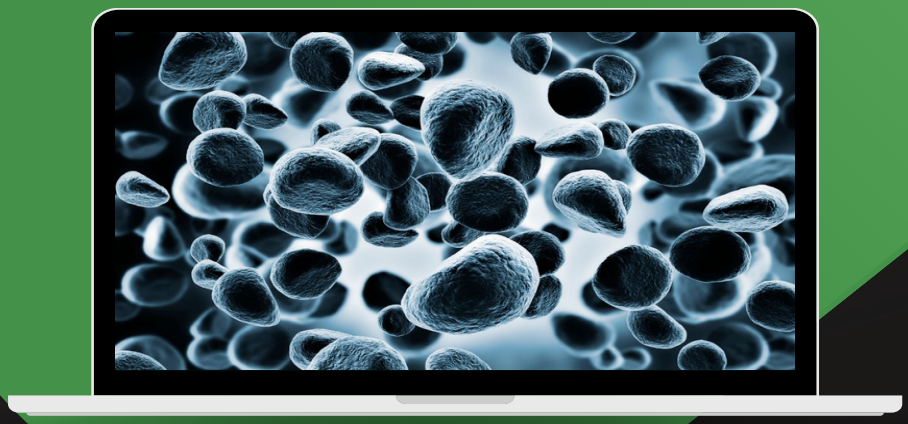

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Dr. S. P. Dwivedi

Fundamentals of Fermentation Technology

EDITED BY

Dr. Surya Prakash Dwivedi

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Fundamentals of Fermentation Technology

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Edited and Compiled by
Dr. Surya Prakash Dwivedi
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Email: rudra.baral@ennobleip.com

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Preface

This book is intended to assist the development, design and production engineer who is engaged in the fermentation industry. Particular emphasis is given to those unit operations most frequently encountered in the commercial production of chemicals and pharmaceuticals via fermentation, separation, and purification. Although it is not stressed, some theory is presented to give the reader the necessary understanding of how the unit operates. The focus is instead on the practical elements of development, design, and operation, such as how to gather design data, what the scale-up parameters are, how to choose the appropriate piece of equipment, where operating issues originate, and how to troubleshoot them.

All of the contributing authors were chosen for the work because of their industry backgrounds and orientations because it is written from a practical and operational perspective. The handbook deals with fermentation, and as fermentation engineers frequently lack knowledge of microbiology, it was deemed prudent to address this topic at the beginning of the book. Similarly, it was thought essential to add some chapters specifically targeted to the creation of sterile products because fermentation deals with the production of antibiotics to a large extent. The engineer reading this manual might have preferred that additional unit operations or equipment types had been chosen instead of the ones that were. The decision was made based on the input from each individual contributor as well as my personal experience working in the field for many years with the equipment and unit operations that have served as the backbone and workhorses of the sector.

Growth kinetics, strain isolation and improvement, inocula generation, fermentation media, fermenter design and operation, product recovery, and the environmental impact of processes are all covered in this book. This edition of this book, which is accurate and understandable, acknowledges the growing significance of animal cell culture, the influence of the post-genomics era on applied science, and the significant contribution that heterologous protein production now makes to the success of the pharmaceutical industry. This book offers crucial and fundamental information about fermentation in a methodical, logical manner, making it perfect for both newbies to the field and seasoned professionals. With a focus on the practical application of theory, Stanbury, Whitaker, and Hall have combined the biological and engineering components of fermentation to make the information accessible to members of both disciplines. This publication compiles all the foundational concepts of fermentation into a condensed reference, making it an important tool for fermentation researchers and students. The main characteristics that have been kept are its successful structure and comprehensive coverage of the fermentation process. discusses the most recent advances and advancements in the discipline while integrating the biological and engineering components of fermentation. Written in a manner that is understandable to readers with backgrounds in biology or engineering, and each chapter is backed up by a sizable bibliography.

Dr. S. P. Dwivedi
Editor

January 2023

CHAPTER 1

Introduction of Fermentation Technology

Padmapriya. G,

Assistant Professor, Department of Chemistry, School of Sciences, B-II,

Jain (Deemed to be University), J C Road, Bangalore-560027.

Email Id- g.padmapriya@jainuniversity.ac.in

The discipline of fermentation technology uses microbes and enzymes to create substances that are used in the food, chemical, pharmaceutical, energy, and material sectors. It is possible to describe biotechnology as the combination of natural sciences or technical methods used to use organisms, cells, or their components for commercial goods and services. As a result, fermentation is a biotechnological technology that employs microbes to generate a variety of industrial goods. A very old method that has been around for a very long time is fermentation. For the first time, it was applied to the brewing of beer. Today, fermentation technology and biotechnology have converged to produce value-added products including hormones, antibiotics, enzymes, and other metabolites. A reduced form of pyridine is produced during the oxidative catabolism of sugars, which must then undergo reoxidation in order for the process to continue. Reoxidation of the repyridine nucleotide takes place in an aerobic environment via the cytochrome system, where oxygen serves as the terminal electron acceptor. But when an organic substance is reduced under anaerobic conditions, decreased pyridine nucleotide oxidation often results as a catabolic byproduct. When yeast is acting on fruit or extracts, NADH is created by the reduction acid, turning it into ethanol. Pyruvate may be reduced by several microbial species to a variety of end products. As a result, the word has been strictly defined in biochemistry to refer to an energy-generation process in which substances serve as both terminal electron acceptors and donors. Below figure shows the Generic methods for several fermentation end products to be produced from glucose by diverse species.

The fundamental idea behind fermentation is to produce energy out of carbohydrates without the need of oxygen. First, glycolysis partly oxidises glucose to pyruvate. The next step involves turning pyruvate into alcohol or acid and replenishing NAD^+, which may then participate in glycolysis to increase ATP production. Only around 5% of the energy produced by aerobic respiration. Fermentation is the metabolic process through which organic molecules (typically glucose) are converted into acids, gases, or alcohol in the absence of oxygen or an electron transport chain. Through fermentation processes, the coenzyme Nicotinamide Adenine Dinucleotide (NAD^+), which is necessary for glycolysis to create energy in the form of adenosine triphosphate, is replenished (ATP). Fermentation only generates a net of 2 ATP per glucose molecule via glycolysis, but aerobic respiration, with the aid of the electron transport chain, generates up to 32 ATP molecules per glucose molecule.

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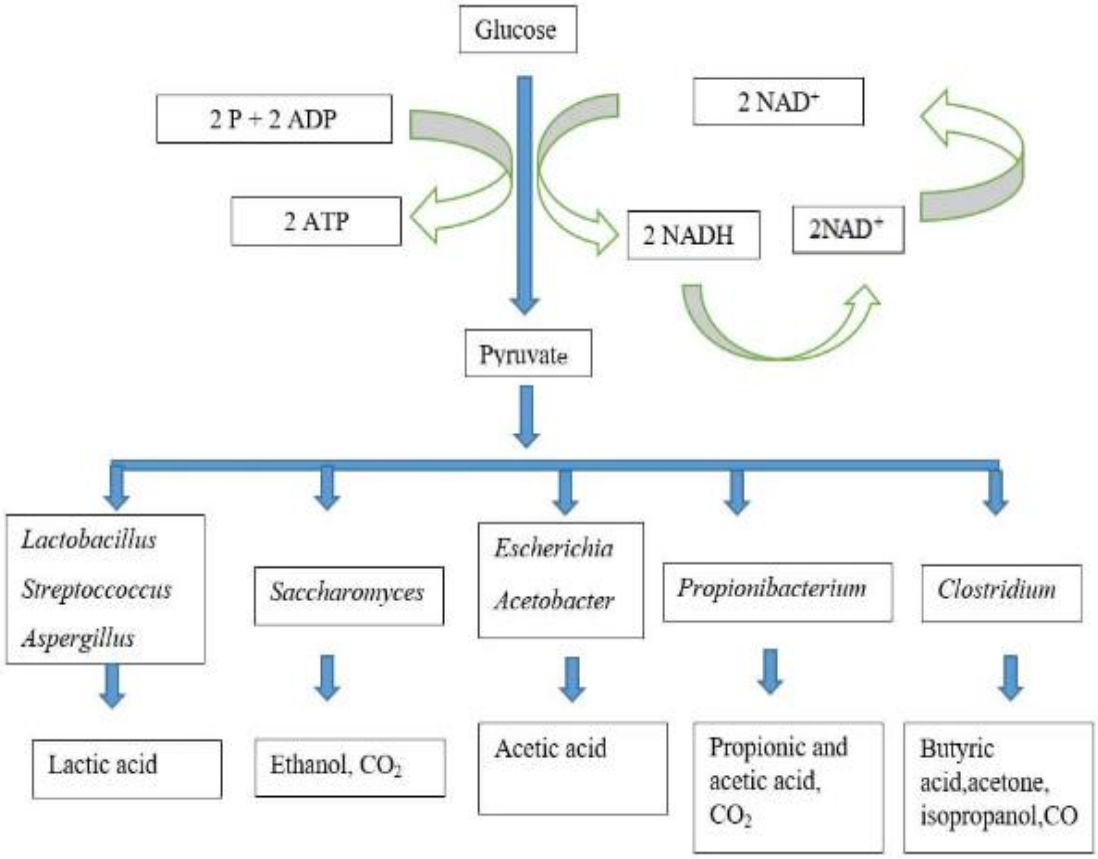

Figure: 1.1 Microbial conversion of glucose into organic acids and alcohols

Zymology was established in 1856 when French scientist Louis Pasteur demonstrated that yeast is the main cause of fermentation. Zymology is the study of fermentation and its uses. Obligatory anaerobes are certain bacteria and fungi that need the absence of oxygen to survive. When oxygen is low, fermentation also occurs in facultative anaerobes like yeast and in muscle cells (as in strenuous exercise). The processes of fermentation are advantageous to the food and beverage industries because they result in the conversion of carbohydrates into ethanol used to make alcoholic beverages, the release of CO_2 by yeast used to leaven bread, and the formation of organic acids to preserve and flavour vegetables and dairy products.

The main goal of fermentation is to convert NADH back into coenzyme NAD^+ so that it may once again be used for glycolysis. Products like carbon dioxide and ethanol (for the fermentation of ethanol) or lactate (for the fermentation of lactic acid) are created as a consequence of the reaction between NADH and an organic electron acceptor (such as pyruvate or acetaldehyde) to make NAD^+ is produced through fermentation.

Fermentation

A metabolic process called fermentation transforms sugar into acids, fumes, or alcohol. It happens in bacteria and yeast, as well as in muscle cells that are oxygen-starved, as in the case of lactic acid fermentation. In a broader sense, the term "fermentation" may also refer to the mass growth of microorganisms on a growth medium, often with the intention of creating a particular chemical

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product, such as an enzyme, vaccine, antibiotic, food product, or food additive. Louis Pasteur, a French microbiologist, is well known for his contributions to our understanding of fermentation and its microbiological origins. The field of zymology studies fermentation. When the electron transport chain is inoperable due to a lack of oxygen, fermentation occurs, and it then serves as the cell's main source of ATP generation. Depending on the type of fermentation, it converts the pyruvate and NADH generated during the glycolysis process into NAD^+ and a variety of other small molecules. NADH and pyruvate are utilised in respiration to produce ATP when O_2 is present. This process, known as oxidative phosphorylation, produces a lot more ATP than just glycolysis. Since of this, cells normally benefit from avoiding fermentation when oxygen is present. Obligate anaerobes are the exception because they cannot tolerate oxygen. Glycolysis is the initial process that all fermentation routes share:

$$C_6H_{12}O_6 + 2\ NAD^+ + 2\ ADP + 2\ Pi \rightarrow 2\ CH_3COCOO^- + 2\ NADH + 2\ ATP + 2\ H_2O + 2H^+$$

Pyruvate is CH_3COCOO. Inorganic phosphate is known as pi. Substrate-level phosphorylation is used to convert two ADP and two Pi molecules into two ATP and two water molecules. Additionally, two NAD+ molecules are converted to NADH. Energy for ATP synthesis in oxidative phosphorylation is produced electrochemically across the inner mitochondrial membrane or, in the case of bacteria, the plasma membrane through the electron transport chain. Substrate-level phosphorylation occurs during glycolysis, and ATP is produced right away. Since the Neolithic period, humans have employed fermentation to create food and drinks. For instance, fermentation is used to preserve food by creating lactic acid, which is present in sour foods like pickled cucumbers, kimchi, and yoghurt, as well as to make alcoholic drinks like wine and beer. Even animals like humans, who have stomachs, may experience fermentation. Fermentation Definitions For far too many people, fermentation solely refers to the alcoholic fermentation of grains and fruits that results in beer and wine. One may describe a meal as "off" or fermented if it soured. These definitions of fermentation are provided. The definitions vary from more casual, generic use to more precise ones.

Techniques for preserving food using microbes (general use). Any procedure that results in the production of alcoholic drinks or acidic dairy products (general use). Any massive microbiological activity that takes place with or without air (common definition used in industry). Any metabolic activity that releases energy and only occurs in anaerobic environments (becoming more scientific). Any metabolic activity that employs an organic molecule as the ultimate electron acceptor, releases energy from a sugar or other organic molecules, and does not need oxygen or an electron transport mechanism (most scientific).

It's not necessary to carry out fermentation in an anaerobic setting. For instance, yeast cells considerably prefer fermentation over aerobic respiration even in the presence of sufficient oxygen as long as carbohydrates are readily accessible for consumption (a phenomenon known as the Crabtree effect). Hops' antibacterial properties also prevent yeast from using its aerobic metabolism. NADH is reacted during fermentation with an organic, endogenous electron acceptor. This is often pyruvate, which is created from the sugar during the glycolysis process. Pyruvate undergoes a number of metabolic reactions during fermentation that result in a variety of chemicals. The process of producing ethanol and carbon dioxide is known as ethanol fermentation

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or alcoholic fermentation. Two processes for creating lactic acid are referred to as lactic acid fermentation. Lactic acid is the only result of homolactic fermentation. Lactic acid is produced during heterolactic fermentation, along with other alcohols and acids. The most frequent starting material for fermentation is sugars, and typical fermentation products include ethanol, carbon dioxide, lactic acid, and hydrogen gas (H_2). However, fermentation may create more unusual substances like acetone and butyric acid. Beer, wine, and other alcoholic beverages that contain ethanol are fermented by yeast, which also produces a significant amount of carbon dioxide. Lactic acid is produced when fermentation takes place in mammalian muscle during times of intensive activity when oxygen supply is reduced.

Alcohol production

The alcohol fermentation of glucose, with the molecular formula $C_6H_{12}O_6$, is shown in the following chemical equation. Two ethanol molecules and two carbon dioxide molecules are produced from one glucose molecule:

$$C_6H_{12}O_6 \longrightarrow 2\ C_2H_5OH + 2\ CO_2\ C_2H_5OH.$$

One glucose molecule is split into two pyruvate molecules prior to fermentation. This process is called glycolysis.

Fermentation of lactic acid

The simplest form of fermentation is homolactic fermentation, which solely yields lactic acid. A straightforward redox process causes the pyruvate from glycolysis to transform into lactic acid. Being one of the few respiration processes that does not result in the production of a gas as a byproduct, it is distinctive. In all, two molecules of lactic acid are produced from one molecule of glucose (or any other six-carbon sugar):

$$C_6H_{12}O_6 \rightarrow 2CH_3CHOHCOOH$$

Animal muscles experience it when they want oxygen and energy more quickly than the blood can provide. Additionally, certain fungi and some types of bacteria (such Lactobacilli) exhibit it. This particular species of bacteria is responsible for the yogurt's sour flavour by converting lactose into lactic acid. These lactic acid bacteria are capable of homolactic fermentation, which results in a mostly lactic acid-containing end product, or.

Heterolactic fermentation, in which a portion of the lactate is further metabolised to produce ethanol and carbon dioxide (via the phosphoketolase pathway), acetate, or even other metabolic products, for example:

$$C_6H_{12}O_6 \rightarrow CH_3CHOHCOOH + C_2H_5OH + CO_2$$

Galactose and glucose, both six-carbon sugars with the same atomic formula, are produced when lactose is fermented (as in yoghurts and cheeses):

$$H_2O + C_{12}H_{22}O_{11} = 2\ C_6H_{12}O_6.$$

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Heterolactic fermentation sits somewhere in between other forms of fermentation, such alcoholic fermentation, or lactic acid fermentation. There are many reasons to proceed and change lactic acid into anything else, including: Because of the lactic acid's acidity, biological processes are hampered. This can be advantageous to the organism that is fermenting because it drives out competitors who are unaccustomed to the acidity, giving the food a longer shelf life (part of the reason foods are fermented in the first place), but after a certain point, the acidity starts to harm the organism that produces it. Le Chatelier's principle states that a high concentration of lactic acid, the result of fermentation, causes the equilibrium to shift, slowing development and reducing the pace at which fermentation may take place.

Because lactic acid is rapidly converted to ethanol, which is volatile and swiftly escapes, the reaction may happen quickly. Additionally, CO_2 is created, however it is considerably more volatile than ethanol and only mildly acidic.

Although acetic acid (another conversion result) is sour and less volatile than ethanol, its synthesis from lactic acid releases a significant amount of extra energy when oxygen is scarce. It is a lighter molecule than lactic acid, more flammable, establishes fewer hydrogen bonds with its environment than lactic acid (as a result of having fewer groups that may form such connections), and will also enable the process to proceed more rapidly. As with ethanol, the quantity of acidity generated per unit of ingested glucose will decrease if propionic acid, butyric acid, and longer monocarboxylic acids are formed (see mixed acid fermentation), enabling quicker development.

1.1.3 Aerobic Breathing

The pyruvate produced during glycolysis is entirely oxidised during aerobic respiration, producing extra ATP and NADH via the citric acid cycle and oxidative phosphorylation. However, oxygen is required for this to happen. Oxygen is poisonous to facultative anaerobes but not to obligatory anaerobes, who do not need it. One of the fermentation processes, lactic acid fermentation, takes place in the absence of oxygen in order to renew NAD+.

1.1.4 Fermentation produces hydrogen gas

To regenerate NAD^+ from NADH, hydrogen gas is created via a variety of fermentation processes, including mixed acid fermentation, butyric acid fermentation, caproate fermentation, butanol fermentation, and glyoxylate fermentation. Ferredoxin receives electrons, which hydrogenase then uses to oxidise it and produce H_2. Methanogens and sulphate reducers use hydrogen gas as a substrate, which keeps hydrogen concentration low and promotes the formation of this energy-rich chemical. However, hydrogen gas may still develop at quite large concentrations, as in flatus.

Bacteria like *Clostridium pasteurianum* ferment glucose to produce butyrate, acetate, carbon dioxide, and hydrogen gas as an example of mixed acid fermentation: The following equation results in acetate:

$$C_6H_{12}O_6 + 4\ H_2O\ 2\ CH_3COO + 2\ HCO_3 + 4\ H^+ + 4\ H_2$$

Although the global reaction only produces a little amount of energy, glucose might hypothetically be transformed into merely CO_2 and H_2.

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Fermentation produces methane gas

Methane and carbon dioxide may be produced by the dismutation reaction of acetic acid:

$$-36 \text{ kJ/reaction for the reaction of } CH_3COO + H^+ + CH_4 + CO_2$$

Methanogenic archaea, as part of their fermentative metabolism, catalyse this disproportionation process. From the carboxylic acid's carbonyl function (e' donor), one electron is transported. The process of fermentation is frequently employed in a wide range of industrial goods, including: The fermentation process results in the bulk production of cells that may be utilized to extract metabolites. An inoculum of microorganisms reaches its maximal growth rate when it is cultivated in an appropriately enriched production medium. The intended product can be extracted using the biomass that was collected. Microorganisms can create either primary or derived metabolites through the use of fermentation technology. Examples of primary metabolites generated by microorganisms during their development phase include ethanol, tryptophan, citric acid, lysine, or threonine. Microorganisms create secondary metabolites while they are stationary in their life cycle. Penicillin or bacteriocins, two types of antibiotics, are examples of secondary metabolites. Fermentation technology may be used to change the metabolic pathways utilizing molecular or cultivation-based methods. Fermentation is a common method used by pharmaceutical firms to manufacture recombinant vaccines, proteins, or hormones.

Industrial fermentation is the purposeful utilisation of microbes like bacteria and fungus to ferment in order to produce goods that are beneficial to humans. Products that have undergone fermentation may be used in both food and general business. Fermentation is used to create a number of common compounds, including acetic acid, citric acid, and ethanol. The number of microorganisms, cells, cellular components, and enzymes, as well as temperature, pH, and oxygen for aerobic fermentation, all affect the pace of fermentation. Concentrating the diluted solution is typically included in the product recovery process. Almost all commercially available enzymes, including rennet, invertase, and lipase, are created by fermentation using genetically altered bacteria. In other circumstances, like the creation of baker's yeast or lactic acid bacteria starting cultures for cheese manufacture, the goal is the production of biomass itself. In general, fermentations fall into one of four categories:

- Production of extracellular metabolites and biomass (viable cellular material) (chemical compounds)
- Intracellular component production (enzymes and other proteins)
- The alteration of the substrate (in which the transformed substrate is itself the product)

These categories provide a foundation for comprehending the variations in approach, however they are not always mutually exclusive from one another. It's possible to utilise bacteria, yeast, mould, animal cells, or plant cells as the organisms. The particular organisms utilised in the fermentation need certain considerations, such as the dissolved oxygen level, nutrition levels, and temperature.

The organisms are typically immersed in a liquid medium during most commercial fermentations, however in other cases, such with the fermentation of cocoa beans, coffee cherries, and miso, the organisms are only exposed to the wet surface of the media. The fermentation process is also

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relevant to industrial factors. For instance, the fermentation medium, air, and equipment are sanitised to prevent biological process contamination. Mechanical foam destruction or chemical anti-foaming chemicals may both be used to regulate foam. Pressure, agitator shaft power, temperature, and viscosity are just a few other variables that need to be monitored and managed. Scaling up is crucial for industrial fermentations. Here, a laboratory operation is transformed into an industrial process. In the area of industrial microbiology, it is well known that what is successful in the lab may not be successful at all when initially used at a big scale. In general, laboratory-tested fermentation conditions cannot be naively applied to machinery designed at industrial scales. Because fermentation processes vary, even though numerous characteristics have been investigated for use as scale-up criteria, there is no universal formula. The two most crucial techniques are to maintain a steady volumetric transfer rate and a constant power usage per unit of soup.

Stages of microbiological development

A chosen growing medium is "inoculated" with a specific organism when a specific organism is added to it. The inoculum grows gradually over time rather than right once. The lag phase, during which adaptation takes place, is at present. Following the lag phase, the organism's rate of development slowly accelerates; this phase, known as the log or exponential phase, lasts for a while. After a particular amount of time in the exponential phase, the rate of development slows down as a result of continually decreasing nutrient concentrations or continuously rising (accumulating) hazardous chemical concentrations. The deceleration phase is the one during which the pace of growth is being slowed down. The culture reaches a stationary phase or steady state after the deceleration phase, when growth stops. Except when certain accumulating substances in the culture lyse the cells, the biomass is constant (chemolysis). The chemical composition of the culture does not change until other microbes pollute it. The cells may become scenescent and start to decompose if all the nutrients in the medium are used up or if the level of toxins is too high. Although the absolute quantity of biomass may not change, fewer living things will exist.

Fermenting Agent

The bacteria that are employed in fermentation grow on (or on) a carefully formulated growth medium that provides the nutrients the organisms need to survive. There are many different types of media, but they all include a source of carbon, a supply of nitrogen, water, salts, and micronutrients. Grape must is used as the medium in the creation of wine. Any cheap carbon source that is accessible might make up the majority of the medium used to produce bioethanol. Typically, carbon sources are sugars or other carbohydrates, however they may also be alcohols or other substances in substrate transformations (such the creation of vinegar). To keep costs down, low-cost sources of carbohydrates like molasses, corn steep liquor, sugar cane juice, or sugar beet juice are employed in large-scale fermentations like those used to produce ethanol. Purified glucose, sucrose, glycerol, or other sugars may be used instead in more delicate fermentations to limit fluctuation and assist maintain the purity of the finished product.

Starch may be given to organisms designed to generate enzymes such as beta galactosidase, invertase, or other amylases in order to choose those that express the enzymes in a significant

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amount. Most organisms need fixed nitrogen sources in order to create proteins, nucleic acids, as well as other cellular components. Nitrogen might well be given as bulk protein, like soy meal, pre-digested polypeptides, like peptone or tryptone, or as ammonia or nitrate salts, depending on the enzyme capabilities of the organism. Cost is a significant consideration when selecting a nitrogen source. Phosphorus is required for the synthesis of nucleic acids as well as the phospholipids found in cellular membranes. The quantity of phosphate that must be added is determined by the nature of the broth, the requirements of the organism, and the intended outcome of the fermentation. For instance, in the presence of phosphate, certain cultures will not create secondary metabolites.

For organisms unable to synthesise all the vitamins they need, growth factors and trace nutrients are added to the fermentation broth. For fermentation medium, yeast extract is a typical source of minerals and vitamins. While unrefined carbon and nitrogen sources generally include inorganic nutrients, such as trace metals like iron, copper, zinc,manganese, molybdenum, and cobalt, using purified both carbon and nitrogen sources may need their addition.

Since fermentation broth often includes a range of foam-reinforcing proteins, peptides, or starches, fermentations that create significant quantities of gas (or that need to add gas) will tend to develop a layer of foam. Antifoaming chemicals may be applied to stop this foam from forming or building up. To keep pH close to ideal, mineral buffering salts like carbonates and phosphates may be utilised. Use of a chelating agent may be required in situations when metal ions are present in large quantities.

Production of biomass

Fermentation may sometimes result in the anticipated outcome of microbial cells or biomass. Examples include baker's yeast, lactobacillus, E. coli, and single cell proteins, among others. Algae are produced in large open ponds, which permit photosynthesis, in the case of single-cell protein. Care must be made to avoid mutations if the biomass is to be utilised as a starter culture for further fermentations.

Extracellular metabolite production

Microbial metabolites may be split into two categories: primary metabolites, which are created during the organism's growth phase, and secondary metabolites, which are produced during its stationary phase. Ethanol, citric acid, glutamic acid, lysine, vitamins, and polysaccharides are a few examples of primary metabolites. The drugs penicillin, cyclosporin A, gibberellin, and lovastatin are examples of secondary metabolites.

Primary metabolites

Primary metabolites are substances produced by an organism's normal metabolism throughout its growth period. Lactic acid and ethanol, which are both products of glycolysis, are frequent examples. Some *Aspergillus niger* strains create citric acid as a byproduct of the citric acid cycle in order to acidify their surroundings and keep out rivals. Some Corynebacterium species and some Micrococcus species produce glutamate, threonine, lysine, tryptophan, and other amino acids, respectively. All of these substances are created by the cell's routine "operations" and discharged

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into the environment. Therefore, the cells do not need to be ruptured in order to extract the product. Subsequent Metabolites Compounds produced in the stationary phase are known as secondary metabolites. Penicillin, for example, inhibits the development of bacteria that may compete with Penicillium moulds for nutrients.

Some bacteria, including Lactobacillus species, have the ability to create bacteriocins, which also inhibit the development of competing bacteria. These substances have clear benefits for those who want to stop the spread of germs, either as antibiotics or as antiseptics (like gramicidin S). In addition, secondary metabolites are formed that include fungicides like griseofulvin. Secondary metabolites, like primary metabolites, are released into the surrounding media without rupturing the cell membrane and are often not created in the presence of glucose or other carbon sources that would drive development.

Development of intracellular components

The microbial enzymes, such as catalase, protease, amylase, pectinase, cellulase, hemicellulase, streptokinase, lipase, lactase, glucose isomerase, and many more, are of particular importance among the intracellular components. This method is also used to produce recombinant proteins including insulin, the hepatitis B vaccine, interferon, granulocyte colony-stimulating factor, streptokinase, and others. The main distinction between this method and the others is the need to rupture (lyse) the cells at the conclusion of fermentation and to manage the environment to increase the quantity of the product. In order for the result, which is often a protein, to be purified, it must also be isolated from all of the other cellular proteins in the lysate. Alteration of the substrate in the case of phenylacetylcarbinol and steroid biotransformation, for example, substrate transformation refers to the conversion of one chemical into another. In the case of food fermentations and sewage treatment, it refers to the conversion of a raw material into a final product.

Fermentation of food

Making bread, cheese, dosa, curds, wine, idli, and other ancient fermented foods may be traced back more than 7,000 years. They were created long before man even knew the microbes in question existed. A consequence of the fermentation process, in this instance the brewing of beer, is certain foods like Marmite.

Alcohol fuel

The primary method of producing ethanol for ethanol fuel is fermentation. Yeast fermentation transforms common crops like maize, potato, cassava, and sugar cane into ethanol, which is then transformed into fuel.

Sewage treatment

Sewage is broken down during the sewage treatment process by enzymes produced by microorganisms. Carbon dioxide and nontoxic, soluble chemicals are produced during the breakdown of solid organic materials. The resulting liquids may be utilised as liquid fertilisers or are cleaned to get rid of germs before being dumped into the sea or rivers. Sludge, also known as

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digested solids, is dried and used as fertiliser. Gaseous waste materials, such methane, may be converted into biogas and used to power electrical generators. Bacterial digestion has the benefit of decreasing the volume and smell of sewage, which minimises the requirement for dumping area. The primary drawback of bacterial digestion in sewage disposal is how slowly it works.

Farm Animal Feed

Fermented waste products from the agroindustrial sector may be used to feed animals, particularly ruminants. Cellulosic wastes have been broken down by fungi to enhance protein content and improve in vitro digestibility. A sort of scientific experiment is a bioassay, often known as a biological assay or evaluation or biological standardisation. In order to ascertain a substance's biological activity, such as a hormone or medicine, a bioassay uses live animals or plants (in vivo) or cultured tissue or cells (in vitro). In order to create novel treatments and to monitor environmental contaminants, bioassays are often used to test how a material affects a living thing. Both methods involve examining a substance's effects on living things in order to assess its potency or nature. A bioassay may also be used to figure out how much of a mixture has a certain constituent that might be hazardous to living things or the environment.

By observing the impact on an organism, tissue, cell, enzyme, or receptor, bioassays may be used to ascertain the concentration, purity, or biological activity of a chemical such a vitamin, hormone, or plant growth factor. Quantitative or qualitative bioassays are also possible. When a substance's physical effects cannot be measured, such as when seeds do not germinate or develop aberrant deformities, qualitative bioassays are performed to evaluate the substance's effects. The well-known experiment with castrated hens conducted by Arnold Adolph Berthold is an example of a qualitative bioassay. According to this investigation, if a chicken's testicles were removed, it would not mature into a rooster since the endocrine signals required for this process were not present.

Estimating the dose-response curve, or how the reaction varies with increasing exposure, is a key component of quantitative bioassays. Because of the dose-response relationship, it is possible to estimate the dosage or concentration of a drug that will cause a certain biological reaction, such as the LC50 (concentration that will kill 50% of the organisms exposed). Biostatistical techniques are often used to examine quantitative bioassays.

In bioassays, the quantity of unknown potency preparation necessary to elicit a certain impact on appropriate test animals, organs, or tissue under standard settings is calculated. A typical effect is contrasted with this one. In order to compare the amount of the test drug needed to have the same biological impact as a certain amount of a standard preparation, a simple formula is used to calculate the potency of the unknown chemical as a percentage of the standard. Using this calculation often does not provide a trustworthy result. As a result, more accurate ways of determining potency based on observations of similar but not identical effects may be required. Statistical approaches may also be used.

The information (obtained from any of the used assay procedures) that forms the basis for bioassays may be categorised as either quantal or graded response. Both of these ultimately rely on predicating or assuming the shape of the DRC. Environmental bioassays often include a thorough toxicity assessment. To identify the relevant toxicants, a toxicity identification

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assessment is carried out. Although bioassays are useful for detecting biological activity inside an organism, they are sometimes difficult and time-consuming. Data that may not be relevant to other members of that species are a possibility due to organism-specific characteristics. For these reasons, different biological methods, such as radio-immunoassays, are often used.

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CHAPTER 2

Fermentative Production Methods

Roopashree Rangaswamy,

Assistant Professor, Department of Chemistry, School of Sciences, B-II,

Jain (Deemed to be University), J C Road, Bangalore-560027.

Email Id- r.roopashree@jainuniversity.ac.in

Due to its benefits for the economy and ecology, fermentation processes have become more important throughout time. The technology of the fermenters has also advanced with new process control devices, while ancient practises have been updated and improved to increase production. Submerged fermentation (SmF) or solid-state fermentation are two common fermentation techniques (SSF). While the first employs solid substrates like bran, bagasse, soya pulp, and rice, the second uses free-flowing liquid substrates or culture broths. Both fermentation techniques have seen improvements in terms of the types of substrates employed, process and instrument controls, and the choice of microorganisms—whether genetically modified or not. It is extremely desired to choose the optimum fermentation process for a given product and facility's availability since one approach may provide better outcomes than the other. Submerged fermentation (7.2) Fermentation refers to microbial activities that take place with or without air. The process of fermentation has been extensively employed to produce a broad range of compounds that are very advantageous to both people and industry. This advancement has led to the emergence of two distinct fermentation methods: SmF and SSF. Microorganisms are grown on both solid (small-scale agar plates) and liquid medium (flasks or bioreactors for large-scale cultivation up to several hundred cubic meters). The growing of microorganisms in a liquid nutrient broth is known as submerged fermentation. Using this method, industrial enzymes and other metabolites may be created. This entails cultivating certain microorganisms (bacteria, yeasts, and fungus) in closed containers with a nutritional broth (the fermentation medium). An appropriate gassing system introduces the necessary oxygen. As the nutrients are broken down by the microorganisms, the required enzymes are released into the culture solution.

A significant amount of the entire output of the biotechnology sector is produced by microbial enzymes as a result of the advancement of large-scale fermentation technologies. Industrial fermentation processes are carried out in large containers (fermenters) with capacities up to 1000 m3. The sterile fermentation medium includes nutrients made from basic sources that are renewable, such as soy, corn, or sugar. To break down the carbon and nitrogen sources, microorganisms secrete the majority of industrial enzymes into the fermentation medium. Fermentation techniques that are batch-fed and continuous are frequent. During the development of the biomass in the batch-fed procedure, sterilised nutrients are supplied to the fermen ter. As a result, throughout the continuous process, the flow rate of sterilised liquid nutrients entering the

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fermenter is the same as the flow rate at which the fermentation broth is being expelled from the system. We refer to this as steady-state production. To optimise the fermentation process, variables like temperature, oxygen consumption, pH, and carbon dioxide generation are analysed and managed. Insoluble products, such as microbial cells or pieces, must be eliminated during the extraction of enzymes from the fermentation medium. Centrifugation is often used to do this. The majority of industrial enzymes are extracellular (secreted by cells into the culture medium), thus even after the biomass has been taken out, they are still present in the fermented broth.

Potential Biotechnological For thousands of years, people have used fermentation to create food and drinks. For instance, fermentation is utilised to make alcoholic drinks as well as lactic acid, which is useful for preservation. These days, submerged fermentation methods are used in commercial settings on a considerable scale (a few thousand cubic metres). They consist of the process of creating industrially useful products from cells, such as microorganisms, or parts of cells, such as enzymes, in industries such chemicals, detergents, paper and pulp, food and feed, textiles, and biofuels. Consumers and the industry now embrace bioconversion through enzymatic routes or fermentation techniques using microorganisms as a sustainable option for the deployment of cleaner processes. These procedures make use of renewable raw resources, which may help reduce greenhouse gas emissions and shift the economy away from petrochemicals. Industrial fermentation helps reduce waste and recover valuable compounds from wastes via bioconversion by employing food industry residues as substrates. Waste reduction or recycling of valuable chemicals, byproducts, and residues are important issues for the global business. To limit the depletion of raw resources, all industrial branches must improve their manufacturing processes. Biotechnological methods do have a huge promise in this regard, if suitable.

Fermentation

The metabolic process of fermentation alters the chemical composition of organic substrates through the action of enzymes. The process of generating energy from carbohydrates without the presence of oxygen is expressly stated as such in biochemistry. If an intended alteration to a food or beverage is produced by the activity of microorganisms, it may more broadly apply to any approach utilized in food production. Zymology is the study of fermentation. Anaerobic breakdown of organic materials occurs during fermentation, which is the main process by which bacteria produce adenosine triphosphate (ATP). Since the Neolithic era, humans have employed fermentation to create meals and drinks [1]. For instance, fermentation is used to preserve food by creating the lactic acid that is present in sour foods like pickled cucumbers, kombucha, kimchi, and yogurt. It is also used to create alcoholic drinks like wine and beer. The process of employing microorganisms to produce chemicals, enzymes, proteins, biofuels, and medications on a big scale is known as industrial fermenting. All animals including humans have digestive systems that undergo fermentation.

History

An anaerobic chemical process called fermentation starts to break down molecules like glucose. Most precisely, fermentation is the process that has existed for at least 10,000 years that results in the foaming that occurs during the production of wine and beer. Although this wasn't realized until

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the seventeenth century, the production of carbon dioxide gas is what causes the foaming. Louis Pasteur, a French scientist, and microbiologist understood that ethyl alcohol or carbon dioxide is not the sole result of fermentation using the phrase with a restricted meaning to describe the changes caused by yeasts and other microbes growing anaerobically without air [2].

The Process of Fermentation:

Fermentation is an enzyme-catalyzed metabolic process that transforms starch or sugar into alcohol or an acid anaerobically and releases energy. The subject area of zymology deals with fermentation. Fermentation is an anaerobic biological process. The generation of pyruvic acid by glycolysis, which leads to the formation of net 2 ATP molecules, is the same first step in fermentation as it is in cellular respiration. In the following stage, pyruvate is changed into lactic acid, ethanol, or other substances. NAD+ is produced here and utilized once more throughout the glycolysis process, Figure 1 shows the process of fermentation technology. Through the activity of numerous enzymes, the metabolic process of fermentation generates energy from sugar or even other organic substrates. Anaerobic biochemical processes are what it is.

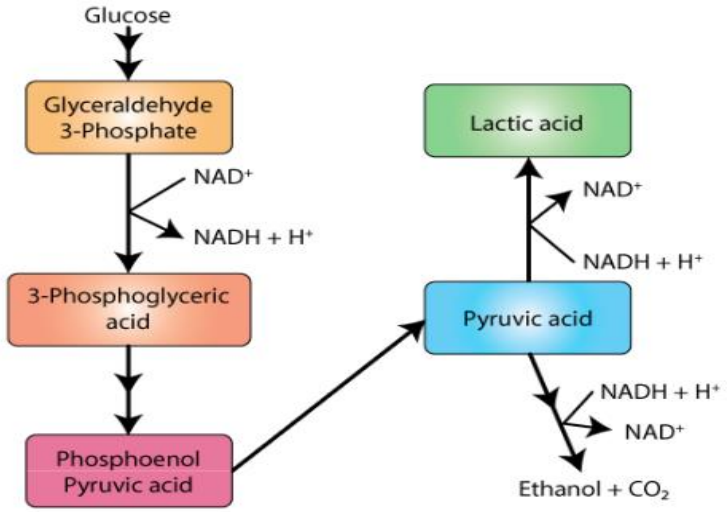

Figure 2.1: Illustration of process for fermentation.

The Value and Advantages of Fermentation:

Fermented foods include bacteria that can assist maintain a healthy gastrointestinal system so it can utilize nutrients from food more effectively. Fermented foods are high in probiotics. They have many positive effects on human health. Probiotics, lactic acid, and enzymes, which are included in fermented foods, can help the body absorb minerals and vitamins. In addition to increasing the number of vitamins, Vitamins B and C, fermentation also improves the absorption of folic acid, niacin, riboflavin, biotin, and thiamin.

In addition, phytic acid, a component of grains, nuts, seeds, or legumes that leads to mineral shortages, can be neutralized by fermentation. Additionally, phytates, the ionization form of phytic acid, reduce the absorption of carbohydrates, proteins, as well as lipids. In addition to generating the antibiotic, antifungal, antiviral and anticancer chemicals found in fermented food, bacteria or

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probiotics may help maintain a healthy gut by promoting an alkaline environment in that pathogens do not thrive.

Uses of Fermentation Everyday

Alcoholic drinks are frequently made by fermentation, such as wine made from fruit juice or beer made from grains. Starch-rich potatoes can be fermented or distilled to create gin and vodka.

Making bread involves the substantial use of fermentation. In the process of creating dough from sugar, yeast, flour, or water, the yeast consumes the sugar and releases carbon dioxide, which makes the bread rise. Yeast and lactobacilli are both used to make specialty pieces of bread like sourdough. This mixture provides the dough with its distinctively elastic texture and sour flavor.

Application of Fermentation in Industry

In the industrial setting, fermentation is utilized to produce ethanol for the creation of biofuel. Because it is made from feedstocks like grains or crops like maize, sugar cane, sugar beets, or cassava, it is a desirable renewable resource. In addition, it can originate from grasses, trees, or agricultural and forestry waste.

Given its availability and low cost, maize serves as the primary feedstock for ethanol fuel in the United States, which is the world's largest producer of fuel. One kilogram of maize may take approximately 0.42 liters of ethanol. Brazil is the second-largest producer, as well as the majority of its ethanol fuel, is produced from sugar cane. In Brazil, the majority of automobiles either operate on pure ethanol or a gasoline and ethanol mixture.

Hydrogen gas may also be produced during fermentation; for instance, in *Clostridium pasteurianum*, glucose is transformed into butyrate, carbon dioxide, acetate, or hydrogen gas. In the process of acetone-butanol-ethanol fermentation, microorganisms break down carbohydrates like starch and glucose to create acetone, n-butanol, or ethanol. During World War I, Chaim Weizmann invented this procedure as the main means of producing acetone.

Types of Bioreactor

Submerged fermentation processes may use a variety of bioreactor types, each of which has benefits and disadvantages depending on the application.

Stirred batch tank

The simplest sort of reactor is a batch stirred tank reactor. The system consists of a reactor and a mixer, which could be a stirrer, a turbine wing, or a propeller, if it is used as a batch reactor. The appropriate organisms or components of those organisms (such as enzymes) are added to the sterile substrate as an inoculant. These organisms then break down the nutrients into products that persist in the bioreactor until the culture is complete. Depending on the fermentation needs, the tank will look like the picture below.

The tank might have gassing equipment at the bottom of the container depending on the needs of the fermentation process. The sterile air intake must be given attention to so that an air filter can guarantee it. The oxygen partial pressure may be managed by adjusting the flow rate and mixer

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speed. By adjusting the temperature of a double jacket that is heated or cooled by water, the process parameter may be controlled in another way. Application, benefits, and drawbacks: High viscosity substrate solutions and mounted enzymes with moderate activity may both benefit from this reactor design. The drawback of this bioreactor is that certain metabolic materials have a propensity to break down when physically stirred.

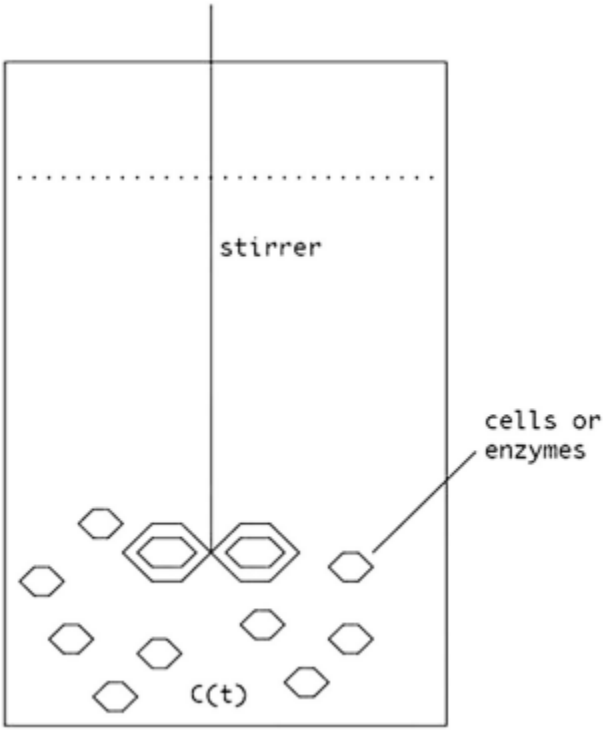

Figure 2.2: Illustrate the Principle of a batch stirred tank reactor.

In general, the batch method is appropriate for the production of relatively modest quantities. In constant-stirring tank the same components of a batch tank are included in a continuous stirred tank reactor, along with a substrate intake and a product exit for continuous harvesting. Although the equipment is somewhat more complex, the continuous stirred tank reactor is more effective than a batch stirred tank reactor. The reactant input assumes a uniform composition throughout the reactor, as well as the product output stream has the same composition as in the tank. The continuous stirred tank is operated at steady state with continuous flow of reactants and products. Federal batch culture another method for running a stirred tank is called a fed-batch culture, in which one or more nutrients are provided to the bioreactor throughout growth and the product(s) is/are kept in the bioreactor during the whole cultivation. The benefit of fed-batch culture is that exceptionally high densities may be attained since the concentration of fed-substrate in the culture medium can be adjusted to the optimum level.

Advantages

It is simple to build, manage, and clean. Low conversion rates per volume and rather strong shear pressures are drawbacks. The bottom of bubble columns serves as a gas entrance. They are

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cylindrical tanks. The gas is introduced as bubbles into a liquid or a suspension of a liquid and solid. When a solid phase is present, these reactors are often referred to as slurry bubble columns.

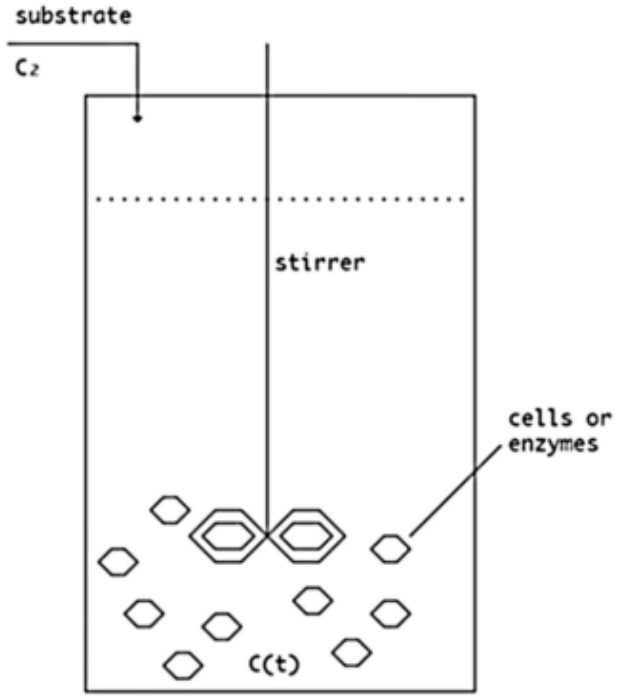

Figure 2.3: Fed-batch stirred tank reactor.

An ideal gas exchange is made possible by the gas intake, which is positioned at the bottom of the column and creates a turbulent stream. Gas sparging is used to combine materials, which uses less energy than mechanical stirring. The liquid may flow concurrently or countercurrently. Hydrodynamic variables such bubble rise velocity, gas residence duration, gas-liquid interfacial area, and gas-liquid mass transfer coefficient are significantly influenced by bubble size. The use of bubble columns as bioreactors for the synthesis of enzymes, antibiotics, proteins, and other substances, as well as in the anaerobic wastewater treatment process, is a significant application field. High heat and mass transmission, cheap investment costs, ease of construction, low energy costs, and the lack of moving components are benefits (low shear forces).

Airlift

Tower reactors for large-scale aerobic cultures called airlift bioreactors use injected gas and an airlift pump to mix the culture broth. At the base of the discharge pipe, which is submerged in the liquid, this pump injects compressed air. The gas-liquid mixture is created when compressed air is added to a liquid, making it less dense than the surrounding liquid. As a result, the surrounding liquid with a greater density pushes the less dense gas-liquid mixture upward through the discharge pipe. Solids may be entrained in the flow and, if they are tiny enough to pass through the pipe, will be released with it at a shallower depth or above the surface. Compressed gas that has been prepared by a compressor serves as the sole source of energy needed. Advantage: Low shear forces and just compressed air (or other gas) are needed. Difficult to regulate and rather expensive to compress flow rate.

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Bed that is fluid

In this kind of bioreactor, the substrate is transported through the pelleted or granular biomass or enzymes at speeds high enough to suspend the solid, and it is then mixed by a gaseous phase added to the reactor's bottom. The liquid-solid dispersion and gas input are separated by a porous plate. The mixing performance is equivalent to an agitated tank once the ideal settings are found, but without shear forces. Advantages: homogeneous mixing of the particles and constant temperature variations. High area needed and solid entrainment are drawbacks. Figure 2.4 shown the fluidized bed bioreactor's basic design.

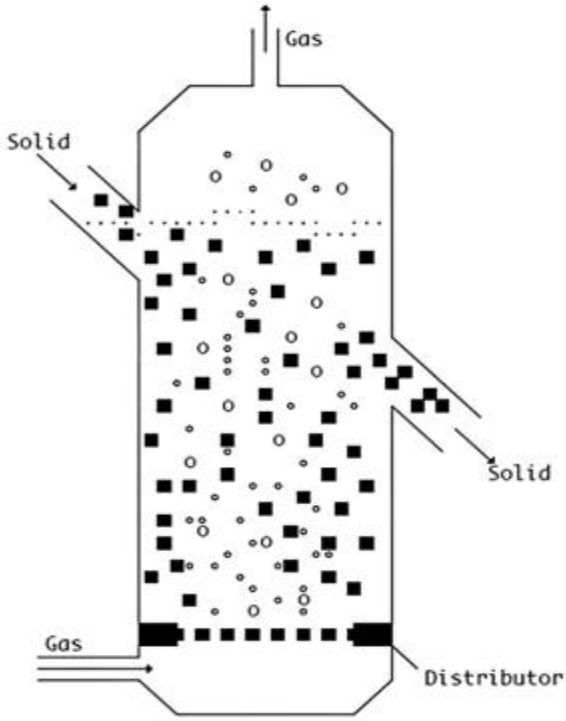

Figure 2.4: Basic design of fluidized bed reactor.

Trickle bed

In a trickling bed reactor, the liquid substrate is pumped downhill through a dense bed of pelleted or granular biomass or encapsulated enzymes, often accompanied by a gas. With just a liquid and a gas in- and outlet as well as a double jacket for temperature regulation, this reactor type is rather simple to build. This reactor type is used for catalytic reactions in the food sector as well as wastewater treatment in refineries (e.g. vinegar production). Limitations in mixing performance due to incomplete understanding of fluid dynamics.

Process Management

Process control is to maintain stable process parameters that enable operating fermentation systems to produce consistent products in large quantities. pH, temperature, dissolved oxygen, off gases,

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substrate concentration in the reaction tank, level, and turbidity may all be monitored throughout the fermentation process, as well as the parameters can be either manually modified or automatically regulated. The system's input variables are those that independently stimulate it and have the potential to affect the process' internal circumstances. The output variables are the ones that can be used to gather data about the system's internal state. The input variables are control variables because they may be changed. In order to regulate the input variables for the best process conditions, additional disturbance factors that cannot be managed independently are taken into consideration.

Microorganisms

Enzymes and other biomolecules are in high demand on the market for use in a variety of industrial applications; among those for detergent development, enzymes for food applications and the bioconversion of lignocellulosic biomass for the production of ethanol are the key application fields. The advantage of using microorganisms like bacteria, fungi, and yeasts for their production is the economical bulk production capacity, even though these molecules can also be extracted from plant material. The properties of the products can be improved by manipulating microorganisms, which is also simple to do. The produced biomolecules can be tailored for the desired properties depending on the strain and the culture conditions.

Substrates

All microorganisms require nutrients for cell activity, growth, and metabolism. Microorganisms have a wide range of metabolic capabilities and can grow on a variety of growth substrates. Because they are nutritionally diverse, bacteria, fungus, and yeasts may thrive in a variety of conditions. The pH control unit analyses the nutrient broth's pH and manages a pump that changes the pH by adding acid or base, as specified by the user, to maintain the ideal pH for the cultivated strain. Microorganisms need a sterile nutritional broth (substrate) that has a specific carbon source, minerals, and trace elements in order to grow if they are to be cultured. a catabolic reaction A physical and chemical environment that is constantly changing is present to bacteria. Within certain bounds, bacteria can respond to environmental changes by altering the patterns of structural proteins, transport proteins, toxins, enzymes, etc., enabling them to adapt to a specific ecological situation. When two different carbon sources are fed to the same bacteria, the first source will be used first because it requires fewer enzymes than the second source does. If a bacterium notices that one nutrient is in short supply in its environment, it can create a complex gathering and transport system. In general, bacteria do not produce degradative (catabolic) enzymes unless their environment contains the appropriate substrates for these enzymes. Different mechanisms have been developed by bacteria to regulate biosynthetic (anabolic) pathways. When the final product of the route is no longer required or can be acquired from the environment, bacteria terminate their biosynthetic process. For instance, it would make sense for a bacterium to shut down its own pathway of amino acid biosynthesis and conserve energy if it could find a preformed amino acid in its environment. Since the environment may alter suddenly and significantly, all of these metabolic pathways' regulation mechanisms must be reversible. The utilization of these less expensive carbon sources, such as processing waste, press cake, and pomace byproducts from the fruit and vegetable processing sector, is intended to cut manufacturing costs. Since they are made

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from plants that absorb CO2 from the atmosphere, renewable by-products from the fruit and vegetable processing industry not only have the advantage of being more environmentally friendly, but they have also recently shown to have more stable price and supply than raw materials made from fossil fuels. Nowadays, purified carbohydrates derived from corn starch, beet, and sugarcane are used in submerged fermentation processes as main raw materials, competing with their uses in food. Accordingly, various types of renewable waste resources, including industrial byproducts and organic material in municipal wastewater, have been used for the fermentation-based production of biomolecules.

Production of Biomolecules

Significant progress has been made over the past few decades in resolving issues related to submerged fermentation's use of raw substrates, such as food byproducts, to produce biomolecules. On that basis, it is possible to produce fuel, chemicals, succinic acid, bioplastics, ethanol, and even enzymes that can be used as food or detergent additives. People today favour foods that are not only nourishing, safe, and aesthetically pleasing but also provide health advantages like disease prevention. Bioactive substances such peptides, polysaccharides, polyphenols, phytosterols, carotenoids, and fatty acids have been recognised by the food industry as potential distinctive qualities when added to food items in a certain quantity. Because submerged fermentation of particular microorganisms (fungi, bacteria, and yeasts) is quicker, easier to control, more effective, and of greater interest to the food and drug industries than traditional field cultivation of plants, it has shown to be a promising alternative for production of various biomolecules on an industrial scale. Benefits and uses of submerged fermentation as previously stated, yeasts, fungus, and bacteria are great providers of active biomolecules. These compounds are simple to manufacture, especially when they are synthesised in the extracellular media. Grow by submerged fermentation, and specialised downstream processing may be used to get the product. Scientists are increasingly interested in SmF since it has been used to produce enzymes, exopolysaccharides, as well as other bioactive compounds with several nutraceutical, pharmacological, and cosmeceutical qualities because fruiting body development is a laborious procedure.

Fermentation in the Solid State

The cultivation of aerobic microorganisms, mostly filamentous mould or single organisms, embedded on wet substrate particles, whose interstitial cavity permits the passive gas diffusion, may be referred to as SSF. A solid substance may be either a natural support or an inert support.

Potential Biotechnological

SSF is a bioprocess that is carried out in the absence or almost complete absence of free water, but with the moisture required to support microbial growth and metabolism. These circumstances mirror the natural surroundings where they are found and where microorganisms are isolated. Because of its potential to produce products with significant industrial value, SSF has recently attracted interest from the academic and business communities. For the generation of various bioproducts, such as enzymes, spores, and secondary metabolites at the laboratory level, SSF offers some benefits over submerged fermentation. However, the use of this method for industrial production has been discouraged due to technical issues with the operation and control of

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fermentation in solid mediums. This is what spurred research into SSF's biochemical engineering components. To effectively deploy this system at the industrial level, bioreactor design and a bioprocess that can regulate the operational variables in SSF are required. Following that, we'll go over some of the bioreactors that can be used for SSF processes along with tried-and-true methods for keeping an eye on and managing operating variables.

Various Bioreactor Types

Typical laboratory glassware like Petri dishes, Erlenmeyer flasks, and Roux bottles are used as bioreactors in the majority of small-scale SSF research projects. These devices are simple, affordable, and enable simultaneous exploration of several experimental settings. They don't work well for scaling up the process and provide little insight into the operational factors.

Moisture in the substrate and water activity

The lack of water flowing freely is one of SSF's key qualities. As a result, the water content (moisture) in SSF relies on the carrier's ability to retain water. Any water loss in these circumstances may have an impact on microbial development. Water serves crucial biological purposes and is required for microbial development. In addition to supplying nutrients in soluble form and enabling the elimination of waste metabolites, water serves as a solvent. Additionally, water has a structural purpose that is important for the stability and performance of biological structures that are arranged at the molecular and cellular levels. The amount of water needed should be specified in terms of the thermodynamic constant Aw, which is linked to the chemical potential of water. The lag phase of microbial development is often prolonged by a decrease in Aw, which results in a poor biomass output. Dehydration of the solid bed or solute buildup in the substrate may both cause a reduction in the water activity of the solid substrate during SSF.

Water losses in lab-scale SSF bioreactors may be reduced by forcing aeration with humid air. Evaporative cooling causes significant moisture losses in large-scale SSF bioreactors, hence it must be paired with water input to provide moisture-content management. Since there are no instruments capable of taking these measurements automatically, online monitoring of moisture and water activity of the solid bed is challenging. Instead, the moisture loss from the solid bed may be calculated using continuous measurements of the water vapour in the gas phase.

A model that makes use of real-time observations of oxygen, carbon dioxide, and water vapour in the gas phase to calculate the extracellular and total water contents of wheat grains during SSF with *A. oryzae*. This model may be used to determine how much water must be added to a 35-L horizontal paddle mixer SSF bioreactor in order to manage the extracellular water content. Heat transmission and temperature When designing, operating, and scaling up SSF bioreactors, temperature management and heat dissipation are crucial considerations. The amount of metabolic activity directly relates to the amount of heat produced. However, microbial development and product synthesis are often temperature-sensitive processes.

Because the majority of solid substrates have poor thermal conductivities, heat removal in SSF is challenging to accomplish. The most significant method for removing metabolic heat in SSF is evaporative cooling. Evaporative cooling, however, results in moisture loss. Therefore, more water

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must be provided to support the regular development of microorganisms. Forced aeration enhances heat transmission in two ways: by directly transferring heat and by affecting the rate of evaporation. However, forced aeration in static bioreactors might result in the development of temperature and moisture gradients along the axis. Heat dissipation is facilitated by the solid bed's continual or intermittent mixing. Additionally, it lessens or inhibits the development of moisture and temperature gradients. However, in fungal cultures, mixing may also have an impact on mycelial growth. A mixture of aeration, water addition, and mixing is utilised in large-scale SSF bioreactors to regulate the temperature and moisture in the solid bed. Kinetics of growth and biomass Even though it is not the finished product, biomass is a crucial factor in the characterization of SSF. For example, developing mathematical models to determine particular yields, studying growth dynamics, and analysing the progress of the fermentation all need biomass estimate. Because it is difficult to distinguish between the biomass and the solid substrate in SSF, direct measurements of biomass are challenging. Therefore, it is usual practise to quantify biomass indirectly. A number of biochemical techniques are based on the detection of a biomass component, such as protein, glucosamine, ergosterol, or DNA.

Substrates

The inexpensiveness and accessibility of the substrate are two benefits of the SSF technique. SSF has used a variety of agro-industrial leftovers to produce a large number of biologic compounds. The most widely used low-cost substrate for the production of enzymes is wheat bran; however, a long list of low-cost substrates can be listed, including apple, banana waste, cassava waste, pomace, aspen pulp, cassava flour, coconut oil, coconut coir pith, oil cake, corn flour, corncobs, gramme bran, grape orujo, maize bran, mustard oil cake, palm oil mill waste, peanut meal, rapeseed cake, rice The solid substrate in SSF acts as both an anchor for microbial growth as well as a source of nutrients for the microbial culture that is developing. In SSF, the solid mass that has to be fermented is where microbial growth and product synthesis take place. In order to maintain the porosity throughout the process, a stiff substrate structure is also required. However, in the event of a very soft material, a mixture of varied texture residues may be the answer to get the highest water-holding capacity or moisture critical point. The majority of agro-industrial residues will cover the desired texture via the particle-sized selection.

Types of Fermentation

It is a metabolic process to ferment food. In the course of fermentation, the appropriate yeast and bacteria devour the carbohydrates necessary for the process and transform them into acid as well as alcohol. That's basically all there is to it! Practically speaking, fermentation is a set of procedures to stop a food product from decaying or decomposing. The food product still spoils, but it does so much more slowly and with more nutritional advantages. Beyond the production of food, various industrial operations, such the creation of sewage treatment, and hydrogen gas, and the creation of biofuels, involve fermentation. The eight primary categories of fermentation are highlighted in the following sections.

The kinds include:

1. Batch fermentation

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2. Continuous Fermentation Feed-Batch Fermentation
3. Anaerobic Fermentation
4. Aerobic Fermentation
5. Surface Fermentations
6. Sunk Fermentations Fermentation in the state.

Batch Fermentation

Because only a small quantity of sterilized nutrient media is initially added to the fermenter, batch fermentations are closed culture systems. An appropriate microbe is added to the medium, and it is then incubated for a certain amount of time to allow fermentation to occur under the best possible physiological circumstances. During the fermentation process, oxygen is introduced in the form of air, an antifoam agent, and an acid or base to adjust the pH. There will be a change in the composition of the culture medium, the biomass, and the metabolites as a result of the microorganism's cells multiplying and going through several stages of growth and metabolism during incubation. The fermentation continues for a certain amount of time or until all the nutrients have been used up. The product is separated once the culture broth has been collected. Under circumstances that promote the quickest growth rate and greatest growth, batch fermentation may be employed to create biomass, primary metabolites, or secondary metabolites. To get the most possible production of primary metabolites, the exponential phase of development should be extended; while, to obtain the highest possible output of secondary metabolites, it should be shortened. The product is removed from the fermenter along with the used medium and microbe cells. The desired product is isolated from the microbe and purified later on after it has generated in the right amounts.

Continuous Fermentation

It is a fermentation system that is locked off and runs forever. This approach involves continually or sporadically adding new nutritional medium to the fermenter while continuously or sporadically withdrawing an equal quantity of spent medium containing microbes in order to recover cells or fermentation products. As a consequence, the volume of the medium and the concentration of nutrients are maintained at their ideal levels. This has been run entirely automatically. The continuous fermenter is most effective when used at its full capacity, which decreases running costs and takes a while to attain high production.

Starting media and inoculum are introduced to the fermenter continuously. After the culture has developed, nutrients are added to the fermenter and broth is taken out at the same pace, keeping the amount of broth in the fermenter constant. The cell mass is reintroduced to the fermenter in continuous mode with cell cycle using microfiltration with bacteria or screens with fungus mycelium. In this method, the fermentation is carried out sequentially while using two or more fermenters at once. Different fermenters are used for the growth and synthesis stages of the fermentation process, respectively. In general, the first fermenter may be used for the growth phase, while the second and subsequent fermenters may be used for the synthetic phase. This method is best suited for fermentations in which the bacteria' growth and synthesis activities do not occur simultaneously. Synthesis, which has nothing to do with growth, takes place after the pace of cell division has reduced.

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Fermentation in Fed Batches

This is a batch fermentation modification. The substrate is always at an ideal concentration in this procedure since it is frequently and incrementally supplied as the fermentation develops. This is crucial because some secondary metabolites may undergo catabolite suppression when there is a high concentration of glucose, or other carbohydrate or nitrogen molecules, in the medium. For this reason, the essential components of the nutritional medium are supplied in modest amounts at the start of the fermentation and are kept there throughout the production stage. Typically, this procedure is used to produce medicines like penicillin. This phrase was initially used to describe supplying substrates to a medium when the nutrients are depleted in order to keep the nutrients at their ideal level.

High cell density production as a result of longer working hours (particularly growth associated products). Controlled conditions for supplying substrates during fermentation, especially in terms of the concentration of certain substrates, such the carbon source. Restrictions on the availability of substrates that are only necessary for product formation lead to control over the synthesis of byproducts or catabolite repression effects. Unlike batch fermentation, the method of operation is capable of overcoming and controlling variations in the organism's pattern of development. Enables water loss to be replaced by evaporation. An alternative way of operation for fermentations working with poisonous chemicals or compounds with limited solubility By manufacturing the corresponding antibiotic throughout the fermentation period, antibiotic-marked plasmid stability is increased. In contrast to batch fermentation, no further specialised equipment is needed. It is a successful approach for producing certain compounds, such as penicillin, which are generated at their best levels when the medium is depleted.

The Four primary types of fermentation are the production

1. Lactic acid,
2. Ethanol,
3. Butyric acid fermentation
4. Acetic acid fermentation

People just going to be concerned with the first two types of fermentation here since the formation of gas isn't very significant to what we want to eat on any given day. They would argue that acetic acid fermentation is actually a subtype of ethanol fermentation, despite the fact that some publications classify it as a separate kind. It will consequently come after our explanation of ethanol fermentation. At the conclusion of fermentation, several end products are produced, but also based on the kind of end product produced, fermentation is classified into various types: Depending on the quantity of finished products, there are two forms of fermentation. Homo Fermentation: Only one product is produced in this type of fermentation. Hetero Fermentation: This type of fermentation produces many end products.

Fermentation of Lactic Acid

The fermentation of lactic acid yields the metabolite lactate, or cellular energy, from the fermentation of glucose as well as other carbohydrates containing six carbon atoms. When there

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is not enough oxygen available to sustain aerobic respiration, this specific type of anaerobic fermentation often occurs in bacteria, mammalian red blood cells, some animal cells, or sporadically in skeletal muscles. Throughout this process, pyruvate, a byproduct of glycolysis, is changed into lactic acid. NAD^+ is created from NADH as lactate dehydrogenase catalyzes the process. Weariness results from the buildup of lactic acid caused by anaerobic respiration during exercise.

Fermentation of Alcohol

Because ethanol is the final result in this process, alcohol fermentation is also sometimes known to as ethanol fermentation. Simple carbohydrates are mostly converted by yeast into carbon dioxide and alcohol. It is extensively employed in the production of items including beer, wine, and biofuel. Acetaldehyde or carbon dioxide are both released during this process, which is carried out by pyruvic acid. Acetaldehyde is produced in the process of making ethanol. Additionally, the production of NAD^+ from NADH is used in glycolysis. The two phases of the process are catalyzed by two enzymes working in tandem. These are alcohol dehydrogenase as well as pyruvic acid decarboxylase.

Alcohol Fermentation

Vinegar is created by the fermentation of acetic acid. The following steps are included in the two-step procedure: Yeast is used to produce ethyl alcohol anaerobically from sugar. acetobacter is used to oxidize ethyl alcohol to produce acetic acid by aerobic respiration.

Fermentation of Butyric Acid

The bacteria responsible for butyric acid fermentation are spore-forming, obligately anaerobic members of the genus Clostridium. Because n-butanol, isopropanol, ethanol, acetic acid, or acetone are also produced depending on the species performing the process, it is also referred to as mixed acid fermentation.

In the first phase, glycolysis uses the oxidation of sugar to produce pyruvate. With the aid of the oxidoreductase enzyme, pyruvate is oxidized to create acetyl-CoA in the next step, which also generates CO_2 or H_2. Finally, butyric acid is produced by further reducing acetyl-CoA. With the production of a net 3 Molecules of ATP, butyric acid fermentation generates more energy.

Fermentation Benefits

Food that has been fermented tastes better, is simpler to digest, and is healthier for you. The following are advantages of ingesting fermented foods: Digestion is aided by fermented food because it keeps the stomach flora healthy. It is anti-carcinogenic in nature. For the immunological system, it is beneficial. People who cannot tolerate lactose might also benefit from it. Fermentation is used in a much wider range of contexts than only commercial and residential. For instance, it is utilized in sewage treatment facilities to create methane. When you exercise, your body employs a variety of metabolic processes to provide the energy your muscles need. For each type to function, specific actions are needed. One thing that both aerobic and anaerobic metabolisms do is

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make ATP, or adenosine triphosphate, which is utilised to power activities. Discover what aerobic and anaerobic metabolism are, how they function, and how exercising affects them.

Metabolic Principles

The processes your body employs to digest food into chemicals that cells may utilise for energy and then use those compounds to power bodily operations are referred to as metabolism. In order to convert food into carbohydrates, proteins, and fats, your body secretes enzymes. Your body's cells may then absorb them and utilise them to create adenosine triphosphate (ATP), the fuel for cells, via aerobic or anaerobic metabolic processes.

Muscle contraction, breathing, blood circulation, regulating body temperature, digestion of food, waste elimination, and brain and nervous system function are all a part of your body's general metabolism. Each cell in the body uses calories from meals to make energy. Your metabolic rate is the rate at which you expend calories. Your respiratory, circulatory, and muscular metabolism all rise as you exercise. To provide oxygen and nourishment to your muscles, you need to breathe and beat more quickly. Additionally, your body has to work harder to sweat and avoid overheating. The body employs two different forms of metabolism to convert fuel (the food you consume) into energy.

Respiratory Metabolism

Your body produces energy during aerobic metabolism by combusting carbs, amino acids, and lipids in the presence of oxygen. The term "burning" comes from the word "combustion," which refers to the act of burning something to produce energy. The body uses energy from aerobic metabolism for exercise and other bodily activities (like breathing). Exercises that use aerobic metabolism include continuous walking, jogging, or cycling.

The Anaerobic Metabolism

Carbohydrates are burned during anaerobic metabolism in the absence of oxygen to produce energy. This happens when your lungs are unable to keep up with the demand for oxygen in your bloodstream from your muscles. It is often only utilised for brief periods of action, such sprinting when running or cycling or lifting large weights. Insufficient blood oxygen prevents glucose and glycogen from completely decomposing into carbon dioxide and water. Instead, lactic acid is created, which accumulates in the muscles and impairs their functionality. Your body will often flip between aerobic and anaerobic metabolism when participating in sports and exercise that call for both steady running and brief sprints, including basketball, tennis, and soccer.

Metabolic Types: Anaerobic vs. Aerobic

In comparison to aerobic metabolism, anaerobic metabolism is less effective. In anaerobic metabolism, a glucose molecule can only generate three ATP molecules; in aerobic metabolism, it can generate. The fuel for the muscles is ATP. Aerobic metabolism can also break down lipids and protein, while anaerobic metabolism can only utilise glucose and glycogen. Anaerobic metabolism will be used to fuel the muscles during prolonged periods of intense activity in the anaerobic zone with a heart rate > 85% of maximal heart rate. You have a choice in how intensely

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you work out, however your body will automatically employ the energy pathways that will best accomplish the task at hand. Training plans are created to maximise the usage of aerobic and anaerobic metabolism for various sports and activities.

Lactic acid and Anaerobic Metabolism

Anaerobic glycolysis and anaerobic metabolism, which take place during hard activity, produce lactic acid as a byproduct. Although the heart uses lactic acid as fuel, having too much of it in your muscles prevents you from performing at your best because it slows down muscle contractions. Lactic acid may diffuse out of muscle cells during moderate-intensity exercise. However, it accumulates with intense muscular contractions. Your muscles burn and get weary as you accumulate more and more lactic acid. This often occurs when weightlifting, but it may also occur while sprinting or going uphill while jogging or cycling. You are compelled to take a step back and go more slowly so that your muscles can rest and lactic acid can leave the cells. The cycle is completed by the liver's subsequent conversion of lactic acid into glucose for energy.

The Effects of Anaerobic Exercise

Lactic acid is produced during anaerobic metabolism, and it may accumulate in the muscles to the point that you can "feel the burn." Anaerobic metabolism produces a common side effect of burning. Although fast contractions need higher anaerobic metabolism, fast-twitch muscle fibres also tyre more rapidly. High-intensity intervals convert an activity that is typically aerobic, such as endurance jogging, into anaerobic activity. Once your maximal heart rate has been surpassed by 90%, anaerobic metabolism is required.

Reducing lactic acid accumulation

With some training regimens, you may alter the lactate threshold, which is the point at which lactic acid starts to accumulate. These are often used by athletes to enhance their performance. In order to get them to their lactate threshold, they use an interval or steady-state training programme. Typically, an athlete's lactate threshold is achieved between 50% and 80% of VO2 max (maximal oxygen uptake). Elite athletes may boost it even higher so they can exert more effort throughout their activities. Maintaining a healthy diet is also crucial for keeping muscles well-stocked with glycogen for fuel.

Energy metabolism and aerobics

In the aerobic metabolic process, the body converts glucose into adenosine triphosphate (ATP) molecules. What powers your muscles is ATP. Anaerobic metabolism, which is employed for powerful muscular contraction, is significantly less effective since it generates a lot fewer ATP molecules per glucose molecule. Your cells generate energy via glycolysis, the citric acid cycle, and electron transport/oxidative phosphorylation as part of aerobic metabolism, which is a component of cellular respiration. The chemistry of how the body generates energy for exercise is complex. The body employs aerobic metabolism all throughout the day to provide energy for the cells, muscles, and organs to perform their normal functions. For this reason, in addition to the calories expended during physical exercise, you have a basal metabolic rate—a rate of calorie

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burning required to sustain normal bodily processes. Even while in rest, a live organism continues to burn some calories.

Additionally, oxygen is taken up by your lungs during aerobic metabolism so that it may be transported to your tissues by haemoglobin in the blood. Aerobic metabolism uses oxygen to oxidise carbohydrates; as a result, oxygen atoms are joined to carbon in the expelled carbon dioxide molecule. Carbon dioxide and water are the sole byproducts of the aerobic breakdown of carbohydrates. These are expelled by your body via breathing, perspiration, and urination. The byproducts of aerobic metabolism are simpler to eliminate from the body than those of anaerobic metabolism, which generates lactic acid. This describes light post-exercise muscular pain with aerobic metabolism. When doing aerobic exercise, the heart rate is kept below 85% of the maximal heart rate and no intense muscular contractions are used. By using aerobic metabolic mechanisms to break down carbs and lipids, your body can maintain a steady flow of energy. You may break down glycogen into glucose and mobilise stored fat to break down for energy while you are exercising at a moderate intensity level since you are breathing enough and your muscles' requirement for ATP is slowly and steadily increasing. Additionally, you may consume carbs that the body can utilise before its reserves run out. When they do this incorrectly, athletes "strike the wall" or "bonk."

Large muscular groups are used during aerobic activities to carry out the same tasks for at least 10 minutes at a time. As a result, your breathing and heart rates increase as your body provides the oxygen your muscles require for aerobic metabolism. For energy, this burns carbohydrates and fats. Walking at a quick speed where you may be breathing a bit harder but can still talk in entire sentences is one of the simplest cardio activities. An aerobic workout may be achieved by running, cycling, rowing, swimming, cross-country skiing, and cardio equipment including elliptical trainers, stair steppers, rowers, and ski machines.

Dancing is a fun aerobic exercise as well. These aerobic exercises may be either moderately strenuous or vigorously strenuous as long as your heart rate doesn't rise over 85% of your maximum. Even while yoga and tai chi employ your aerobic metabolism, they often don't cause your heart rate to increase sufficiently to be categorised as moderate-intensity aerobic exercise. Aerobic metabolism is helpful if your objective is to reduce weight via exercise since it removes fat from fat cells and burns it to provide energy for the muscles. Additionally, it burns up the sugars (carbohydrates) that are present and stored in your cells, preventing any surplus from being converted to fat. Your body replaces its energy reserves with eating. You won't retain additional food calories as fat if you don't consume more calories than you expend. However, exercise helps to develop muscle, so in addition to decreasing weight, you can also be adding muscle mass.

Aerobic fermentation and domestication:

Numerous businesses depend on aerobic fermentation, which led to the domestication of various yeast strains by humans. Throughout the course of human history, beer and other alcoholic drinks have contributed significantly to civilization via drinking rituals, the provision of food, medicine, and clean water. The transition from diverse and varied natural habitats to simple, stable ecosystems with a consistent substrate occurs during domestication. This often encourages

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adaptations for specialisation in domesticated microorganisms and is linked to lax selection for genes involved in pathogenicity or different metabolic methods. The characteristics of industrial species that encourage aerobic fermentation may in part be a result of domestication. In domesticated strains of Saccharomyces, introgression and HGT are frequent. Significant amounts of the DNA in many commercial wine strains are the result of HGT with non-Saccharomyces species. In contrast to what is seen under domestication forces, HGT and introgression are less frequent in nature. For instance, the significant industrial yeast strain Saccharomyces pastorianus is a cross between the cold-tolerant *S. eubayanus* and *S. cerevisiae*. This combination is often used in the lager-brewing process, which calls for a low-temperature, slow fermentation.

Cancer cells

Alterations to metabolism or the deregulation of cellular energetics are characteristics of cancer. In order to undertake lactic acid fermentation in the presence of oxygen instead of transporting the pyruvate produced during glycolysis to the mitochondria, cancer cells often altered their glucose metabolism. The Warburg effect is characterised by a high rate of glycolysis and a high rate of glucose intake. These cancer cells often exclusively produce ATP via the process of glycolysis, and the fermentation process in the cell's cytoplasm breaks down pyruvate. Since cancer cells need more energy owing to their ongoing growth and respiration generates substantially more ATP than glycolysis alone, this occurrence is sometimes seen as being paradoxical (fermentation produces no additional ATP). Normally, the glycolysis pathway's glucose transporters and enzymes are up-regulated. There are several similarities between aerobic fermentation in tumour cells and Crabtree-positive yeasts. A relevant model for understanding aerobic fermentation in tumour cells may come from further study of the development of aerobic fermentation in yeast like *S. cerevisiae*. This may help us comprehend cancer and cancer therapies better.

Aerobic fermentation in species other than yeast

In anaerobic environments, plants often employ alcoholic fermentation to create ATP and replenish NAD+ so that glycolysis may proceed. There are a few exceptions to the rule that fermentation only occurs in anaerobic environments for most plant tissues. No matter the oxygen content, the fermentation enzyme ADH is prevalent in the pollen of tobacco and maize. PDC is likewise widely expressed in this tissue in tobacco pollen, and transcript levels are unaffected by oxygen content. Similar to Crabtree-positive yeast, tobacco pollen also performs high levels of fermentation that are reliant on the presence of sugar rather than oxygen. The simultaneous occurrence of respiration and alcoholic fermentation in these tissues is accompanied by a high sugar availability. Acetaldehyde and ethanol, which are poisonous and may accumulate significantly during pollen formation, are products of fermentation. Acetaldehyde has been proposed as a pollen component causing cytoplasmic male sterility. Inability to generate viable pollen is a characteristic of plants such as maize, tobacco, and other ones that exhibit cytoplasmic male sterility. This feature is thought to be caused by the buildup of hazardous aldehyde and the much earlier than usual expression of the fermentation genes ADH and PDC during pollen formation.

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CHAPTER 3

Industrial Production of Amino Acids by Fermentation

Suhas Ballal,

Assistant Professor, Department of Chemistry, School of Sciences, B-II,

Jain (Deemed to be University), J C Road, Bangalore-560027.

Email Id- b.suhas@jainuniversity.ac.in

The identification of an effective producer of glutamic acid, *Corynebacterium glutamicum*, has made the commercial manufacture of amino acids by fermentation possible (synonym *Micrococcus glutamicus*). Since the bacteria was discovered at a period when monosodium glutamate was in high demand as a flavouring ingredient, a lot of study has been done on the synthesis of amino acids by microorganisms. The main motivation behind these efforts was the expectation that adding essential amino acids would increase the nutritional content of affordable vegetable proteins. Following the discovery of *C. glutamicum* via the screening of natural isolates, similar efforts resulted in the identification of bacteria that produce DL-alanine or L-valine.

However, it was discovered that the majority of wild type strains obtained from nature were unable to synthesise any additional amino acids in industrially relevant quantities except from the three previously mentioned. The prevention of over-synthesis is one of the key causes, which is why cellular metabolism is regulated. At the time *C. glutamicum* was isolated, the presence of these regulatory events was just beginning to be understood. When grown on a limited supply of the necessary nutrient, an auxotrophic mutant that is unable to generate the regulatory effector or corepressor (often the end product or a derivative of the end product) overproduces and excretes the precursor or the associated metabolite of a blocked process. This is the basic idea behind using an auxotrophic mutant to increase the synthesis of amino acids by microbes.

Since the 1950s, there have been ongoing efforts to use this phenomenon for the commercial production of microbial metabolites, and auxotrophic mutants are currently used to synthesise various amino acids. It is plainly futile to utilise an auxotrophic mutant to accumulate the byproduct of an unbranched route, such as arginine and histidine. The usage of a regulatory mutant is required to produce such a metabolite. By choosing an analog-resistant and prototrophic revertant from the auxotroph harbouring a defect in a regulatory enzyme, a mutant that has lost some biosynthetic control may be created. Mutants with several indicators, such as auxotrophy and analog-resistance, which contribute to the creation of the specified amino acid, are chosen to increase the yield of an amino acid. By preventing reverse mutation during fermentation, several markers also help to stabilise production, which helps to increase yield. The permeability barrier is another mechanism, along with metabolic control, that prevents microbes from releasing organic chemicals into the environment. It enables cells to store intermediates and large compounds required for microorganism survival.

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The majority of microorganisms' cells generate approximately 20 amino acids. They are used to create the proteins and other vital compounds that the cell needs. The identification of the glutamic acid-producing bacteria *Corynebacterium glutamicum* led to the beginning of the fermentation of amino acids (*Micrococcus glutamicum*). Since then, a great deal of study has been done on the fermentation-based generation of amino acids. Numerous microorganisms, particularly from auxotrophic bacteria, have been isolated from nature that are capable of generating amino acids via fermentation in economically viable amounts, as shown in Table 1.

Table 1.1: Illustrate Production of Amino Acids by Fermentation.

Amino acid	Strain used	Genetic characteristics	Yield (gl^{-1})	Carbon source
D-L-Alanine	*Microbacterium ammoniaphilum*	ArgHx	60	Glucose
L-Arginine	*Serratia marcescens* AT 428 (aru argR2 arg A2)	Transduction Canavanine	50	Glucose
L-Glutamic acid	*Corynebacterium glutamicum* *Brevibacterium flavum* *Arthrobacter paraffineus*	Wild type	100 98 82	Glucose Acetate n-Alkanes
L-Glutamine	*Corynebacterium glutamicum*	Wild type of glutamic acid producer	58	Glucose, with high biotin and NH_4Cl content
L-Lysine	*B. lactofermentum* Aj 11204 *B. flavum*	AEC^r Ala^- $CCL^rML^rFP^s$ Hom^{leaky} $Thre^-$	70 75	Glucose Acetate
L-Proline	*C. acetocidophylum*	Uncharacterized mutant	108	Glucose + Glutamic acid
L-Threonine	*E.coli* VL 344 (pYN7)	$_R$DNA	55	Sucrose
L-Tryptophan	*E.coli JP 4114*	$_R$DNA	23	Glucose
L-Valine	*Brevibacterium lactofermentum* No. 487	TA^r	31	Glucose

L-glutamic acid or L-lysine are the two amino acids that are most in demand for commercial manufacturing. These two amino acids are produced by fermentation with the aid of auxotrophs. The majority of the amino acid buildup takes place following the microorganism's log phase of development. The concentration of a certain growth factor is particularly important for the product's maximum production since concentrations that are above or below the optimum significantly lower the output. In general, microorganisms don't manufacture excess or more amino acids than they need. They manage cellular metabolism to do this. Microorganisms can, however, overproduce amino acids by manipulating the intricate regulatory mechanisms. This is accomplished by breeding mutant auxotrophs. The regulatory enzymes or repressors for an amino acid are made inactive as a result of mutation, causing the amino acid to accumulate in commercially feasible proportions and be utilised for fermentation. Aside from fermentation, several amino acids may also be produced rather cheaply by chemical means. Moreover, the D-

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isomers of amino acids that are often produced by these chemical methods are physiologically inert and cannot be employed as food additives or flavourings. Since only L-isomers are effective as food additives or flavouring agents, fermentation is the primary method used to create the majority of L-isomer amino acids.

Fermentation

Like other amino acids, phenylalanine is successfully produced by managing the synthesis process and planning cellular metabolism. Control is important for two reasons. The first stage is to optimally distribute the carbon flux among the four main products of glucose conversion—acetic acid, biomass, phenylalanine, as well as CO_2. The second factor is that when E. coli grows, its cellular physiology changes and tends to create acetic acid, which has a significant detrimental effect on the process' efficiency. Feeding sugar, regulating oxygen levels, sugar intake, or biomass concentration can all stop the creation of acetic acid. Glucose should be added at stage two of fermentation, when it has fully run out. As a result, feeding rate is a compromise that allows the process to operate at its maximum feeding rate.

L-Lysine

Microorganisms produce L-lysine, 2, 6-diaminohexanoic acid, either through the aminoadipic acid route or the diaminopimelic acid pathway. However, only one of the two other paths is used by each given creature. The DAP (Diaminopimelic Acid) pathway is used by bacteria, cyanobacteria (blue-green algae), certain phycomycetes, actinomycetes, and protozoa, whereas the aminoadipic Acid system is used by some phycomycetes, all ascomycetes, basidiomycetes, as well as eukaryotic algae.

Glutamic Acid

L-glutamic acid is a dicarboxylic amino acid that also has an amino-group connected to the -carbon atom or two carboxyl groups. Following the identification of *Corynebacterium glutamicum* in 1957, the fermentation-based synthesis of L-glutamic acid was initiated. Prior to then, it was made by chemical synthesis, and the final product included a combination of D and L-glutamic acid. Later, it was discovered that a large range of bacteria, including *Brevibacterium, Corynebacterium, Arthrobacter,* and *Microbacterium,* produce L-glutamic acid. According to reports, yeasts, streptomycetes, or fungi may also produce up to 30 g of L-glutamic acid per litre.

L-Phenylalanine

E. coli or *C. glutamicum* can be used to make L-phenylalanine. L-phyenylalanine is synthesised along a route that also produces L-lyrosine and L-tryptophan. Commonalities among these three aromatic amino acids. Erythrose-4-phosphate or phosphoenol pyruvate condense to form deoxyarabinoheptulosonate phosphate (DAHP), which is then converted to choristmate in six stages. Lastly, L-Phenylalanine is created in three more stages.

Economic Significance

Aspartame (L-aspartyl-L-phenylalanine methyl ester), a low-calorie sweetener used in soft drinks, is mainly composed of phenylalanine and L-aspartic acid. In medicine, a lot of amino acids are

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employed, especially as components of infusion solutions for post-operative therapy. Amino acids are utilised as raw ingredients in the chemical industry to create polymers including polyalanine fibres or lysine-isocyanate resins.

Polymethylglutamate is used as an upper layer in the production of synthetic leather. Some amino acid N-acetyl derivatives are used in the manufacture of cosmetics and as surface-active compounds. Urocannic acid, which is used as a sun-tanning agent, is produced from histidine. Glycine is necessary for the production of the herbicide glycophosphate.

Another crucial element for the synthesis of amino acids is permeability. In actuality, it was discovered that C. glutamicum's excessive L-glutamic acid synthesis was mostly caused by the permeability shift brought on by reducing the amount of biotin the bacteria needed. The majority of amino acids can now be produced by the so-called "direct fermentation" process, which refers to microbial production from a cheap carbon source by fermentation, even though certain specific environmental conditions make it possible to exploit a single enzymatic process or a process using a precursor. Commercial production of methionine, alanine, glycine, and cysteine does not include fermentation. Methionine, alanine, and glycine are formed in their racemic forms by chemical synthesis, while L-methionine and L-alanine may be created from the racemic form through the action of certain enzymes. Less than 1000 MT (1100 tonnes) of other amino acids are generated via fermentation annually.

L-glutamine and L-glutamic Acid

Producing Glutamic Acid from Carbohydrates

Kinoshita and Tanaka's review of L-glutamine and L-glutamic acid production (1972). A genus of bacteria headed by *Corynebacterium glutamicum* produces glutamic acid from carbohydrate in high yields (synonym Micrococcus glutamicus). *Corynebacterium glutamicum* (Micrococcus glutamicus), Brevibacterium flavum, B. lactofermentum, B. divaricatum, B. thiogenitalis, Corynebacterium callunae, C. herculis, Microbacterium ammoniaphilum, and others are among the species that make up this genus. The DNA of these bacteria contains guanine + cytosine (G-C) in a very small range (51.2 to 54.4 moles%). Their tight affiliations are also supported by morphological and physiological traits, and they may be categorised as a Corynebacteriaceae family of bacteria. These bacteria will henceforth be referred to as "glutamic acid bacteria" for convenience. The unique requirements for biotin and the absence or extremely low concentration of -ketoglutarate dehydrogenase in these bacteria enable them to excrete huge levels of glutamate. The primary regulating component in the fermentation is the need for biotin. The organism creates lactate when the amount of biotin required for optimum development is provided. Under less-than-ideal development circumstances, glutamate is excreted.

Approximately half of the given carbohydrates are converted into L-glutamic acid by glutamic acid bacteria under ideal growing conditions, with no byproduct production. The carbon source might be any kind of carbohydrate substance. Particularly suited are glucose and sucrose. Hydrolyzed starch solutions, cane molasses, and beet molasses are favoured for industrial uses. Additionally, acetic acid and ethanol are employed as sources of carbon. With the addition of penicillin during logarithmic growth or of fatty-acid derivatives like polyoxyethylene sorbitan-

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monooleate (Tween 60) before or during logarithmic development, carbon sources with a high biotin content, including cane molasses, are utilised. Nitrogen sources include ammonium sulphate, ammonium chloride, ammonium phosphate, aqueous ammonia, ammonia gas, and urea. Although a significant quantity of ammonium ion is required, a high concentration of it inhibits both organism development and glutamic acid synthesis. Ammonium ions are therefore added as the fermentation develops. The majority of industries employ ammonia water or gaseous ammonia.

Biotin, a crucial component of the glutamic acid bacteria's growth media, is crucial for the glutamic acid fermentation process. For development, the biotin concentration must be less than ideal. The strain, type, and concentration of the carbon supply all affect the optimal biotin concentration for glutamic acid fermentation, however it is typically less than 5 g per litre of medium. Some strains need biotin in addition to thiamin or cystine. Since iron-chelating compounds are created during autoclaving, they are not required when the carbohydrate is autoclaved with other medium components. However, certain iron-chelating compounds are essential for the development of glutamic acid bacteria.

Pathway for Biosynthesis from Glucose

There are at least 16 enzymatic steps in the main pathway, which is shown by the strong arrows. Reductive amination transforms -ketoglutarate into glutamate. The NADP-specific glutamate dehydrogenase is the enzyme responsible for catalysing this reaction. This enzyme's presence is necessary for the production of glutamate. Glutamate does not increase when resting cells are treated with glucose in the absence of ammonia; instead, -ketoglutarate does. The previous isocitrate dehydrogenase process provides the NADPH needed for glutamate dehydrogenase to function. Isocitrate dehydrogenase activity requires NADP, which is supplied by glutamate dehydrogenase. The production of glutamate is favoured by a low level of -ketoglutarate dehydrogenase. E. coli, which doesn't need biotin and isn't a glutamate excreter, has been used to illustrate the significance of the absence of this enzyme. Even in the absence of the biotin requirement, it was discovered that a mutant lacking -keto-glutarate dehydrogenase excreted 2.3 g of glutamate per litre whereas its parent did not.

Glutamic acid bacteria also employ the HMP route to transform glucose into 3- and 2-carbon compounds in addition to the EMP pathway. The TCA cycle may then be fed with these molecules. According to estimates of how much glucose each of the two routes uses on an individual basis, the EMP pathway is more prevalent during fermentation. When 14CO2 is used in glutamate fermentation, the radioactivity is fixed into the a-carboxyl group of glutamate. Oxalacetate carboxylase and the NADP-linked malic enzyme, which catalyses the fixation of carbon dioxide to pyruvate to produce malate, have both been discovered in glutamate excreters as participants in the fixation of carbon dioxide. Malate dehydrogenase oxidises malate to oxaloacetate, and oxaloacetate is subsequently changed into citrate. Isocitrate's two antagonistic responses are significant for this reason. The isocitritase process is required for the synthesis of intermediates for biosynthetic reactions as well as energy during the growth phase. After the development phase, however, the generation of glutamate would be better without the isocitritase process being active. This would suggest that the ideal environment for each phase growth and glutamate production should be different.

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L-glutamic acid cannot be produced from n-paraffins using biotin auxotrophs or oleic acid auxotrophs. When penicillin or cephalosporin was added, L-glutamic acid was excreted together with phospholipid and N-acetylglucosamine, which are components of the cell membrane and cell wall, respectively. This finding led to the isolation of a *Corynebacterium alkanolyticum* glycerol auxotroph. The quantity of glycerol available in the auxotroph controlled the amount of cellular phospholipid synthesis. In the culture with 0.01% glycerol but without penicillin, the mutant generated roughly 40 g of L-glutamic acid per litre from n-paraffins. Studies using the auxotroph reveal that phospholipid content regulates the permeability of L-glutamic acid in membranes rather than always being regulated by the amount of unsaturated fatty acyl residues in cells.

Penicillin causes a quick 97–99.5% decline in viability in *C. glutamicum* log phase cultures, which is followed by a rapid rate of glutamate excretion. After a little initial rise, cell mass as measured by optical density and total cell count stay mostly stable. However, during the period of declining viabilty, the packed cell volume reduces by 60–80%, suggesting a change in the surface characteristics of the cells. Following the addition of penicillin, the cells continue to generate glutamate for 40–50 hours with no lysis occurring throughout the whole fermentation. It seems that the cells are able to maintain their shape and undergo permeable "resting" entity metabolism in the absence of an excess of powerful mucopeptidases. *C. glutamicum* produces glutamate during development in a medium with a high biotin content up until the cell reaches saturation at 25–35 g per mg of dry weight. It is believed that some kind of feedback regulation of glutamate toward its own synthesis is what caused the production to stop. The feedback effect is released and additional synthesis is permitted when the cells become more permeable and glutamate travels from the cells into the media.

Under some circumstances, glutamic acid bacteria may also create L-glutamine and N-acetyl-L-glutamine in addition to glutamic acid. Maintaining the medium's pH at a slightly acidic level will promote glutamine synthesis, while maintaining a neutral to weakly acidic pH level will boost N-acetylglutamine production. 20% of glucose is converted to glutamine under ideal conditions, and over 20% is converted to N-acetylglutamine. Growth and the synthesis of glutamic acid are both hindered by high ammonium ion concentrations. Growth is stimulated and glutamine synthesis is increased by increasing the biotin supply and adding natural nutrients like corn-steep liquor and meat extract to the medium. The synthesis of N-acetylglutamine is suppressed by these nutrients or Zn++ when present in concentrations above those needed for development. A appropriate strain (KY 9003) was chosen, and restricting the amount of Zn++ in the medium allowed for preferential N-acetylglutamine synthesis. Gastric ulcers may be treated with L-glutamine and N-acetylglutamine.

L-lysine: Fermentation-based L-lysine production:

Nakayama reviewed the L-lysine synthesis by microorganisms (1972B). The first microbial method for producing L-lysine was combining the manufacture of diaminopimelate by an *Escherichia coli* lysine auxotroph with the decarboxylation of the resulting chemical by *Aerobacter aerogenes* or wild type *E. coli*. First, a homoserine-auxotroph of *Corynebacterium glutamicum* (or threonine plus methionine) was used to directly produce L-lysine from carbohydrates. A homoserine auxotroph of *Brevibacterium flavum* was found to undergo the same

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kind of procedure. Because extra threonine hindered growth and methionine addition relieved the inhibition, the leaky homoserine auxotroph was identified as a threonine-sensitive mutant. This occurrence is brought on by threonine's feedback suppression of residual homoserine dehydrogenase. However, the yields were smaller than those from the homoserine auxotroph of coryneform bacteria. Homoserine auxotrophs of other bacteria were also discovered to make L-lysine. Leucine and threonine auxotrophs of *C. glutamicum* generate considerable quantities of L-lysine, but they fall short of the homoserine auxotroph in this regard. The homoserine auxotroph of *C. glutamicum* was also superior to other auxotrophs of this bacterium and other bacteria.

It has been discovered that double auxotrophs, which need at least one of the amino acids threonine, isoleucine, or methionine in addition to homoserine for development, are strongly stable, with minimal inclination to return to homoserine independence. Since many of the microorganisms are double mutants in the homoserine pathway, it is conceivable to not only avoid reversion of the cultures to a wild type condition but also to manufacture lysine in larger quantities. In the commercial manufacture of lysine, cane molasses is currently typically utilised as a carbon source, while other carbohydrate sources, acetic acid, and ethanol can also use. By adding ammonia or urea throughout the fermentation, the pH of the medium is kept close to neutral. Generally speaking, ammonium salts are excellent suppliers of nitrogen, and urea may be employed for species that have urease activity. One homoserine dehydrogenase that is threonine-sensitive and methionine-repressible, as well as one aspartokinase that is concerted feedback-inhibited by threonine plus lysine, are both present in *Corynebacterium glutamicum*. However, *E. coli* possesses two homoserine dehydrogenases, one of which is threonine-sensitive and threonine-repressible whereas the other is methionine-repressible and insensitive. In addition, three isoenzymes of asparto-kinase are involved in the phosphorylation of aspartate in *E. coli*, one of which is repressible by methionine and insensitive to threonine and forms a complex with the homoserine dehydrogenase repressible by methionine; the second is multivalent, repressible by threonine and isoleucine and inhibited The contribution of methionine, lysine, and isoleucine deficits to threonine overproduction in E. coli is explained by these regulatory mechanisms. Isoleucine auxotrophy also helps to prevent the production of threonine from being metabolised.

To acquire the optimal microbial performance after strain creation, the culture conditions must be tailored for each individual strain. Basic research must be effectively transferred into industrial operations for a process to be commercially realised. Scale-up is a process in which a large-scale system's design, testing, and implementation are based on the findings of smaller-scale studies. The traditional batch fermenter, an agitation jacketed pressure vessel with cooling coils, baffles, as well as a sparger ring to provide vapour to the fermentation process, is the workhorse of the fermentation business. Using the top-performing mutants, a batch-fed technique is used to synthesise the majority of the amino acids. The following stages are at least involved in the fermentation process: (1) a culture medium charge and sterilisation are performed on a fermentation tank. The medium includes the necessary sources of nitrogen, sulphur, and phosphorus in addition to certain trace elements. A appropriate carbon source (such sugar cane syrup) is also present. (2) The fermentation tank is then filled with a seed culture of the production strain that was previously produced in a smaller fermenter, and it is agitated while meeting the required temperature, pH, and aeration requirements. (3) In order to get the best yields, extra

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nutrients are carefully given throughout the fermentation as needed, depending on the needs of the culture. (4) The microbe releases the amino acid into the fermentation solution, which is then separated by ion exchange and crystallised to isolate it. The amino acids generated by microbial processes are the L-forms, which are presently manufactured in large facilities for industrial purposes. When compared to synthetic procedures, the process is favourable because of its stereo specificity.

L-glutamic acid production

The market for amino acids' major product category is glutamate. A powerful flavour enhancer, monosodium glutamate is essential to the flavour of cheese, seafood, meat broths, and other meals. Two more umami compounds, inosinate and guanylate, are generated from primary metabolites in addition to glutamate. Despite the fact that glutamate is a naturally occurring substance in many meals, it is regularly added to improve taste. Several organisms from numerous taxonomically similar genera, such as *Micrococcus*, *Brevibacterium*, *Corynebacterium*, or *Microbacterium*, are able to produce too much glutamate. *Brevibacterium flavum* & *Brevibacterium lactofermentum* have been classed as *C. glutamicum* subspecies. Due to feedback control, glutamic acid overproduction is often not anticipated to happen. PEP carboxylase, citrate synthase, or nicotinamide adenine dinucleotide phosphate (NADP)-glutamate dehydrogenase are three enzymes that are suppressed by glutamate as part of the glutamate feedback regulation. Glutamate may, however, be pumped out of the cell by reducing the efficiency of the barrier to outward passage, enabling its manufacture to continue unhindered. Up until a very high amount is accumulated, glutamate excretion releases the glutamate pathway from feedback regulation; commercial.

L-amino acids are important biological building blocks that are employed commercially as food additives, feed supplements, infusion ingredients, therapeutic agents, precursors for the manufacture of peptides, or agricultural-based chemicals. Following antibiotics, amino acids are the second-most significant group, with fermentation products showing the fastest growth rates (1). The first commercially manufactured amino acid was L-glutamic acid. The chemical was discovered and named by German scientist Karl Heinrich Leopold Ritthausen in the year 1866. Due to the development of racemic mixture, the chemical route of synthesis is not often favoured for the production of L-glutamic acid, which was mostly generated by microbial fermentations (2). *Corynebacterium species* are used in biotechnological procedures to economically produce glutamic acid by submerged fermentation (3). Coryneform bacteria are used to manufacture L-glutamic acid on an annual basis. Several fermentation processes have been utilised to produce glutamic acid (4–6). One of the main carbon sources used in the synthesis of glutamic acid is glucose. With the use of submerged fermentation, glutamic acid was made from a variety of raw materials, including sugar cane bagasse (6), palm waste hydrolysate (7), cassava starch (8), and date waste.

Microbial cell immobilisation in biological processes may take place either naturally or by an artificial technique. Cells were immobilised using the techniques of adsorption, cross-linking, covalent bonding, and encapsulation. These are all frequently used techniques for enzymes and microbial cells, and their use relies on the cultures and environmental factors (10). Cells that have

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been artificially immobilised grow more slowly and the manufacturing process is facilitated. Immobilization and co-immobilization of cells and enzymes have been shown to make two- or multi-step conversions possible in a single step in biotechnology. Co-immobilized cells result from the binding of the insufficient enzyme from an external source to free or immobilised microorganisms or from the immobilisation of mixed cultures capable of performing two- or multi-step conversions in a single step. Co-immobilized cells may provide new opportunities for synergistic action that increase yield and conversion in ways that independently immobilised cells cannot. In order to produce glutamic acid, entire *C. glutamicum* cells were immobilised, along with a mixed culture of *C. glutamicum* and *P. reptilivora* grown in an optimal medium. The current work also focused on the reusability of the immobilised cells for the synthesis of glutamic acid.

L-glutamate

By deliberately altering the growth conditions, glutamate excretion can be affected in the following ways: (1) biotin deficiency causes glutamate overproduction in *C. glutamicum* by lowering the cell membrane permeability barrier that prevents glutamate from being excreted (all glutamate overproducers are naturally occurring biotin auxotrophs); and (2) the addition of penicillin or fatty acid surfactants (such as Tween 60) to an exponentially growing culture changes the All of these adjustments, it seems, leave the cytoplasmic membrane lacking in phospholipids, favouring active glutamate drainage from the cell. The finding that oleate restriction of an oleate auxotroph and glycerol limitation of a glycerol auxotroph likewise result in glutamate excretion further supported this theory. In addition, it was subsequently shown that glutamate-excreting cells had very low levels of cell lipids, particularly phospholipids. Also shown was the fact that the mycolic acid layer of the cell membrane becomes more permeable as a result of the numerous treatments that promote glutamate overproduction. A unique cell envelope made of mycolic acids that surrounds the whole cell as a structured layer and is hypothesised to be involved in solute penetration distinguishes glutamate-overproducing bacteria from other types. The second lipid layer of the cell is made up of the noncovalently bound mycolic acid derivatives and mycolic acids esterified with arabinogalactan, the first being the cytoplasmic membrane. The cytoplasmic membrane's chemical and physical characteristics are changed, and glutamate efflux is significantly altered, by overexpression or inactivation of lipid synthesis-related enzymes.

Manufacturing of L-lysine

An economically significant and necessary amino acid utilised as a food and feed supplement is lysine. In the creation of diets with a balanced amino acid content and in amino acid infusions, it also has certain pharmacological uses. Lysine has been produced using chemical, enzymatic, and fermentation techniques. This study summarises the work of several scholars who have contributed to our understanding of how bacteria produce lysine via fermentation. It also describes several techniques, such as the creation of fresh auxotrophic mutants and the improvement of culture conditions that may be used to raise lysine's overall production and quality.

The section of amino acids with the quickest growth is lysine. The majority of cereals eaten worldwide are lacking in the amino acid L-lysine. This is a crucial component of the billion-dollar animal feed business and is necessary for the development of animals. Cereals may be made into

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balanced diet or feed for animals, such as pigs, poultry, and other livestock, by adding lysine to them. Amino acids are nutritional ingredients that are necessary for all living organisms. The 20 primary amino acids each have distinct chemical properties and different degrees of commercial interest. L-lysine is an essential amino acid that is very crucial commercially since it is required in animal and human feed to meet their nutritional demands. Due to the increase in demand for it, L-lysine must be manufactured on a large scale. Lysine is largely created when bacteria, particularly *Corynebacterium glutamicum*, digest carbohydrates. Metabolic engineering and genomic approaches were used to create the concept of genome breeding. The systematic insertion of desired genotypes that increase industrial efficiency into the wild-type genome is now possible because to the accessibility of complete genome sequences. This approach was originally used in the commercial production of l-lysine to improve the overall manufacturing process and yields. The present review's major focus is the expansion of l-lysine production.

One of the necessary amino acids that the body cannot natively produce is lysine. Lysine is essential for the production of bones, hence children and developing animals have higher lysine requirements. According to universal consensus, lysine is the amino acid that is most lacking in both human and domestic meat-producing animal diets. Cattle, poultry, and other live stocks cannot manufacture lysine because animal feeds like grain and defatted oil seeds only contain trace amounts of this amino acid. In order to offer a sufficient diet, it must be added to these feed ingredients. The excretion of minor amounts of histidine, alanine, glutamic acid, and aspartic acid in an E. coli culture. Additionally, they discovered that adding more ammonium salt than was necessary for growth boosted the synthesis of amino acids. The concepts of the fermentative process were readily accepted, and systematic work on the synthesis of additional amino acids soon followed. The business of fermenting amino acids was therefore born (Aida, 1972).

Numerous microorganisms, including bacteria, yeast, filamentous fungus, and actinomycetes, accumulated amino acids in culture when a secondary source of nitrogen was present, according to research on the potential use of wild strain. The processes of microbial amino acid synthesis have lately received a lot of attention. The most notable findings relate to amino acid transport and metabolic control. Now that the biosynthesis routes for the majority of amino acids are well known, the emphasis has shifted to metabolic regulation and its breakdown, including the genus and species uniqueness of the phenomena (Aida, 1972). Numerous labs have investigated the creation of protein by microorganisms that are abundant in essential amino acids, both as a dietary supplement and as a source of amino acids. The cell hydrolyzate included fifteen amino acids, with arginine (1.14 g L-1) and L-lysine (0.4 g L-1) being the most prevalent.

Due of numerous metabolic regulatory systems, the majority of wild strains cannot generate industrially significant levels of L-lysine in the culture broth. L-lysine buildup, however, may result from changes to these mechanisms. There are two different biosynthetic routes that may produce L-lysine. The carbon skeleton of L-lysine is produced in certain actinomycetes, fungi, and algae through biosynthetic processes that also contain -amino adipic acid from acetate and -ketoglutarate. Bacteria, higher plants, blue-green algae, certain fungus (some phycomycetes), and protozoa have all been shown to use the other method. A crucial intermediary in the synthesis of the L-lysine carbon chain from pyruvate and aspartate is -diaminopimelic acid.

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The aspartate family of amino acids includes lysine. It is produced through a branching process in bacteria that also produces methionine, threonine, or isoleucine. In species like Escherichia coli, which has three aspartate kinases (AKs) that are each controlled by a distinct end product, this route is carefully regulated. Additionally, the beginning enzymes at each branch point are blocked by the corresponding end products, so no overproduction often takes place. The bacteria *Corynebacterium glutamicum*, which is utilised to make L-lysine for human consumption, has a single AK that is controlled by threonine and lysine by coordinated feedback inhibition. Depending on the amino acid being made, the proportional contribution of the carbon flow via the pentose phosphate route varies. For instance, although it contributes only 20% of the overall flux in the case of glutamate generation, it provides 60-70% in the case of lysine production. This is undoubtedly caused by the substantial amount of nicotinamide adenine dinucleotide phosphate (NADPH) needed for lysine synthesis. The low levels of aspartase, dihydrodipicolinate synthase, PEP carboxylase, and feedback inhibition of AK by lysine plus threonine are the mechanisms that greatly restrict the overproduction of lysine, according to research using rDNA technology.

Making of Aromatic Amino Acids

L-tryptophan and L-phenylalanine are two aromatic amino acids that have a variety of uses in the food business. An essential amino acid called tryptophan is employed as a feed addition as well as in the food, beverage, pharmaceutical, and cosmetic industries. In 2010, there was an annual need of 5000 tonnes worldwide. 3-Deoxy-D-arabino-Heptulosonate 7-Phosphate Synthase (DAHPS) is feedback inhibited concertedly by phenylalanine and tyrosine and moderately repressed by tyrosine in *C. glutamicum* subsp. flavum. Phenylalanine, tyrosine, and tryptophan do not inhibit the other enzymes of the common route but they do suppress the following ones: shikimate dehydrogenase (SD), shikimate kinase (SK), and 5-enolpyruvylshikimate-3-phosphate synthase, as shown in figure below. When the aromatic amino acid absorption mechanism in *C. glutamicum* is removed, unregulated strains produce more aromatic amino acids.

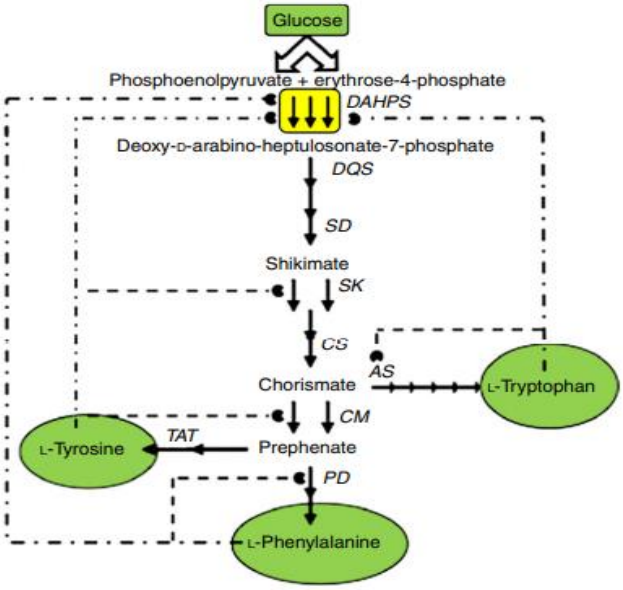

Figure 3.1: Biosynthesis of aromatic amino acids (Sikmic Acid Pathway)

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L-tryptophan is an important amino acid that is added to low-protein diets for pigs who may be lacking in it when their grain intake is high. It is very useful for enhancing feed intake, growth, and feed efficiency in young piglets. Tryptophan may also be broken down by the body into nicotinic acid or nicotinamide and also plays a role as a precursor for serotonin or melatonin. The fermentation-based microbial synthesis of amino acids serves a market with promising development prospects and greatly improves our current standard of living. Microorganisms have the ability to transform affordable Amino acids, which may be added to food as a flavouring agent or to boost its nutritional content, are useful metabolites that are produced from the transformation of carbon and nitrogen sources. Additionally, a few amino acids are proven to be quite useful as biosynthetic precursors for the production of pharmaceuticals.

The strain being use's capability for overproduction determines how well a fermentation process can create amino acids. Early fermentation systems relied only on classical strain breeding, which included rigorous rounds of random mutagenesis followed by an equally demanding programme of screening and selection. More logical methods for strain improvement have been made possible by recent advances in molecular biology and the creation of new tools in functional genomics, metabolomics, and proteomics. The majority of amino acids are typically synthesised using high-performance mutants in a batch-fed method, then separated using ion exchange chromatography in order to crystallise them. The importance of amino acids thus, in turn, microbial fermentations is expected to increase as we move into a new age when the utilisation of renewable resources is seen as a pressing need.

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CHAPTER 4

Lactic Acids Fermentation

Swarupa. V

Assistant Professor, Department of Chemistry, School of Sciences, B-II,

Jain (Deemed to be University), J C Road, Bangalore-560027.

Email Id- v.swarupa@jainuniversity.ac.in

A metabolic process called lactic acid fermentation turns six-carbon sugars like glucose or other six-carbon sugar disaccharides like sucrose or lactose into the metabolite lactate, which is lactic acid in solution, and cellular energy. In certain bacteria and animal cells, such muscle cells, an anaerobic fermentation process takes place. Many organisms will skip fermentation and go straight through cellular respiration if oxygen is available; however, facultative anaerobic species will both ferment and go through respiration in the presence of oxygen. In certain cases, even when oxygen is available and aerobic metabolism is taking place in the mitochondria, fermentation will still occur if pyruvate is accumulating more quickly than it can be digested. Pyruvate and lactate are converted into one another by lactate dehydrogenase, which also catalyses the conversion of NADH and NAD+. One glucose molecule is finally split into two lactic acid molecules during homolactic fermentation. Contrarily, heterolactic fermentation, which is also known as the phosphoketolase route, also produces carbon dioxide and ethanol in addition to lactic acid.

Glucose and other six-carbon sugars, as well as six-carbon sugar disaccharides like sucrose or lactose, are converted into cellular energy and the metabolite lactate, which is lactic acid in solution, through a metabolic process known as lactic acid fermentation [3]. An anaerobic fermentation process occurs in various animal and bacterial cells, including muscle cells. If oxygen is present, many organisms will skip fermentation and proceed directly to cellular respiration; however, facultative anaerobic species will ferment and undergo respiration simultaneously. In certain situations, fermentation will continue take place even when oxygen is present and aerobic metabolism is occurring in the mitochondria if pyruvate is building up faster than it can be digested. For many years, it has been discovered that lactic acid bacteria (LAB) fermentation is used in the dairy sector, the manufacture of wine and cider, the manufacturing of fermented vegetable products, as well as the meat industry. The importance of eating in promoting health or avoiding illness is now widely recognised as a key component of leading a healthy lifestyle. Consequently, there is a growing trend toward meals that contain probiotic microorganisms. To extract the important biomass from LAB cultivations that may be used commercially as a probiotic element in different products, high cell density is essential. The worldwide dairy federation advises probiotic food items to include at least 106 to 107 CFU/mL of probiotics at the time of intake to

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ensure its positive effects. However, the main issue with using LAB culture as probiotics is the end product inhibition, which results in lower growth and biomass concentration.

Lactic acid is the main metabolic byproduct of the fermentation of LAB through carbohydrate metabolism. Because of the pH change into an acidic state caused by lactic acid buildup, LAB development is inhibited. The end product inhibition in LAB fermentation is brought on by the acidification of the cytoplasm and the failure of the proton motive forces. The amount of undissociated lactic acid in the medium increases when the lactate concentration rises or the medium's pH falls. While the dissociated lactate is insoluble, the undissociated lactic acid is cytoplasmic membrane soluble and may pass through the bacterial membrane by simple diffusion. This will eventually have an impact on the transmembrane pH gradient, making it impossible to sustain and disabling cellular activities. Additionally, when energy is consumed to maintain the transmembrane pH gradient, less energy is available for cellular development. To overcome the product inhibition, it will be helpful to create fermentation techniques that can keep the culture's lactate content below harmful levels. Numerous studies on fed-batch fermentation have been done to get around the end product inhibition in LAB fermentation, which in turn increased biomass output. Due to strong osmotic pressure and the presence of acid anions, fed-batch and pH-controlled fermentations are often ineffective in LAB fermentations for overcoming end product inhibition. Therefore, lactic acid must be carefully eliminated in situ from the culture to lessen its inhibitory impact throughout the fermentation phase.

Fermentation of Lactic Acid

You may not have known that the cells in your muscles can ferment. The process of creating ATP only by glycolysis during fermentation takes place in the absence of oxygen. Recall that a glucose molecule is split into two pyruvate molecules during glycolysis, yielding a net gain of two ATP and two NADH molecules. When you work your muscles hard and quickly, they produce lactic acid fermentation, a kind of anaerobic respiration used by yoghurt bacteria (Lactobacillus and others) as well as by your own muscle cells. In order for glycolysis to continue producing ATP under low oxygen circumstances, lactic acid fermentation transforms the 3-carbon pyruvate to the 3-carbon lactic acid ($C_3H_6O_3$) (see figure below). This process also regenerates NAD +. In order for ATP synthesis to continue, this electron acceptor must be renewed since each cell has a finite amount of NAD +. To accomplish this, NADH gives the pyruvate molecules their additional electrons, renewing NAD +. Pyruvate is reduced to produce lactic acid. Due to growing global concerns about the depletion of fossil fuels and environmental protection, interest in the development and use of more sustainable energy and chemical resources has surged over the last several decades. The world's chemical and energy demands have been satisfied by the petrochemical sector since the middle of the 19th century. However, research into other methods of satisfying the energy and chemical needs of a continually expanding population was stimulated by the limited nature of oil, its price volatility, and the environmental effect of its consumption. Industrial biotechnology is a growing area that has a specific emphasis on employing microorganisms to manufacture chemicals and energy using renewable resources as substrates.

There are now a number of well-established single product bioprocesses that demonstrate viability from an economic and environmental standpoint. However, it was suggested that multi-product

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methods may enhance commercial single-product biotechnological processes by the valorization of residues and byproducts. One such is the creation of value-added compounds using the cellulose and hemicellulose from sugarcane bagasse, a byproduct of the manufacturing of bioethanol that is typically utilised to generate electricity. Similarly, glycerol, a biodiesel industry byproduct with minimal market value, is aimed at as a molecule of interest in fermentation processes. Biorefineries are multi-product manufacturing facilities that use renewable resources to create biobased goods.

The two metabolic routes for hexose fermentation—homofermentative and heterofermentative—are used by LAB to get energy since they lack a functioning respiratory system. In contrast to the second route, known as the pentose phosphate pathway, which is characterised by the generation of CO2 and ethanol or acetate in addition to LA, the first pathway, as illustrated in Figure 1, is based on glycolysis and produces primarily LA.

The majority of LAB belong to the class Bacilli, phylum Firmicutes, and order Lactobacillales. Six families—Aerococcaceae, Enterococcaceae, Leuconostocaceae, Lactobacillaceae, and Streptococcaceae—more than 30 genera, and more than 300 species make up the order Lactobacillales, a figure that keeps growing as new species are found. Despite being a member of the phylum Actinobacteria, the genus Bifidobacterium (family Bifidobacteriaceae) is also categorised as part of the LAB group. In addition to phylogenetic analysis based on 16S ribosomal RNA (rRNA) gene sequences, LAB identification is based on the criteria first stated by Orla-Jensen in 1919, which include morphology, manner of glucose fermentation, growth temperature ranges, and sugar usage patterns [6]. Furthermore, several housekeeping genes are employed as alternative markers since some LAB species within the groupings have substantial sequence similarity. Whole-genome sequencing is a popular method for LAB identification in laboratories right now. In order to modify the genome of food-grade LAB using clustered regularly interspaced short palindromic repeats (CRISPR) technologies, it is crucial to first identify the species. The creation of medicinal probiotics and virus-resistant strains are two potential uses for these technologies.

Animals, food, feed, people, plants, and soil are just a few of the many nutrient-rich environments where lactic acid bacteria may be found. Composts, fermented foods, gastrointestinal and vaginal tracts, plant surfaces, and silages, to mention a few, have all been described as sources of LAB isolation [8]. Because LAB have a flexible metabolism and may produce a variety of healthy metabolites in addition to LA, they are widely employed in biotechnology, food, and pharmaceutical items. The use of LAB for starter cultures, probiotics, antifungal and anti-mycotoxigenic agents, bacteriocin manufacturers, nutraceutical producers, and extra barriers for pathogenic and spoilage microorganisms are only a few applications. Following a safety evaluation, the European Food Safety Agency (EFSA) and the United States (US) Food and Drug Administration (FDA) added a number of LAB species and food additives derived from them to the generally recognised as safe (GRAS) inventory or, in turn, granted the qualified presumption of safety (QPS) status. The goal of this study is to highlight the many industrial applications of LAB, including their usage as probiotics and as biofactories for valuable metabolites. It also looks at the possibility of a homofermentative LAB producing numerous important compounds at once. A multi-product biorefinery method is then suggested.

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Lactic Acid Bacteria: Potential Applications

In fermentation systems, which may be carried out in solid (SSF) or liquid forms, lactic acid bacteria are primarily utilised (SLF). SLF is the method that has received the greatest research and practical use. It is used to isolate organic acids (mostly lactic acid), ethanol, and bioactive peptides, among other things. These substances need to be isolated and purified after fermentation in order to be extracted from the biomass phase or the supernatant. However, certain LAB biomass, which is often employed in single-product fermentation procedures, may potentially have probiotic potential in addition to the supernatant products. Therefore, adopting a multi-product process strategy, their separation and subsequent usage might enhance the product's value.

Product and By-Product Inhibition of Lab Fermentation

One of the primary issues with the fermentation process is the existence of inhibitors known as substrate and product inhibitions that prevent cell development and decrease the activity of product production. The main cause of batch fermentation's poor biomass output is usually product inhibition in LAB culture. Lactic acid often induces either competitive or non-competitive inhibition. It has been shown that the inhibitory impact of lactic acid on cell development is larger than the influence on fermentation activity. According to one study, the growth of *Lactobacillus plantarum* in a fed-batch culture was completely inhibited when the osmotic pressure reached 2416 m Osm kg^{-1} because of the continuous accumulation of various metabolites. This suggests that the inhibitory effect of lactic acid on cell metabolism and proliferation may be caused by the increase in medium osmotic pressure as well as other fermentation by-products like acetic acid, formic acid, or sodium formate that causes According to some reports, lactic acid inhibited bacterial growth when it started to be formed quickly after the exponential phase of development.

History of lactic Acid Fermentation

Throughout the 19th century, a number of scientists produced significant advancements in the field of organic chemistry. For instance, Justus von Liebig, one of his most important students, and the French scientist Joseph Louis Gay-Lussac had a fondness for fermentation processes. The molecular structure of the contemporary lactic acid molecule was defined by each of them, who were a few years apart from one another. They understood the fermentation process entirely from a chemical perspective, which implies that it can only be aided by chemical catalysts and cannot be seen under a microscope. Louis Pasteur, a French scientist, was the first to identify lactic acid as a result of microbial fermentation in 1857. Pyruvic acid is transformed into lactic acid by certain bacteria. This metabolic pathway is also present in the muscle cells of animals. Muscle cells' oxygen supply for breathing during exercise could not be enough. In anaerobic conditions, pyruvic acid is changed into lactic acid. This occurs when lactate dehydrogenase is present. Reoxidation of antioxidants like NADH+H+ to NAD+ also occurs. During the fermentation process, a sizable percentage of the energy is not released. In this condition, less than 7% of the energy in the glucose is released. Furthermore, not all of it is included in the high energy bonds of ATP.

Biological Process Anaerobic

When oxygen is not present, cellular respiration known as anaerobic respiration occurs. Lactic acid fermentation or alcoholic fermentation are the two kinds of anaerobic respiration.

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Fermentation Yeasts or bacteria are frequently the sites of fermentation. Animal muscle cells also exhibit lactic acid fermentation when there is insufficient oxygen supply. In yeast, partial glucose oxidation takes place in an anaerobic setting. In this process, pyruvic acid is transformed into both carbon dioxide and ethanol. Some bacteria use lactic acid fermentation to convert pyruvic acid into lactic acid, as shows the process of lactic acid fermentation in below figure.

The Process of Fermenting Lactic Acid

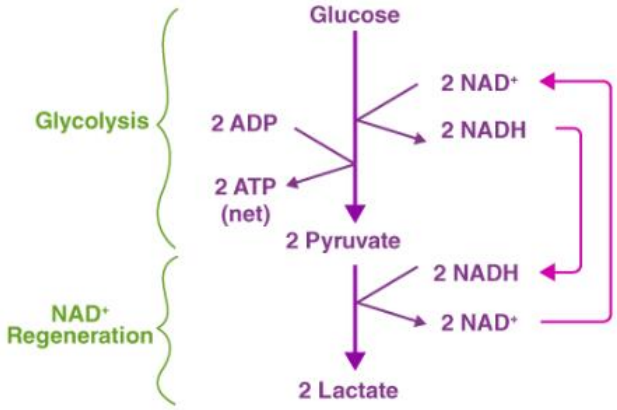

Figure 4.1: Process of Fermenting Lactic Acid

In the breakdown of the glucose or 6-carbon molecule, glyceraldehyde 3-phosphate and 3-phosphoglyceric acid are produced. NAD+ undergoes this transformation into NADH+H+. When combined with 3-phosphoglyceric acid, phosphoenol pyruvic acid eventually yields pyruvic acid. In this procedure, net 2 ATP molecules are produced (glycolysis). NADH+H+, a solution containing that reduces pyruvic acid to lactic acid and then reoxidizes to NAD+, aids in this process. Two pyruvic/ pyruvate acid molecules are converted into two lactic/ lactate acid molecules by this procedure. The enzyme enzyme that catalyzes is present throughout this process.

Lactic Acid Fermentation Applications

People now understand that people require lactic acid fermentation when their bodies urgently want a lot of energy. It also acts as an alternate way for muscle cells to produce energy when they are under stress from demanding exercises. Additionally, lactic acid fermentation enables certain bacteria, including Lactobacilli, to breathe even in the absence of oxygen. So, in bacteria, animals, and people, lactic acid fermentation acts as a rescue mechanism. The commercial usage of this sort of fermentation is significant. In the business, it is frequently used to create fermented food items and drinks, including yogurt, fermented pickles (like kimchi or sauerkraut), and cheese, soy sauce, or sourdough bread. The greatest technique for food preservation is lactic acid fermentation. The bacterium most frequently employed for this procedure in industry is lactobacillus. They are employed in the creation of sour beer, yoghurt, fermented salmon, pickles, and other products.

Bacteriocins are tiny peptides produced by ribosomes that have antibacterial capabilities. These substances, which are often produced by LAB, are effective against other Gram-positive bacteria or closely similar microbes. Some bacteriocins, like nisin and pediocin, are employed as preservatives in food items due to their antibacterial capabilities, which prevent the development

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of spoilage and harmful microbes. Bacteriocins may also help the producer cells become more competitive, which is a crucial quality for certain LAB employed as starting cultures in fermented foods. Bacteriocins' general antibacterial action is based on the breakdown of the cell membrane by the development of pores or, in the case of nisin, a "detergent effect".

Bacteriocins generally consist of 20 to 60 amino acid residues and are low-weight, cationic, and hydrophobic compounds. The leader sequence that is typically generated with them is cleaved during maturation and liberated from the cell. Bacteriocins may be divided into three groups based on their common structure and characteristics:

Peptides that include lanthionine belong to

- Class I: (lantibiotics) (a non-canonical amino acid). These may be globular with a net negative charge or elongated with a net positive charge (sub-class A, for example, nisin) (sub-class B, e.g., mersacidin).
- Class II: Peptides that are heat-stable but do not include lanthionine. Their subclasses are mostly dependent on the activity (sub-class A, e.g., pediocin; sub-class B, e.g., lactococcin, plantaricin; sub-class C, e.g., acidocin).

Large, heat-labile peptides of class III that are poorly described. The lytic proteins are often categorised as murein-hydrolases (e.g., helveticin).

Only nisin and pediocin, which are mostly employed as food additives, are commercially accessible despite the range of antimicrobial peptides generated by LAB, particularly in dairy products. However, one of the key areas of focus in the LAB biotechnology industry is the study and development of new antimicrobial peptides because to the rise in antibiotic-resistant strains and the rising demand for minimally processed foods and clean labelling. Every year, new bacteriocins or bacteriocin-like peptides from LAB are found, yet obstacles to their commercialization still stand in the way of their use. Studies to create improved procedures for bacteriocin synthesis and the subsequent purification of the compounds are still required before taking into account the industrial production of bacteriocins.

The majority of investigations on the generation of bacteriocin coupled bacterial growth with antibiotic production. The medium composition, acidity levels, and incubation temperature are some of the fermentation parameters that have been described as being the most important aspects so far. Additionally, several research revealed modelling and optimization techniques for microbes to determine the ideal circumstances for bacteriocin production. Multi-Product Process Perspectives

Many research projects were geared at creating environmentally friendly methods for producing organic compounds via biorefinery or white biotechnology utilising carbohydrate sources like corn syrup. However, alternative energy sources were thoroughly examined for the production of these compounds, which are typically used in the automotive, textile, pharmaceutical, beverage as well as food, plastic, and many other industries. These sources included agro-industrial by-products and other solid food waste. Such procedures are feasible because agro-industrial by-products provide additional nutrients, such as vitamins, minerals, and amino acids, in addition to serving as a source

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of carbohydrates. The fact that by-products do not compete with grain crops grown for human feed on arable land is one of the most significant benefits of adopting them. As previously indicated, lactic, succinic, and butanediollactic acids, fructo-oligosaccharides, bioethanol, biodiesel, bioactive peptides, enzymes, volatile chemicals, and many other metabolites are derived through green processes. Parallel to this, the industrial sector is being pushed toward more cost-effective procedures while still satisfying consumer demand by the existing strict restrictions surrounding the application of by-products as well as the need for renewable chemicals and fuels.

White biotechnological processes are usually created and improved based on a specific-metabolite route including chosen starter cultures or microbial consortia, regardless of the microorganism utilised. Although this method was highly efficient in producing particular metabolites like l-(+)-LA, researchers are always looking for bacterial processes that leverage possible by-products to make many bio-products at once while improving the efficiency of the process. A recent integrated approach for the cost-effective, high-yield manufacture of d-mannose and bioethanol from a coffee waste is an example of a multi-product process. Environmentally friendly methods may be used to carry out the pretreatment, enzymatic hydrolysis, fermentation, recuperation, and purifying processes in this process.

The so-called "reverse food engineering" approach, which entails at least five key processes, may be applied in efforts aimed at the creation of metabolites. When every stage is understood, it is required to build a cell factory to serve as a productive platform for refining bacteria into a range of natural goods. As a result, extensive research is needed to establish the prerequisites for a balanced multi-product process. Utilizing several streams produced by the downstream process in the instance of LA might potentially increase the process' value.

In Bacteria

Lactic Acid Bacteria are often used to describe bacteria that do lactic acid fermentation (LABs). These bacteria use this process as a means of survival since it gives them the energy they need for metabolism and lowers the pH of their surroundings by creating lactic acid. By making the environment inhospitable for other microbes, such an acidic situation lessens the competition for development. While some bacteria only have anaerobic metabolisms, others can alternate between aerobic or anaerobic respiration depending on the availability of oxygen.

In Cells of Animals

Anaerobic respiration occurs when muscle cells don't get enough oxygen during intense activity and need to produce energy. Lactic acid, a consequence of this anaerobic respiration, builds up in the muscles and causes weakness as well as a burning feeling. Red blood cells (RBCs) also undergo lactic acid fermentation because they lack mitochondria, which are necessary for aerobic energy production.

History

Several scientists made important contributions to the field of organic chemistry throughout the 19th century. One of them, for instance, was the French scientist Joseph Louis Gay-Lussac, who had a particular passion in fermentation processes and shared it with Justus von Liebig, one of his

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greatest pupils. Each of them, separated by a few years, working with colleagues characterised the modern lactic acid molecule's chemical structure. They had a completely chemical knowledge of the fermentation process, which implies that it can only be improved by chemical catalysts and cannot be seen under a microscope. Lactic acid was initially identified as a byproduct of microbial fermentation in 1857 by French scientist Louis Pasteur. He was employed at the University of Lille at the time, when a nearby distillery sought him counsel on some issues with fermentation. By happenstance, and using the shoddy laboratory he had at the time, he was able to determine that in this distillery, two fermentations were occurring: one producing lactic acid, and the other, alcohol. Both of these fermentations were triggered by microbes. In Paris, where he also published his views that offered a stable contradiction to the merely chemical version advanced by Liebig and his colleagues, he then continued his investigation on these findings. Liebig rejected several ideas that Pasteur had articulated but which are now widely accepted. But even Pasteur acknowledged that he was "drawn" to a totally different comprehension of this chemical phenomena in his own writing. Even though Pasteur didn't identify every aspect of this procedure, he nonetheless figured out how the microbial lactic acid fermentation works in general. It was he who coined the phrase "form of life without air" to describe fermentation.

Although microbial lactic acid fermentation for food production had been used for a very long time before to Pasteur's study, this chemical process had not yet been thoroughly defined. The Neolithic Revolution is likely when the earliest uses of milk fermentation first appeared, according to chemical analyses of archaeological discoveries. The discovery of the fermentation process was relatively obvious since milk naturally contains lactic acid bacteria and because it occurs spontaneously at an appropriate temperature. These early farmers faced the issue that adults had a hard time digesting fresh milk, thus they were interested in learning about this process. In actuality, the enzymes required to digest lactose are present in lactic acid bacteria, and during fermentation, their numbers grow rapidly. Since milk contains enough enzymes to break down lactose molecules once it enters the human body, even briefly fermented milk may be consumed by adults. A prolonged fermentation, used in the production of cheese, was even safer. Recipes for making cheese may be found on the earliest known written records, Cuneiform scripts, as well as subsequently in Babylonian and Egyptian literature, which further attest to the lengthy history of this procedure. The argument behind fermented milk products' competitive advantage is intriguing. According to this argument, the greater lactose absorption from drinking milk would enable the women of these early settlers' farming clans to have fewer children more often. They may have had a significant competitive advantage over hunter-gatherer tribes as a result of this aspect.

These civilizations acquired a lactase persistence via epigenetic inheritance as a result of their increased use of dairy products, which allowed them to consume unfermented milk as adults since the lactase-digesting enzyme was always present in their systems. Regional variations in the concentration of this mutation still allow us to see the early adaptation to lactose intake in the initial settler civilizations. It is said that 65% of people worldwide still lack it. The gene is more prevalent in those locations, as well as North America, where Europeans originally arrived, since these early cultures originated in areas around eastern Turkey or central Europe. Because of this mutation's predominance, Western cultures mistakenly think that lactose intolerance is uncommon

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whereas in reality it is more widespread than the mutation. Contrarily, Asia has a far higher prevalence of lactose intolerance.

Lactic acid fermentation

The lactic acid theory was developed in the 1990s to explain why individuals reported burning or cramping during and after strenuous exercise. Lactic acid fermentation was a consequence of a deficiency of oxygen within the muscle cells. This is due to the cell requiring oxygen as a terminal electron acceptor to make ATP. The cells had to find an alternative way to produce energy because there was no oxygen available. As a byproduct, lactic acid, or lactate, and H+ were produced. Leg cramps and pain are brought on by the lactic acid accumulation that generates a burning sensation within the muscle cells. Another modification to the lactic acid theory is that the host experiences more weariness after exercise when sodium lactate is present in the body. The procedure is known as lactate because lactate is just lactic acid that has undergone deprotonation. Both the bacteria that create yoghurt and the red blood cells in your body, which lack mitochondria and are unable to undertake cellular respiration, do lactic acid fermentation, as shown in below figure.

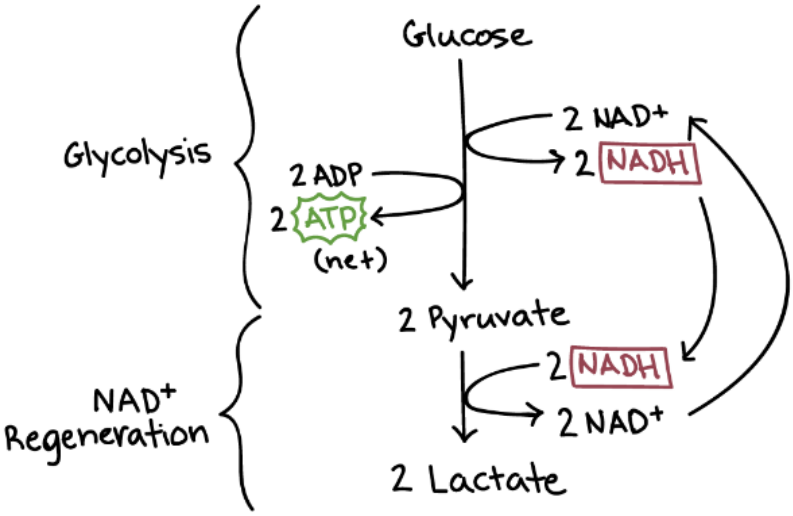

Figure 4.2: Lactic acid fermentation

The production of lactic acid is crucial to the physiology of muscle cells. Muscle cells need energy fast when they are engaged in vigorous action, such as running. Only a few seconds' worth of running can be accomplished using the ATP that is stored in muscle cells. Due to their anaerobic surroundings, the cells subsequently go back to fermenting lactic acid. Even during vigorous exercise, muscle cells may continue glycolysis by producing ATP and NAD+ via lactic acid fermentation. Lactic acid-producing bacteria have a significant impact on the environment in the vagina. The vaginal canal's resident Lactobacilli spp. aid in pH regulation. More lactic acid will be generated to raise the pH back to a more acidic level if the vagina's pH becomes too basic. Through the formation of hydrogen peroxide or antibacterial chemicals, lactic acid-producing bacteria also serve as a protective barrier against potential pathogens including bacterial vaginosis or vaginitis species, various fungi, and protozoa. It is unknown whether lactic acid is still being used in the vaginal canal after fermentation. Lactic acid is beneficial to the body in modest doses because it gives it energy and substrates to move through the cycle. In modest experiments, it has

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been shown that lactose intolerance may be alleviated by the conversion of lactose to lactic acid. The quantity of lactose that is accessible is limited by fermentation. Reduced lactose results in less accumulation inside the body, which lessens bloating. Yogurt cultures were where lactic fermentation was most successful. Additional research is being done on other milk products, such as acidophilous milk.

In certain mammalian cells and bacterial species, lactic acid is fermented. In animal cells, aerobic respiration which doesn't entail lactic acid fermentation is often favoured. However, cells must engage in anaerobic respiration in situations when the oxygen supply is insufficient primarily in muscle tissues during times of intense exercise in order to appropriately fulfil the need for energy. Lactic acid is a consequence of this anaerobic respiration process, which builds up in the muscular tissues. The burning and weakening you experience in your muscles when exercising are caused by the increased acidity, and this is thought to assist you avoid overworking your muscles. But contrary to common belief, delayed-onset muscle soreness is not brought on by lactic acid i.e. the soreness you might feel the day after an intense workout.

Lactic acid is expelled from the muscle tissue via the circulation once the muscular action is finished. After that, it is transported to the liver, where it engages in chemical processes to create pyruvic acid, which is subsequently utilised to create ATP, an extra type of energy. Red blood cells, in addition to muscular tissue, also go through this process as they lack mitochondria and cannot produce energy aerobically. Lactic acid fermentation has many uses in bacterial organisms. Lactic Acid Bacteria are often used to describe bacteria that do lactic acid fermentation (LABs.) While fermentation is essential for LABs to produce energy under anaerobic circumstances, it also enables them to lower the pH of their surroundings by producing lactic acid. The lower pH lessens competition for the LABs by making their habitat unfavourable for the majority of other microbes. Depending on the availability of oxygen, certain LABs may alternate between the two processes whereas others are purely anaerobic.

In the preservation and creation of healthy meals, lactic acid bacteria play a crucial role. The lactic acid fermentations are often cheap and frequently need little to no heat during preparation, making them also fuel-efficient. Foods that have undergone lactic acid fermentation are crucial in providing food for everyone on every continent. This vital task is carried out by lactic acid bacteria in the preservation and production of a wide variety of foods, including fermented fresh vegetables like cabbage (sauerkraut, Korean kimchi), cucumbers (pickles), fermented cereal yoghurt sourdough bread and bread-like products made without wheat or rye flour (Philippine puto, Indian idli,), fermented milks (yoghurts and cheese (e.g., salami). On artificial medium, lactic acid bacteria are often picky, although they grow well in most dietary substrates and quickly drop the pH to a point where competing species cannot thrive. Before preventing their own development, leuconostocs, lactic streptococci, and certain lactobacilli and pediococci often reduce the pH to between 4.0 and 4.5. The oxidation of reduced nicotinamide adenine dinucleotide (NADH) by flavin nucleotide, which interacts quickly with gaseous oxygen, allows lactobacilli to create hydrogen peroxide in addition to lactic acid. In addition to producing hydrogen peroxide, flavoproteins like glucose oxidase also have antibiotic effects on other microorganisms that might lead to food degradation. Lactobacilli themselves are rather resistant to hydrogen peroxide. The

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polypeptide antibiotic nisin, produced by Streptococcus lactis, is effective against gram-positive organisms like S. cremoris. S. cremoris then creates the antibiotic diplococcin, which is effective against gram-positive organisms like S. lactis. Thus, despite preventing the development of other gram-positive bacteria, these two species compete for the fermentation of milk products. Foods may benefit from the cleansing action of the carbon dioxide that heterofermentative lactobacilli create, which can potentially cause anaerobiosis if the substrate is not well protected.

Because they are inexpensive, use little energy to process and prepare food for consumption, and provide a wide range of tastes, brining and lactic acid fermentation remain highly favoured techniques of processing and preserving vegetables. The quantity of pectinolytic and proteolytic hydrolysis that happens is constrained by salting, which controls softening and prevents putrefaction by reducing the amount of hydrolysis that occurs. Other noteworthy benefits of lactic acid fermentations include the foods' increased resistance to microbial deterioration and toxin production. Acid fermentations often increase nutritional value while also changing the taste of the original materials. For the hundreds of millions of economically disadvantaged and hungry people around the world for whom canned or frozen food is frequently either unavailable or unaffordable, acid fermentation combined with salting continues to be one of the most practical methods of preservation, frequently enhancing the nutritional and organoleptic qualities of fresh vegetables, cereal gruels, and milk-cereal mixtures. Utilizing microorganisms' capacity to eat, digest, and release hydrogen from biomass is a key component of microbial biomass conversion processes. This study may lead to commercial-scale systems in the mid- to long-term, depending on the route taken. Microorganisms, including such bacteria, break down organic materials to create hydrogen in fermentation-based systems. Refined sugars, unprocessed biomass sources like maize stover, and even wastewater may be considered organic material. These techniques are commonly referred to as "dark fermentation" techniques since no light is needed.

Circular bioeconomy is a relatively new concept, and it is anticipated that it will continue to develop as a result of sustainability issues including population growth, resource depletion, and climate change. The industry is strongly encouraged to see the manufacture of bio-based chemicals as a promising area for investment due to its compatibility with technical processes as well as economic and environmental evaluations. Accordingly, biorefineries must to include procedures that make it easier to fully separate and value every component of biomass. A crucial factor to support research, development, and industrial application is the development of multi-product integrated processes.

Some of the most promising microorganisms for converting biomass into useful commercial products are said to be lactic acid bacteria. LA is a remarkably adaptable biotechnological molecule that is crucial in the production of several interesting compounds as an intermediate metabolite. In addition to LA, LAB fermentation may produce a number of items with intriguing properties, including bacteriocins, probiotics, and LTA. The titers for these auxiliary products aren't yet optimal, however. They do, however, have potential and commercial importance, which might raise the value of conventional LA fermentation. In fact, greater research into the successful co-production of LA and other interesting metabolites may pave the way for the development of long-lasting and more effective multi-product bioprocesses. In a process called direct hydrogen

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fermentation, bacteria create hydrogen on their own. These microorganisms can degrade complex compounds in a variety of ways, and enzymes can combine the results of some of those methods to generate hydrogen. Researchers are looking at ways to increase the pace at which hydrogen is produced by fermentation systems and how much hydrogen can be produced from the same quantity of organic matter (increasing the yield).

Microbial electrolysis cells (MECs) are machines that use a modest extra electric current, together with the energy and protons generated by bacteria consuming organic materials, to make hydrogen. Since this technology is somewhat recent, researchers are attempting to improve a variety of system components, from locating more affordable materials to figuring out the best kind of microorganisms to utilise. As a plentiful home resource, biomass has been effectively broken down by several bacteria to create hydrogen and other compounds. Researchers studying hydrogen production can now concentrate on the difficulties specific to producing hydrogen because fermentation has already been used as an industrial technology to produce biofuels and other products, and many of the problems with scaling up systems have been solved for different products. MEC-based systems might lower the significant energy required for wastewater treatment while providing a valuable fuel in the form of hydrogen from materials that would not otherwise be suitable for the manufacture of fuel. To increase the hydrogen output from the beginning biomass fuel, these two methods may be combined. Although there has been advancement in the study of microbial biomass conversion for hydrogen generation recently, there are still a number of issues that need to be resolved, according to the US Department of Energy.

Enhancing the rates and yields of hydrogen production from fermentation processes using a variety of techniques, including the development of better microbial strains, the optimization of reactor systems, and the discovery of feedstock sources and processing techniques that produce the highest yields. Creating MEC systems that can be expanded to sizes that are applicable to the commercial market while preserving the output rates and system efficiency observed at the bench scale and reducing the cost of the reactor's component parts In the West, China, and Korea, lactic acid fermentation of cabbage and other vegetables is a typical method of preserving fresh vegetables (where kimchi is a staple in the diet). Simple food preservation involves slicing or shredding the fresh vegetable and adding around 2% salt. As the salt draws liquid from the vegetable, lactic acid bacteria may develop on the liquid, acting as a substrate. In order to avoid the development of bacteria that might lead to spoiling, anaerobic conditions should be maintained as much as feasible. Following is the order in which the organisms grow during a normal sauerkraut fermentation: In the shredded cabbage, *Leuconostoc mesenteroides* starts the growth process at a variety of temperatures and salt concentrations. It generates carbon dioxide as well as lactic and acetic acids, which swiftly reduce pH levels and prevent the growth of bacteria that may otherwise ruin crispness. The fermentation's need for anaerobiosis is made easier by the carbon dioxide that is created in lieu of the air. *Lactobacillus plantarum* and *Lactobacillus brevis* finish the fermentation in that order. High acidity is a result of *Lb. plantarum*. *Pecicoccus cerevisiae* grows and helps produce acid if the fermentation temperature or salt concentration are high.

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CHAPTER 5

Alcohol Fermentation

Malathi. H

Assistant professor, Department of Life Science, School of Sciences, B-II,

Jain (Deemed to be University), J C Road, Bangalore-560027.

Email Id- h.malathi@jainuniversity.ac.in

A biological process known as alcoholic fermentation, which also produces the byproducts ethanol and carbon dioxide, turns carbohydrates like glucose, fructose, and sucrose into cellular energy. Alcoholic fermentation is regarded as an anaerobic process since yeasts carry out this conversion without the presence of oxygen. It also occurs in certain fish species, such as goldfish and carp, where it works in conjunction with lactic acid fermentation to provide energy when oxygen is in short supply. Alcoholic drinks, ethanol fuel, and bread dough rising all depend on ethanol fermentation.

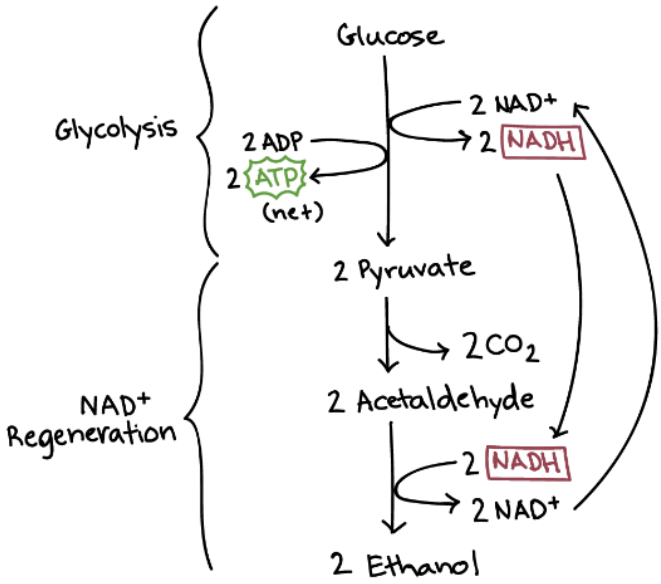

Figure 5.1: Alcoholic fermentation.

Effect of oxygen

Oxygen is not necessary for fermentation. Some yeast species (such as *Kluyveromyces lactis* or *Kluyveromyces lipolytica*) may totally oxidise pyruvate to carbon dioxide and water in a process known as cellular respiration if oxygen is available. As a result, some yeast species will only create ethanol in an anaerobic environment (not cellular respiration). The Pasteur Effect is the name given to this phenomenon. Even in the presence of oxygen, many yeasts, including the widely used

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baker's yeast *Saccharomyces cerevisiae* and the fission yeast *Schizosaccharomyces pombe*, ferment rather than breathe. This is referred to as the counter-Pasteur effect while producing wine. If these yeasts have the proper nutrients, they will still be able to create ethanol even in an aerobic environment. The rate of ethanol generation per milligramme of cell protein is maximum for a short time early in the batch fermentation process and gradually decreases as ethanol builds up in the surrounding broth. The drop-in metabolic rate is caused by physiological changes (including potential ethanol damage) rather than the presence of ethanol, according to studies showing that the removal of this stored ethanol does not instantly restore fermentative activity. The reason for the decrease in fermentative activity has been looked at from a number of angles. Throughout batch fermentation, viability maintained at or above 90%, internal pH was close to neutral, and the specific activity of the glycolytic and alcohologenic enzymes (measured in vitro) remained high. None of these elements seem to be directly responsible for the decline in fermentative activity that occurred during batch fermentation. Alcoholic drinks include ethanol that is created by yeast-induced fermentation. Cider and perry are made by simulating the fermentation of the natural sugars found in apples and pears, respectively, while other fruit wines are made by simulating the fermentation of the sugars found in any other sort of fruit.

Beer, whisky, and sometimes vodka are made by fermenting grains whose starches have been changed into sugar by the enzyme amylase, which is found in malted grain kernels (i.e. germinated). Since the amylase will also operate on other sources of starch, such as potatoes and unmalted grains, these may be added to the mixture. In certain nations, it may also be fermented with saliva while being amylase-induced. Whiskey and vodka are also distilled, and flavourings are added to a feedstock that is similar to vodka during the distillation process to create gin and other drinks. Rice wines, including sake, are made by the fermentation of grain starches that the mould *Aspergillus oryzae* has transformed into sugar. The end result of this fermentation is used to make baijiu, soju, and shach. Sugarcane is fermented and then distilled to create rum and several other alcoholic drinks. Molasses, a byproduct of sugarcane, is often used to make rum. All fermentation processes must be carried out in a container that excludes outside air while allowing carbon dioxide to escape. This is done to lower the possibility that the beer may be contaminated with undesirable germs or mould and because a buildup of carbon dioxide increases the possibility that the vessel would fail or burst, which might result in property damage or injury. Sugars like glucose, sucrose, and fructose are transformed into energy molecules during the complicated biotechnological process known as alcohol fermentation, which also results in the production of carbon dioxide, ethanol, and other metabolic byproducts. The chemical makeup and sensory qualities of the fermented food are influenced by these components. Yeast or other microbes like bacteria may metabolise alcohol during the fermentation process, which is also known as ethanol fermentation. This is how they produce ATP. Although yeast usually works in an aerobic or oxygen-rich environment, it may also operate in anaerobic or oxygen-free environments.

Saccharomyces cerevisiae is the yeast species that predominates in the manufacturing of alcoholic drinks globally, and the specific strains of this species used in fermentation have a significant impact on the flavour and aroma properties of various beverages. Pure cultures of chosen *S. cerevisiae* strains are often employed for large-scale beverage fermentations, such as those involved in brewing, winemaking, and the manufacture of distilled spirits. These strains are either

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obtained internally or from suppliers of yeast. Spontaneous fermentations that depend on local microbiological flora (wild yeasts and bacteria) existing in the raw material and in the production facility may be permitted to happen in smaller-scale (artisanal) procedures. For instance, this would be usual in small distilleries in Mexico (for the manufacturing of Tequila and Mezcal) and Brazil (for the production of Cachaça). Non-*S. cerevisiae* yeasts may be used in various forms of alcoholic beverage fermentations, either as starter cultures or organically. For instance, the native yeast flora associated with the grapes may replace the *S. cerevisiae* yeast strain used to start fermentation in winemaking.

Brewing yeasts belong to the Saccharomyces genus, although two distinct species are used in the production of ale and lager. In terms of the former, they are *S. cerevisiae* strains that often carry out "top fermentations," in which yeast colonies assemble on the surface of the fermenting wort. If centrifuges are utilised in the yeast harvesting process, certain non-flocculent ale yeasts may be used. Ale yeasts come in a wide variety of strains, each with unique aneuploid or polyploid genetic traits. Lager yeast strains are currently known as Saccharomyces pastorianus, a hybrid of the species *Saccharomyces cerevisiae* and *S. eubayanus*. These yeast cultures engage in "bottom fermentations" and often flocculate. This makes it easier for yeast to crop near the conclusion of fermentation, often in the cones of cylindro-conical containers on the bottom of a fermenter. In the essay that follows in this Special Issue, Stewart discusses the functions of yeast in the brewing of beer.

S. cerevisiae's Nutritional Needs

The nutritional makeup of the fermentation medium is vital for yeast growth and metabolism, and therefore, for the quality of the finished beverage, when *S. cerevisiae* is used to produce alcoholic drinks. Raw materials make up a significant amount (often more than 50%) of the total expenses of producing fermented beverages, therefore the cost of the medium is also crucial. It should be mentioned that other factors also affect the effectiveness of yeast fermentation. In addition to water, various major, minor, and trace nutrients are also necessary for yeasts to successfully carry out fermentation. When given glucose, ammonium salts, inorganic ions, and a few growth stimulants, the majority of *S. cerevisiae* strains are capable of growing. Sources of carbon (in the form of sugars), free amino nitrogen (in the form of amino acids, short peptides, and ammonium salts), oxygen, sulphur, phosphorus, potassium, and magnesium are all macronutrients that need to be given at millimolar quantities. Micronutrients, which include trace elements like calcium, copper, iron, manganese, and zinc, are only required by yeast at micromolar quantities. The following is a description of several typical media sources of nutrients used in the preparation of fermented drinks.

Growth factor sources

To carry out certain catalytic or structural functions, *S. cerevisiae* needs growth factors at extremely low concentrations. These factors include vitamins, purines and pyrimidines, nucleotides and nucleosides, amino acids, fatty acids, and sterols. These auxiliary growth nutrients for alcohol fermentations should be accessible in complex media, such malt wort or wine must, although commercially supplied yeast "foods" may also be used to augment media. These are used

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in alcohol fermentations to maintain regular yeast activity and are based on mixes of yeast extract, ammonium phosphate, and minerals (such as magnesium and zinc). There may sometimes be a pantothenic acid and inositol deficiency in blackstrap molasses used to make rum.

Fermentation media's nutrient composition

For beverage fermentations, sugars may either be derived directly from plants that are high in sugar (such as sugarcane in the case of molasses or fruits in the case of wine must) or from plants that are high in starch (for example, following pre-hydrolysis of cereal starches from barley, maize, and wheat). Malt amylase enzymes hydrolyze starch, releasing higher saccharides such oligosaccharides (like maltodextrins) that are often not used by Saccharomyces yeasts used to make fermented beverages.

The cellular barrier known as the yeast plasma membrane controls nutrient entrance into cells and is crucial in controlling the rates of yeast growth and fermentation. Through processes including simple net diffusion (a passive or free method), facilitated (catalysed) diffusion, diffusion channels, and active (energy-dependent) transport, nutrients are transferred into yeast cells through the plasma membrane. In the latter method, plasma membrane ATPases function as directional proton pumps to create pH gradients that stimulate the transport of nutrients either via proton symporters (as is the case with certain sugars and amino acids) or through proton antiporters (as is the case with potassium ions).

Yeast species, fermentation circumstances, and the kind of sugar being utilised will all affect how precisely *S. cerevisiae* translocates sugars. For instance, maltose is carried through active transport, but glucose is transferred by facilitated diffusion. The process known as catabolite suppression occurs when glucose inhibits *S. cerevisiae*'s ability to assimilate other sugars (such as maltose) in fermentation medium like malt wort. This might lead to incomplete or sluggish fermentations and the development of bad flavours in drinks [9]. For instance, Berry and Slaughter have observed a "maltose lag" in brewer's wort that is caused by glucose inhibiting the absorption of maltose and may lead to "stuck fermentations" if malt wort is supplemented with glucose adjuncts.

Since amino acids are progressively digested by yeast cells, they are the preferred nitrogen sources for *S. cerevisiae* in fermentation medium. However, the presence of ammonium ions may impede their absorption owing to nitrogen catabolite suppression. Two classes of energy-dependent amino acid uptake systems function in *S. cerevisiae*: one is broadly specific and affects the uptake of all naturally occurring amino acids, while the other includes several transporters that display specificity for different amino acids. Additionally, *S. cerevisiae* may digest amino acids to produce ammonium, glutamate, and higher alcohols by decarboxylation, transamination, or fermentation.

Due to its non-diazotrophic nature, *S. cerevisiae* cannot fix atmospheric nitrogen and instead depends on a source of easily assimilated organic nitrogen (such as amino acids) or inorganic nitrogen (such as ammonium salts) for growth and fermentative metabolism. Although urea may be used by yeast, it shouldn't be added to beverage fermentation medium due to the potential for the creation of the carcinogenic compound ethyl carbamate. The creation of structural and functional proteins (enzymes) and nucleic acids in yeast fermentation medium is aided by nitrogen, whereas the generation of fermentation flavour congeners such higher alcohols is facilitated by

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nitrogen. Free alpha-amino nitrogen (FAN) concentrations may restrict the development of distillery yeasts, however Ingledew found that the growth of distilling strains of *S. cerevisiae* rises essentially linearly with FAN concentrations up to 100 mg/L.

Inorganic Nutrient Sources

Yeasts need the proper amount of inorganic ions in addition to the sugar and nitrogen sources in fermentation medium. Despite the fact that the type and quantity of metal ions given in growth medium may have a major influence on yeast fermentations, minerals, particularly critical metal ions, are often disregarded as crucial drivers of yeast fermentation performance. Key examples of "bulk" minerals needed at millimolar concentrations are phosphorus, sulphur, potassium, and magnesium, while "trace" minerals needed in micromolar or fewer quantities include sodium, calcium, iron, cobalt, zinc, molydenum, copper, manganese, nickel, and selenium.

Malt wort, molasses, wine must, and cheese whey are examples of complex fermentation media used in the manufacturing of fermented beverages. These materials often contain sufficient quantities of inorganic ions for yeast growth, although supplementation with extra minerals may sometimes be required (for example, zinc may be deficient). Also, precipitation, chelation, or absorption may impair the bioavailability of metal ions in intricate industrial fermentation medium. Regarding zinc, it is crucial for ethanol (alcohol) dehydrogenase, the last alcohologenic enzyme, to function properly during alcoholic fermentations. As a consequence, zinc-deficient medium may cause fermentations to be slow or blocked, which has long been acknowledged as a rare issue in the brewing business. Regarding magnesium, it is crucial to maintain high levels of bioavailable magnesium to guarantee optimal fermentation efficiency since yeast definitely need it to produce ethanol.

Growth of *S. cerevisiae* During Fermentation

In order for cells to expand in biomass and ultimately divide, nutrient transport and absorption is followed by their integration into a variety of cellular components during yeast development. Instead of producing alcohol, a yeast cell's main goal is to proliferate. However, during the fermentation of beverages, the development of yeast and the generation of alcohol are inexorably related. Cells create ethanol in an effort to keep their redox balance in check and produce enough ATP to support ongoing growth. In actuality, yeast cell proliferation is essential for the effective production of ethanol. Only enough sugar will be fermented by dormant yeast cells to provide energy for cell maintenance. Therefore, the challenge faced by distillers, brewers, and winemakers is to supply yeast cultures with enough nutrients to carry out fermentation while also preventing excessive yeast growth that would result in alcohol loss. By using continuous or semi-continuous fermentations, immobilised yeast bioreactors, high yeast cell densities, and cell re-cycle systems, compromise attempts may be made to reduce yeast growth during alcoholic fermentation.

S. cerevisiae divides vegetatively by means of a process known as multi-lateral budding, in which daughter buds protrude from various areas of the mother cell surface. When mother cells reach a crucial cell size at the same time that DNA synthesis begins, yeast buds begin to form. When yeast cells divide, chitin a polymer of N-acetyl glucosamine rich scar tissue is left on the yeast cell surface. These scars, which are referred to as the bud and birth scars, are still visible on the mother

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and daughter bud cells, respectively. When *S. cerevisiae* cells are added to new fermentation media and incubated under ideal physical growth conditions, a traditional batch growth curve will develop. This curve consists of a stationary phase, an exponential phase, and a lag phase during which no cells grow but instead adapt physiologically to their new environment. Growing yeast cells create ethanol 33 times quicker than non-growing cells during fermentation, when the period of maximal sugar intake and alcohol production corresponds with the logarithmic phase.

Certain yeasts, often known as wild yeasts, may impair the organoleptic properties of fermented drinks both during the fermentation process and thereafter as contaminating microorganisms. These include several non-Saccharomyces yeasts as well as wild strains of *S. cerevisiae* and other members of the Saccharomyces genus. In general, it is believed that the presence of bacteria during yeast alcoholic fermentations is harmful since it reduces ethanol yields and jeopardises product quality, notably in beer. Lactic acid bacteria are the main source of contamination, however acetic acid bacteria and wild yeast contamination may also sometimes cause issues. In finished drinks like beer and wine, these bacteria may lead to a range of flavour (organic acids, diacetyl) and fragrance (sulphurous smell) flaws.

Particularly during malolactic fermentation, which lowers acidity and produces a smoother-tasting wine, some bacteria in winemaking may be helpful. This happens after the primary yeast fermentation is over, and it causes l-malic acid to be decarboxylated into l-lactic acid. The malolactic enzyme in lactic acid bacteria, such as Oenococcus oeni, the predominant bacterial species in charge of the malolactic fermentation, catalyses this process. This might happen on its own or could be regulated by adding pure O. oeni malolactic starting cultures. Late-stage lactic acid bacteria are thought to be advantageous in the fermentation of Scotch malt whisky because they produce flavour components (such lactones) that are acceptable and pass through to the final distillate. Other distilled spirits, like sour-mash bourbons, promote the development of lactic acid bacteria during certain stages of the distillation process to lower the pH of the wort and add the necessary flavour congeners to the finished distillate.

In order to make wine, grape juice (or "must") must be extracted and then fermented with yeast using either natural yeasts or *S. cerevisiae* commercial starter cultures. Malic acid may also undergo bacterial malolactic fermentation, which results in lactic acid, before being aged, clarified, and packaged. Although *S. cerevisiae*-based wine yeast starter cultures predominate, certain commercial non-Saccharomyces starter cultures, such Candida stellata, may be used to provide wine particular flavour and aroma qualities. In this Special Issue, Pretorius discusses the function of *S. cerevisiae* in the manufacture of wine. Specific strains of *Saccharomyces cerevisiae* carry out the fermentations necessary for the production of whisky and other distilled spirits made from cereals. These strains turn the mash sugars into ethanol, carbon dioxide, and a variety of secondary fermentation metabolites, which together serve as flavour congeners in the finished spirit. Therefore, it's crucial to choose a yeast strain that might greatly impact the organoleptic properties of spirits. Maltose and maltotriose make up the majority of the sugars that are extracted when cereal is mashed, as opposed to the glucose, fructose, and sucrose that are released after the crushing of grapes to make wine must. Yeast uses the anaerobic route to produce alcohol when

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there isn't any oxygen present. The production of alcoholic drinks like beer and wine depends greatly on this process. Without oxygen, the process takes occur in the cytoplasm of yeast.

More information on Anaerobic and Aerobic Respiration

In the absence of oxygen, a metabolic activity called fermentation takes occur. Fermentation is a process through which several advantageous bacteria impart favourable modifications to foods and drinks. As a consequence of being preserved, the end goods have improved taste and longer shelf lives. They also have a lot of positive health effects. Before discussing alcohol fermentation, let's first have a basic understanding of the many forms of fermentation. Based on the pyruvate end products, there are three different kinds of fermentation.

Fermentation of lactic acid

Without the use of heat, bacteria and yeast strains ferment various carbohydrates into lactic acid in this fermentation. Pyruvic acid utilises NADH to create lactic acid and NAD+ in anaerobic chemical processes. Lactic acid fermentation also takes place in the human body's muscle cells during hard exercise. The pace at which oxygen is given to muscle cells is slower than the rate at which muscles consume energy in the form of ATP. The muscles' oxygen supply is reduced during vigorous activity. They switch to fermentation of lactic acid. Muscle cramps are brought on by lactic acid buildup in the muscles. Healthy foods like yoghurt and pickles, among others, are produced and preserved in large part thanks to lactic acid bacteria. These dietary items play a crucial role in the digestive process.

1. **The fermentation of alcohol:** During this fermentation, yeast converts the molecules of pyruvate (the glycolysis byproduct of the metabolism of glucose into alcohol and carbon dioxide. Beer and wine are made by alcoholic fermentation.
2. **Fermentation of acetic acid** Grain and fruit starch and sugars fermented into acetic acid and vinegar throughout this fermentation. Apple cider vinegar, wine vinegar, and other vinegars are made by acetic acid fermentation.
3. **Oxygen's impact on fermentation:** Since fermentation is an anaerobic process, oxygen is not needed. Some yeast species will totally metabolise pyruvate to water and carbon dioxide molecules if oxygen is available. However, due to a phenomenon known as the Pasteur Effect, yeast species will only create ethanol in an anaerobic environment.
4. **Fermentation Rate**: The rate of ethanol generation during fermentation is highest early on. However, when ethanol builds up in the environment, the rate of fermentation gradually decreases. It has been shown that eliminating this ethanol buildup does not restore fermentation activity, and a steady metabolic decrease starts instead. The origins of this drop in fermentation activity have been looked at from several angles. It is thought that this is because the fermentation process harms the yeast cells.
5. **Fermentation by-products**: Fermentation of ethanol results in the production of unharvested byproducts such as heat, carbon dioxide, methanol, fuels, water, alcohol, and fertiliser. The unfermented grain remains may be fed to animals or utilised to make biogas, which is shown in below figure. According to the fundamental equation for alcohol

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fermentation, the procedure starts with glucose (sugar) and finishes with carbon dioxide or ethyl alcohol. It is broken down into a number of phases for easier comprehension.

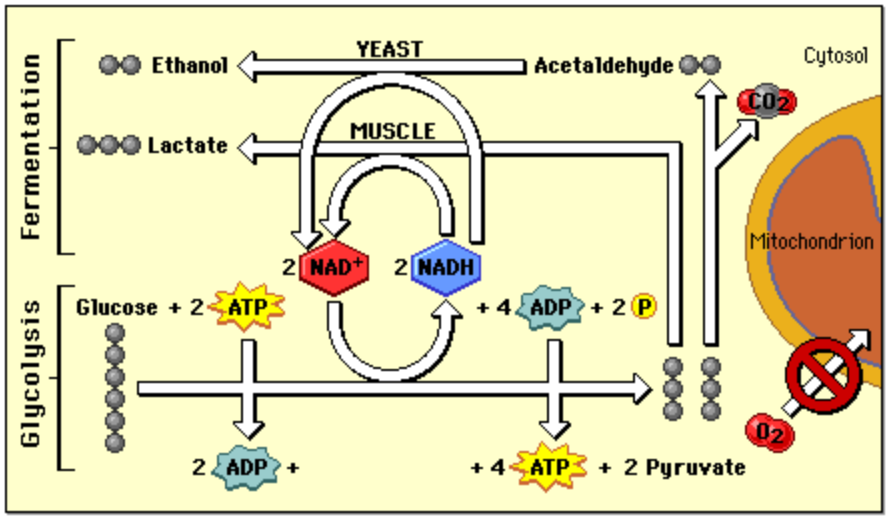

Figure 5.2: Fermentative reactions within the cell.

Advantages of fermentation

The significant advantages of fermentation to humans conclude that by removing nutrients from food, probiotics and helpful bacteria found in fermented food aid in maintaining a healthy gut. Antinutrients like phytic acid found in grains, nuts, seeds, and legumes are also neutralised by fermentation. If left untreated, it may result in a mineral shortage in the body. The gut bacteria's production of lactic acid aids in the transformation of ammonia into ammonium ions. It protects the body against ammonia's harmful effects on the brain. Hepatic encephalopathy may be avoided in large part because to this fermentation process. The following alcoholic drinks are made via the industry's fermentation of alcohol technique. The natural sugars found in grapes are fermented to create wine. A similar fermentation procedure is used to create perry and cider from the natural sugar found in pears and apples. Beer, whisky, and vodka are made by the fermentation of grain starches that have been amylated into sugar. Molasses, a byproduct of sugarcane, is fermented and then distillated to create rum. In each of these procedures, fermentation must occur in a container that lets carbon dioxide out but keeps air out. As carbon dioxide increases the chance of a vascular rupture, it will assist in lowering the danger of contamination by undesirable bacteria or mould.

In the current research, people compared species from different phyla that illustrate the gradual acquisition of distinguishing features from bryophytes to angiosperms in order to evaluate the conservation of ethanol fermentation in land plants from an evolutionary viewpoint. Our findings demonstrate that, whereas this metabolic pathway seems to have persisted throughout plant evolution, seed plants have only recently learned to put transcriptional control on the genes that encode fermentative enzymes. We discovered that acetaldehyde synthesis, rather than its consumption through ADH, is necessary to improve life under low oxygen circumstances by taking advantage of Arabidopsis mutants defective in fermentative metabolism. Surprisingly, ethanol generation was nevertheless visible in plants without ADH1 expression, and Marchantia

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polymorpha, the most primitive lineage of land plants, does not seem to have an ethanol-processing ADH. Together, these findings refute the conventional wisdom that ethanol synthesis only depends on ADH-like enzymes and their critical function in anaerobic glycolysis.

Glycolysis is an anaerobic (without oxygen) mechanism that muscles use to get the majority of their energy during hard or extended activity. A somewhat similar process known as alcoholic fermentation is used by yeast cells to produce energy in anaerobic environments. The chemical conversion of glucose to lactic acid is known as glycolysis. Through this process, adenosine triphosphate, a high-energy phosphate molecule, becomes accessible for use by cells as energy (ATP). The only difference between glycolysis and alcoholic fermentation is the last stage. Pyruvic acid is converted into ethanol and carbon dioxide during the alcoholic fermentation process. The byproducts of alcoholic fermentation have been utilised in baking and brewing for ages; lactic acid from glycolysis causes fatigue.

Anaerobic fermentation processes that start with the sugar glucose include alcoholic fermentation and glycolysis. Enzymes are needed for glycolysis, which converts glucose to lactic acid. The first 10 stages of alcoholic fermentation use the same enzyme pathway. In alcoholic fermentation, two enzymes take the place of lactate dehydrogenase, the last enzyme of glycolysis. In the course of alcoholic fermentation, the two enzymes pyruvate decarboxylase or alcoholic dehydrogenase transform pyruvic acid into both ethanol and carbon dioxide. According to the evolutionary theory that is most widely accepted, life originally appeared in an oxygen-deficient environment. The most ancient method of energy production is said to have originated with anaerobic fermentation. However, treating fermentations as prehistoric energy collecting systems created by chance and time has a number of scientific drawbacks. A kind of fungus, yeast is a single cell creature. It includes the catalyst for the process, the enzyme zymase. Other fruit juices, such as those from apples, plums, pears, and others, may be fermented to create wines with a variety of flavours. The following prerequisites must be met for the fermentation reaction:

Temperature - The temperature must fall between 25 and 50 degrees Celsius. Temperature has a significant impact on enzymes. When it's too cold, the enzymes can't reach the substrate quickly enough for a reaction to happen. However, the rate of reaction also rises with temperature. This is due to the fact that heat energy increases the number of times the enzyme and substrate collide. All enzymes are proteins, however, and proteins degrade at very high temperatures. The enzyme is considered to be denatured when the substrate no longer fits in the enzyme's active site, which results in the reaction not happening. Substrate (the glucose solution): Enzymes function best when the process they catalyse has a substrate concentration that is high enough. If there isn't enough accessible substrate, the reaction's pace slows and eventually stops growing.

Absence of Oxygen - Air must be kept out of the container where fermentation is taking place. Acetobacter, a kind of bacterium, is widely present in air. Wine's ethanol is oxidised by Acetobacter bacteria using ambient oxygen from the air, creating a diluted solution of ethanoic acid (vinegar). Yeast - Without yeast, the fermentation of the glucose solution into ethanol is not possible. Zymase, an enzyme found in yeast, serves as a catalyst for the process. Ethanol in wine may range in content from 14 to 15%. This is so that fermentation won't continue over this point since the ethanol kills the yeast.

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Large-scale fermentation is utilised to manufacture ethanol for use as fuel in nations like Brazil with little natural oil sources but great conditions for sugar cane cultivation. A renewable fuel is the ethanol that fermentation produces. This is so that new sugar cane can be cultivated in its stead. Additionally, since plants create the glucose needed for the fermentation by collecting carbon dioxide from the atmosphere, it is a more environmentally benign source of fuel. The carbon dioxide is released into the atmosphere when the ethanol is burned. The long-term effect of utilising sugar cane as a fuel source in Brazil is that as demand rises, it's possible that rainforests will be removed to make room for the vast amount of land needed to grow sugarcane. The local ecosystems will be impacted, and the sugarcane may not be as effective at removing carbon dioxide from the atmosphere as the rainforests it replaces.

The yeast strains used in fermentations undergo a lot of changes during the creation of alcoholic beverages. Research aims to choose novel yeasts to impart acceptable flavours and use various carbohydrate source materials, as well as increase the efficiency of sugar conversion to alcohol. We've found favourable qualities in distilling yeast strains for Scotch whisky manufacturing. Rapid developments in molecular biology have had an impact on the use of new yeast strains in wort and grape juice fermentations, respectively, in brewing and winemaking. These innovations, particularly recombinant DNA technology, have the potential to enhance fermentation efficiency and product quality. Due to more favourable regulatory difficulties and market acceptance, yeast-yeast genetic alteration, also known as self-cloning, provides an appealing technology . However, it is crucial to remember that genetically modified (GM) *S. cerevisiae* strains for food and beverage fermentations have not yet gained widespread acceptance and are only permitted for use in a small number of nations, not in Europe.

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CHAPTER 6

Ethanol Fermentation

Renuka Jyoyhi

Assistant professor, Department of Life Science, School of Sciences, B-II,

Jain (Deemed to be University), J C Road, Bangalore-560027.

Email Id- j.renuka@jainuniversity.ac.in

During alcoholic fermentation, glucose and fructose (sugars) are anaerobically transformed into ethanol or carbon dioxide. A few numbers of bacteria and yeasts perform the process (*Zymomonas mobilis*). Through the metabolism of hexose, alcohol fermentation replenishes the NAD+ that was depleted during glycolysis and provides yeast with a boost of two ATP molecules. While fermenting grape juice, the yeast *Saccharomyces cerevisiae* primarily uses the pyruvate to produce ethanol to replace the NAD^+ lost during the glycolysis process. This phenomenon is referred to as alcoholic fermentation. Humanity has been using fermentation, a well-known natural process, for thousands of years primarily to produce alcoholic drinks, bread, and byproducts. A carbohydrate, such as starch or sugar, is converted by an organism into an alcohol or an acid during fermentation, which is seen solely from a biochemical perspective. For instance, yeast uses fermentation to create alcohol from sugar in order to get energy. Before the biochemical process was completely understood, fermentation processes occurred on their own. When Louis Pasteur, a French chemist and microbiologist, showed that fermentation was carried out by live cells, he became the first scientist to research fermentation in the 1850s and the 1860s. The most prevalent and widely accessible yeast strains, *Saccharomyces cerevisiae* strains, are typically used in fermentation procedures to create wines, beers, and ciders. They are widely renowned for their technical traits and fermentative activity, which enable the production of goods with consistent and standardised quality. Fermentation produces several additional significant industrial goods, including yoghurt, cheese, bread, and coffee. Yeasts are essential for the treatment of wastewater and the creation of biofuels. From a biological perspective, fermentation occurs when pyruvate produced by the metabolism of glucose is broken down into ethanol and carbon dioxide by yeasts (and certain bacteria).

The organism most usually used in the fermentation of alcoholic drinks is *S. cerevisiae*, as is well known. This yeast is often used as a microbial starter in several fermentation-related industries. *S. cerevisiae* emerges as the dominant species during the alcoholic fermentation of fruits and juices as a result of their strong selective environment, given the low pH, high ethanol and sugar concentrations, and anaerobic conditions. During the fermentation of alcohol, one mole of glucose is converted into two moles of ethanol and two moles of carbon dioxide, producing two moles of ATP in the process. Fermentation of alcohol is a difficult process. As the reaction progresses, grape juice is transformed into vine by a variety of biological, physical, and chemical processes.

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The continued expansion of the world economy has raised both energy demand and worry about the buildup of greenhouse gases in the atmosphere and its impact on climate change. Many nations are developing renewable energy in response, including the manufacturing of biofuels. Any fuel made from biomass, such as organic waste materials, is referred to as a biofuel. When compared to conventional fossil fuels, biofuels may have a substantially less ecological impact. Bioethanol is one such biofuel, with global output expected to reach 130 billion litres per year, with the United States and Brazil providing the majority of the world's ethanol. Alcohol (ethanol) produced by the microbial fermentation of plant or algal sugars is known as bioethanol (e.g., corn, sugarcane, wheat, lignocellulosic biomass, etc.).

A natural method for dissolving more complex organic compounds into simpler ones is microbial fermentation. Pretreatment procedures may be necessary to get the biomass ready for extraction and fermentation before alcoholic fermentation. Enzymatic hydrolysis may then produce fermentable monosaccharide and disaccharide sugars after preparation. Yeast subsequently undergoes metabolic activities that may take place both aerobically and anaerobically to transform these sugars (such as glucose, galactose, and fructose) into ethanol, carbon dioxide, and other byproducts. For instance, during glycolysis, two molecules of pyruvate are created from one molecule of glucose. Under anaerobic circumstances, pyruvate may be converted to acetaldehyde with the emission of carbon dioxide. The two molecules of pyruvic acid are subsequently reduced to two molecules of ethanol and carbon dioxide. Acetaldehyde may then be converted to ethanol by alcohol dehydrogenase after that.

Food crops, such as wheat, maize, potatoes, beets, and sugarcane, have been employed in traditional alcoholic fermentation (first-generation bioethanol production) as feedstocks because they are better sources of the readily available starch and sugar needed for fermentation. However, there has been growing worry about fuel generation from food crops as the world's population rises and the quantity of arable land remains constrained. In order to produce ecologically friendly bioethanol, non-edible biomass sources as lignocellulosic materials and algae are being investigated. As a consequence, an expanding variety of feedstock materials may be used to produce bioethanol. A wider variety of biomass raw materials may now be used to make ethanol thanks to advances in ethanol production technology. A new generation is often used to describe fermentation technology that makes it possible to produce bioethanol from a biomass resource that has not previously been used. Additionally, strategies for biomass generation are categorised according to elements affecting the fermentation environment. Fermentation technology is categorised based on the amount of water and sugar present in the fermentation medium and whether batch or continuous methods are utilised. To increase ethanol production, additional methods may be used with the fermentation medium. This review's objective is to summarise current understanding of fermentable substances and fermentation processes utilised in the manufacture of bioethanol. This assessment also takes into account a number of other elements that affect ethanol output.

First-generation bioethanol is produced by fermenting biomass with high quantities of starch and/or sugar, such as wheat and maize (e.g., sugar cane, sugar beet). Although the chosen feedstock varies, the industrial manufacture of gasoline and drinkable ethanol using first-generation

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technologies is extensively used commercially in many nations. Maize is the most extensively used feedstock in the United States, although both corn and wheat are widely utilised in Canada. In Brazil, sugarcane is a popular feedstock, whereas in Europe, potatoes, wheat, and sugar beets are used by the ethanol sector most often. This high-quality feedstock may produce reasonably high ethanol yields with minimum preprocessing. The use of crops that might otherwise be utilised as food for humans or as animal feed in the production of ethanol using first-generation technologies and feedstocks is questioned. However, the manufacture of bioethanol may provide a way to transform crops into valuable products and to salvage damaged grain that would otherwise be discarded. For instance, grain infected with Fusarium head blight may be hazardous to humans or other animals owing to the presence of mycotoxins (such as deoxynivalenol). However, by utilising insects (such as black soldier fly larvae; *Hermetia illucens*) and lactic acid bacteria and yeast-based fermentation, tainted grain may be detoxified. Economic loss may be reduced, particularly as a result of this fungal disease in cereal grains like wheat and barley, by strategies that recover grain products that would otherwise be lost. Insects and lactic acid bacteria may be employed to produce protein feed additives for domestic animals after detoxification. Edible crop fermentation leftovers are also thought of as enhanced feed materials. In comparison to the original grain, wet distillers' grain has a substantially greater protein level on a dry basis. Wet distillers' grain may either be used directly to animal feed or combined with another fermentation byproduct called distillers' solubles, dried further, and then marketed as a cheap livestock feed.

Production of Bioethanol and Biorefineries

Approximately 75% of fossil resources are still utilised to produce heat and energy, 20% are used as fuel, and just a small percentage are used to create chemicals and materials (1). The carbon cycle naturally regenerates fossil fuels at a pace that is far slower than the rate at which they are currently used. The majority of fossil fuel reserves are concentrated in a limited number of nations, which further contributes to the unsustainable nature of their production. Additionally, increasing greenhouse gas emissions occur from the burning of fossil fuels and changes in land use brought on by human activity, which exacerbates the global warming situation (2, 3). With the following basic objectives in mind, governments in the majority of industrialised nations encourage the use of renewable energies and resources: I to assure access to energy, (ii) to mitigate climate change, (iii) to develop/maintain agricultural operations, and (iv) to ensure food safety. The three pillars of sustainability—affordable energy, climate change mitigation, and social stability are closely tied to the key objectives listed above (1, 4). By substituting fossil fuels with renewable resources, which are more evenly distributed and raise fewer environmental and social issues, it is possible to effectively change the current state of global warming and all problems resulting from the usage of fossil fuels (3).

Liquid biofuels (biodiesel or bioethanol) were the subject of intense research throughout the later decades of the 20th century as potential replacements for fossil fuels. Biomass from plants is used to make biofuels, which are sources of renewable energy. Utilizing this feedstock would decrease the usage of fossil fuels and the resulting harm to the environment (5-7). The creation of a biorefinery attempts to meet the sustainability requirements for the production of biofuels. Using biomass for the sustainable manufacture of various intermediates and products as well as the fullest

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possible use of all feedstock components, biorefinery is an integrative and multifunctional concept (8). The idea calls for the selective conversion of the many compounds present in the biomass into biofuels, as well as into other products like food or cow feed, medicines, pulp, paper, and polymers (9, 10). Biomass resources may be broken down into its component parts, such as carbs, proteins, lipids, etc., using a broad variety of methods (3). A excellent example of a biorefinery is a plant that produces raw materials that include lignocellulose, where cellulose and hemicellulose are converted into simple (fermentable) sugars and lignin is converted into target chemicals (e.g. polymers, resins, pesticides, levulinic acid and other materials). For the synthesis of the desired molecule, there have recently been significant attempts to increase the selectivity and efficiency of lignin depolymerization and upgrading methods. The most promising method for producing target compounds from lignin is the catalytic hydrodeoxygenation process.

The following phases are often included in the biorefinery process: pretreatment and preparation of the biomass, separation of the components of the biomass, and further conversion and product purification operations. The implementation of the biorefinery idea may be done in one of two ways: top-down or bottom-up. The spread of current biomass processing facilities into a biorefinery with the goal of obtaining a wider range of products and/or an increase in usable biomass fractions through the connection to additional technologies is known as a "bottom-up" biorefinery approach. These facilities currently produce only one or a few products. The wheat and maize starch biorefinery in Lestrem, France, which began as a straightforward starch mill, is an example of a bottom-up biorefinery. It steadily increased the variety of goods available, including chemicals, fermentation products, and starch derivatives and alterations. Additionally using a bottom-up strategy are a wood lignocellulosic biorefinery in Austria (Lenzing) and a maize starch biorefinery in the United States.

The new top-down strategy was developed as a highly integrated system for the usage of diverse biomass fractions and the production of various goods for the market (zero-waste generation). Getting biomass used completely is the goal (e.g. wood lignocellulose, grain and straw from cereals or green grasses). Austrian Green Biorefinery is a prime illustration of a top-down strategy. In order to produce biobased goods like proteins, lactic acid, fibres, and biogas from the leftover biomass, green grass silage is used as the feedstock. *Wautersia eutropha* also used silage juice and green grass juice, sources of complex nitrogen and phosphate, as components of its cultural medium for growth and the manufacture of polyhydroxyalkanoates. Demonstration facilities for top-down biorefineries are mostly located in the United States, Europe, and other developed nations. Top-down biorefineries are currently in the research and development phases.

The two most significant sugar-producing plants in the world are sugar cane and sugar beet. The world produces two thirds of its sugar from sugar cane and one third from sugar beet (28). The majority of Saccharomyces species produce the enzyme invertase, which is readily able to hydrolyze them. As a result, producing bioethanol from feedstocks containing sugar (sucrose) does not need pretreatment, making this bioprocess more practical than using feedstocks containing starch (28). To extract sugars from sugar crops and turn them into a fermentation medium, just a milling procedure is required. In this situation, juice or molasses may be used to directly generate ethanol. Since sugar cane is a semi-perennial crop that doesn't need as many agricultural operations

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as are often required to prepare raw crops, and since its biomass can be used to generate energy and heat, it has certain benefits as a raw material for bioethanol production. Because it is simpler to process and more productive than other raw materials used to produce bioethanol (12), sugar cane is less costly (30). However, several initiatives continue to focus on enhancing sugarcane-based bioethanol production. This involves creating new strains of sugar cane that have higher sugar content, resistance to illnesses, better yields per hectare, and longer life spans.

Therefore, the algae species and mix employed in retreatment methods are crucial. Large kinds of algae (macroalgae), which naturally grow attached to the bottom and may reach lengths of several metres, often contain fibrous material that needs substantial physical/chemical processing in order to release starches or sugars. Microalgae, on the other hand, may have significantly greater sugar concentrations and are often much smaller and unicellular. However, unlike macroalgae, they are difficult to recover from water, which might result in considerable inputs to dewater the algal biomass before processing. Ethanol yields per gramme of algal dry matter may vary, and some researchers have found that second-generation bioethanol feedstocks produce less bioethanol than first-generation feedstocks. Although third-generation bioethanol production is less substantial than the previous two, the technology is more modern, and algae species are being studied and modified to find and breed more productive species or mixes of species than the first two generations. The production of third-generation bioethanol may increase in the future thanks to technological advancements or in conjunction with other uses, such as making bioethanol from algae that are also used to clean wastewater.

The first-generation biofuels are now the preferable alternative for commercial biofuel production, according on technoeconomic analyses of the three generations of biofuel processes (e.g., economic advantages, process design, etc.). Processes for producing second-generation biofuels are becoming competitive. Further process improvements will reduce production costs (for example, reduced enzyme costs) and boost coproduct usage (for example, increased use of power). Additionally, combining first- and second-generation biofuels may increase ethanol yields and earnings while also requiring less capital expenditure to include lignocellulose processes into an industrial design. The greatest option for producing biofuels, according to experts, is third-generation biofuels made from microalgae since they need less space and have a high potential for CO2 collection. However, in order to lower prices and boost economic sustainability, the usage of these feedstocks has to be improved.

Fourth-generation bioethanol production techniques are now being developed, and they combine high-yielding biomass with little lignin and cellulose content with other means of enhancing fermentations, such as the use of genetically modified organisms (yeasts, algae, etc.). Although there are many different fourth-generation bioethanol production techniques, some of them use oxy-fuel combustion, which burns fossil fuels with an oxygen-enriched gas mixture rather than with air to collect CO2 emissions throughout the manufacturing process. This results in flue gas mixes that are predominantly made up of CO2 and water. By physically compressing and chilling CO2 in this way, for example via the distillation process, it is possible to directly extract CO2. Unfortunately, present commercial techniques are both commercially unviable and energy intensive. The process of electro-fermentation, which controls the respiration of genetically

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modified algae by transferring electrons, is another illustration of fourth-generation bioethanol synthesis. Industry does not yet use these techniques, which mark a significant departure from the more conventional ways for producing bioethanol.

Liquid-State Fermentation and Submersion

When a fermentable substrate is extensively liquefied and bacteria are cultivated in that liquid substrate, the process is referred to as submerged fermentation. This form of fermentation is often utilised in the synthesis of first-generation bioethanol because it allows for the further liquefaction of starchy materials by heating and enzymatic hydrolysis of ground up starch/sugar-rich materials. As a consequence, a liquid medium is created in which different nutrients and carbohydrates are suspended as particle solids or dissolved. Since submerged fermentation may swiftly produce a large output of bioactive metabolites, it is used in various bio-industrial processes, such as enzyme manufacturing. Unfortunately, there are certain drawbacks to this process, such as the need for energy and water inputs, the need for large bioreactors or distillation columns, and the production of a lot of trash or low-value byproducts (such as thin stillage and wet distillers' grains). Fortunately, the extra thin stillage from the waste by-product wet distillers' grains can be removed using centrifugation. The thin stillage can then be dried to distillers' solubles with a minor amount of efficiency, and the solids may then be dried to distillers' dried grain. Three products that are utilised as feed components result from these drying procedures are distillers' solubles, distillers' dried grains, and distillers' dried grain with solubles (the latter being a combination of the former two products). Thin stillage may also be given to calves in neighbouring feed lots as a water alternative, or it can be further processed by microbial fermentation to provide a high-quality protein feed. The transformation of low-value glycerol into the more valuable molecule 1,3-propanediol is one advantage of the latter method.

Solid-State Fermentation

In the solid-state fermentation (SSF) process, organisms develop on solid or non-soluble substrates with little or no free water. In addition to producing bioethanol, solid-state fermentation is presently employed to make a variety of products, including enzymes, antibiotics, bioactive substances, organic acids, and biodiesel. Numerous variables, such as the kind of microbe, substrate, water activity (to stop the development of unwelcome organisms), temperature, aeration, and bioreactor employed, have an impact on the SSF process. Since solid matrices more closely resemble certain fungal's natural environment, filamentous fungi (such as Trichoderma and Aspergillus) are the most popular species employed for SSF. However, SSF is also used to single-celled species like bacteria and yeast. Solid-state fermentation using trash and other feedstocks is a common step in the synthesis of second-generation bioethanol. Except for agave, all of the feedstocks for second-generation bioethanol are fermented utilising SSF technology. Large amounts of waste from agricultural-based sectors that may have poor nutritional value (e.g., low digestibility, crude protein, and mineral content) are commonly processed using SSF. These leftovers are often disposed of by burning or dumping, which may have an adverse effect on the environment by releasing greenhouse gases. Many of these substrates include molecules of lignin, cellulose, and hemicellulose that, when fermented, may be utilised to create ethanol. However, compared to starchy materials, the lignocellulosic properties of these materials render

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saccharification of them into substrates for fermentation far more labor-intensive. While hemi-cellulose is a polysaccharide made up of d-xylose, d-mannose, d-galactose, d-glucose, l-arabinose, 4-O-methyl-glucuronic, d-galacturonic, and d-glucuronic acids connected by -1,4 and sometimes -1,3 glycosidic bonds, cellulose is generated from connections of d-gluco The refractory structure of lignocellulosic must be broken down by mechanical or physiochemical pretreatment procedures (such as steam explosion and acid/alkaline treatments) in order to make these sugar bonds accessible. The substrate must next undergo acid prehydrolysis, followed by enzymatic hydrolysis. As the composition of cellulose, hemicellulose, and lignan depends on the kind of agro-industrial waste utilised, the implementation of these pretreatment techniques is reliant on the feedstock.

The usage of enzymes is another distinction between submerged fermentation and SSF. For saccharification, submerged fermentations normally need significant starting enzyme dosages, while SSF systems use enzymatic cellulose hydrolysis to constantly produce reducing sugars. In a process known as simultaneous saccharification and fermentation, reducing sugars are fermented to ethanol while enzymatic hydrolysis and fermentation take place in the same step. This method increases ethanol yields by lowering product inhibition and eliminating the need for separate saccharification and fermentation reactors. To completely implement this hybrid approach, it is crucial to determine a temperature range that is suitable for both enzymatic hydrolysis and fermentation since the optimal temperature for enzymatic hydrolysis is often higher than the fermentation temperature.

A combination of filamentous and thermotolerant fungus (such as Trichoderma and Aspergillus) or bacteria (such as Streptomyces) and yeast (such as Saccharomyces cerevisiae) is often used to accomplish simultaneous saccharification and fermentation. Higher temperatures are required to enhance enzymatic hydrolysis, which is often the rate-limiting step during the SSF process. Thermotolerant yeasts and bacteria can withstand these temperatures. Although longer incubation durations may be necessary, regulating the interior temperature and maintaining the right process conditions might be difficult. Microbial saccharification and simultaneous fermentation can eliminate the requirement for costly enzymes. Agricultural wastes may be used to produce bioethanol using solid-state fermentation techniques, and for many feedstocks, simultaneous saccharification and fermentation can lower costs and increase SSF ethanol yields. Without the addition of extra nutrients, solid-state fermentation has proven successful.

Simultaneous saccharification and co-fermentation is another hybrid strategy. Some microbes use this technique to simultaneously consume two distinct substrates. This strategy presents difficulties since many organisms use substrates in a sequential manner. An organism that is growing in the presence of both xylose and glucose, for instance, may initially metabolise glucose more quickly than xylose and may start eating xylose only when glucose concentrations are low. Fermentation may be slowed down by the progressive removal of substrates. The bacterium may be forced to use both substrates concurrently and is first acclimated to a low glucose substrate to ameliorate this problem. In order to evaluate this possibility for producing biofuels, genetic engineering has also been studied.

During batch fermentation, the microbes are typically introduced to a predefined volume of medium in the fermenter. When nutrients are metabolised and bacteria multiply, byproducts

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accumulate. When the nutrients are gone, the fermentation is complete. Because the initial nutrition input is fixed and bacteria continuously use resources, the culture environment is constantly changing. For this kind of fermentation, the usual growth curve typically consists of a lag phase, exponential phase, stationary phase, and death phase. The lag phase, or first important step of microbial growth in batch fermentation, is when the organism begins to adapt to the new environment. During the exponential phase, the rate of reproduction stays constant, leading to an exponential increase in microbial growth (logarithmic growth phase). The pace of cell development is often substrate constrained and is influenced by substances that are either absent from the medium (like nutritional imbalance) or present in excessive quantities (like excess sugar).

Fermentation periods are extended as a consequence, and ethanol yields are lowered. The microorganisms enter the stationary phase after the exponential phase, when the ratio of live to dead cells equalises as a consequence of the medium's loss of nutrients (such as sugar) or the accumulation of harmful byproducts (such as ethanol toxicity). After fermentation, there may be a death phase when the number of viable cells decreases. However, some businesses pre-grow yeasts in smaller, more appropriate tanks before adding a significant innoculum to the main fermentation to reduce lag times. Shortening the exponential period might improve fermentation efficiency. However, batch fermentation has the advantages of typically being less expensive, having a low risk of contamination, and being easier to sterilise and manage feedstocks when compared to other fermentation methods. However, compared to fed-batch and continuous fermentations, batch fermentations have lower cell densities because nutrients are not provided during the exponential growth phase. Since the containers must often be cleaned and sterilised in between fermentation periods, there is also greater downtime. The bulk of batch fermentation techniques are solid-state, long-term, and moderate in size.

With the exception of nutrients being progressively provided to the fermenter over time, fed-batch fermentation is identical to batch fermentations. Because nutrients are continuously supplied, cell density rises throughout the exponential phase, increasing product yields. For instance, stationary phase yeast cells may receive continual sugar inputs to boost ethanol generation. The maximum operating capacity of the fermentation vessel, however, can be a limiting factor for the amount of new media and nutrient input. While reducing the likelihood of excess metabolism and the risk of excreting metabolic byproducts that may be used for catabolism or anabolism, nutrient additions support yeast alcohol production. This kind of fermentation is very beneficial when the intended product, such as bioethanol, is linked to microbial growth.

Finally, during continuous fermentation, fresh medium and nutrients are continuously added to the fermenter at the same rate as ethanol, byproducts, and toxic metabolites are removed from the culture. During these operations, yeast is often removed and reinserted into the fermentation tank. If the medium is continuously added at an acceptable pace, the fermentation broth eventually reaches a steady state. Continuous fermentations may keep a constant volume unlike fed-batch fermentations, therefore the amount of fresh medium that can be added to the culture during the fermentation is not limited by the bioreactor's maximum operating capacity. Less downtime between batches is needed since cultures in a steady state may last for a long time, and economic yields are higher because the culture is kept in the ideal state for making ethanol. Because of the

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lengthy fermentation duration and potential processing difficulties, continuous fermentation systems are vulnerable to contamination. Traditional techniques of distilling ethanol from fermented medium by heating would kill the microbial culture; thus, processes including settling and/or filtering yeast from the product stream are utilised prior to distillation. Because lignocellulosic biomass is renewable and non-competitive with food crops, it is advantageous and sustainable to produce bioethanol from raw materials that include lignocellulose. Additionally, using bioethanol produced from lignocellulosic biomass is linked to a significant decrease in greenhouse gas emissions. Compared to fossil fuels, lignocellulosic biomass is nearly evenly distributed over the planet, ensuring supply security by using indigenous energy sources.

In the typical lignocellulosic biomass, cellulose makes up 43% of the material, lignin 27%, hemicellulose 20%, and miscellaneous components 10%. The compositional diversity of lignocellulosic biomass may be both a benefit (more products are available than are produced in petroleum refineries, and a wider range of feedstocks) and a drawback (requires a wide range of technologies). When compared to the homogenous and consistent raw materials required by the chemical industry, the heterogeneous nature of lignocellulosic biomass necessitates more sophisticated chemical processes. Further complicating matters for biomass providers is the fact that harvesting of lignocellulosic crops is often not year-round. Therefore, in order to be accessible for long-term storage and to guarantee that the biorefinery operates continuously throughout the year, this issue must be resolved through biomass stabilisation.

Before microbes to digest lignocellulosic biomass, it must be hydrolyzed into monomeric sugars. This procedure is often carried out using acids, alkalis, or enzymes. Factors related to physicochemistry, structure, and composition may significantly delay this process down. Consequently, an alkaline pretreatment step is sometimes required to provide the right conditions for an effective enzymatic hydrolysis. To ensure the successful enzymatic hydrolysis of lignocellulosic biomass during pretreatment, it is necessary to decrease polymerization degree and crystallinity index, break lignin-carbohydrate connections, remove lignin and hemicelluloses, and enhance material porosity. Pretreatment selection has a significant effect on all following steps in the synthesis of bioethanol and is influenced by the nature of the raw material and the creation of byproducts during the chosen pretreatment.

Toxic substances such furans (such as 2-furaldehyde (furfural) and 5-hydroxymethylfurfural (HMF)), carboxylic acids (such as acetic, formic, and levulinic acids), and phenolic compounds are created when harsh conditions are applied during pretreatments (aldehydes, ketones, p-coumaric and ferulic acids). The following strategies (to lessen their impact on the performance of the bioprocess) were suggested because these substances have the potential to inhibit yeast growth: I removal of inhibitors by solvent extraction, ion exchange, overliming, use of zeolites, or enzyme laccase; (ii) use of yeast strains that are very tolerant of inhibitors; and (iii) selection of efficient pretreatment that results in little sugar degradation and inhibitor formation. Most detoxification techniques only completely eliminate inhibitors, and they also cause sugar loss, which raises the overall cost of the procedure. Recently, Burkholderia cepacia and Burkholderia sacchari used lignite as an adsorbent for the detoxification of spurce sawdust hydrolyzates in the synthesis of polyhydroxyalkanoates (PHA). While using lignite instead of activated carbon for detoxification

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has a stronger favourable effect on bacterial growth and PHA output, it is less effective in removing inhibitors. Additionally, lignite is a much more affordable adsorbent than activated carbon, which might increase the viability of a bioprocess economically. Lignite used in detoxification may help offset some of the energy and heat requirements for fermentation.

Byproducts of alcoholic fermentation

Pyruvate is initially transformed into ethanal by pyruvate decarboxylase. The enzyme requires magnesium or thiamine pyrophosphate as cofactors. Alcohol dehydrogenase as a result turns ethanal into ethanol and utilizes NADH to create NAD +.

In Saccharomyces cerevisiae, alcohol dehydrogenase has three isoenzymes, although isoenzyme I is principally in charge of converting ethanal to ethanol. Zinc is a cofactor in the process that alcohol dehydrogenase is involved in. Ethanol and carbon dioxide are the end results of the fermentation process for alcohol. Simple diffusion is the mechanism that moves both to the cell's outside. In addition to ethanol, ethanol fermentation also produces additional substances such esters, succinic acid, glycerol, higher alcohols, diacetyl, 2, 3-butanediol, diacetyl, and acetoin. In this procedure, the NADH gives its electrons to the derivative of pyruvate, resulting in the production of ethanol [4]. Pyruvate is converted into ethanol in two stages. Acetaldehyde is produced when the carboxyl group in pyruvate is removed and released as carbon dioxide. A two-carbon molecule called NADH then transfers its electrons to acetaldehyde, replenishing NAD+ and forming ethanol. The ethanol found in alcoholic beverages is produced by yeasts during the fermentation of alcohol. Anaerobic respiration, sometimes referred to as alcoholic fermentation or lactic acid fermentation, does not require oxygen. Because of the energy released during the partial degradation of organic food, the final products are never wholly inorganic. Two molecules of pyruvate are produced by glycolysis first, followed by an oxygen-free fermentation that yields lactic acid and ethyl alcohol [5].

More Alcoholic fermentation byproducts

In addition to the aforementioned major products of ethanol and glycerol, fermentation also results in the production of various additional chemicals as a result of its intricate process. These factors, which are typically combined in synergistic ways, can prevent proper alcoholic fermentation from occurring. When fermentation eventually finishes, the yeast has to be reinoculated. As a result, alcoholic fermentation is a sophisticated process that involves turning carbohydrates into ethanol or other byproducts.

Bioethanol generated from renewable feedstock is a profitable and environmentally benign substitute for non-renewable fuels as the world's need for energy rises. However, lignocellulosic non-edible biomass (second-generation bioethanol) and algal sources (third-generation bioethanol) are becoming more and more alluring feedstocks for bioethanol production as worries about the world's food supply rise. To reach the fermentable sugars in the second- and third-generation feedstock, the refractory lignocellulosic structure and algal cell wall must be disrupted under pretreatment conditions. The feedstock, cultivar, and organism being employed all have an impact on the efficiency of fermentation and bioethanol output. To guarantee optimal fermentation rate and extent, biotic (e.g., microbial contamination) and abiotic variables (e.g., nutritional, trace

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metal, and vitamin deficits) must also be taken into consideration. Different fermentation techniques, such as fed-batch and continuous fermentation, may be used to solve some of these issues and assist reduce yeast stress.

Additionally, additional inputs (like Mg2+ and other micronutrients) and adaptive reactions may boost yeast organisms' capacity to withstand stress (like heat shock and ethanol shock) and enhance fermentation efficiency. In order to choose a feedstock option, industrial bioethanol producers should consider the necessary pretreatment conditions, take into account the various fermentation technical designs, and identify any possible fermentation-related difficulties. These actions improve fermentation efficiency and increase ethanol output when combined. To explore the viability and financial effects of integrating these technologies in the future production of biofuels, particularly in examining and encouraging the use of third-generation biofuels, technoeconomic considerations should also be assessed.

Enzymatic hydrolysis is more environmentally friendly and less energy-intensive than acid hydrolysis of lignocellulose. It also occurs at gentler reaction conditions (such as pH=5 and temperatures below 50 oC). Additionally, it produces a high glucose yield with less byproduct production, which is advantageous for using hydrolysate in fermentation later on. The issues with corrosion are not brought on by enzymatic hydrolysis (95). In order to lessen its effect on the hydrolysis kinetics, the end product of enzymatic hydrolysis (glucose) must be eliminated as soon as it is formed since it suppresses the activity of the enzyme. Numerous methods have been investigated to lessen the inhibition of glucose by hydrolysis, including the use of high enzyme concentrations, the inclusion of -glucosidases, the removal of sugar by ultrafiltration during hydrolysis, or simultaneous saccharification and fermentation. On both the starting rate and the yield of cellulose enzymatic hydrolysis, substrate concentration is a key factor. Yield and hydrolysis rate increase with low substrate concentration. Even though the cost of cellulase has decreased more than 10-fold in recent years, it still accounts for more than 20% of the expenses of producing bioethanol from lignocellulosic feedstocks. Since cellulases continue to function after hydrolysis, recycling them could be a beneficial and practical strategy.

A cascade of continuous bioreactors where ethanol inhibition is decreased typically makes up continuous bioethanol production systems. This theory is supported by the observation that ethanol produced in the first bioreactor is readily transmitted to the following bioreactors, which reduces ethanol inhibition. Continuous ethanol removal from broth throughout the bioprocess using vacuum or membrane devices is another way to boost productivity, although the associated capital expenditures are higher. Increasing the air supply may enhance the productivity, concentration, and vitality of yeast cells in continuous systems that produce bioethanol. The benefits of continuous bioprocesses over batch bioprocesses for the generation of bioethanol include cheaper construction costs for bioreactors, lower plant maintenance and operating costs, better bioprocess control, and greater productivities.

Because of its benefits in terms of practicality on an industrial scale, the majority of bioethanol production facilities in Brazil still utilise the fed-batch operating mode. However, owing to their benefits associated with the greater yeast cell concentrations, continuous bioprocess systems are used in 30% of Brazil's industrial facilities for the manufacture of bioethanol. The yeast cell density

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may be increased via immobilisation, recovery and recycling of yeast cells, or restriction of yeast development. In continuous bioprocesses for the generation of bioethanol, the concentration of immobilised cells is relatively high, and at greater dilution rates, the bioprocess can be readily managed, leading to better bioprocess productivities. There are four categories of immobilisation techniques:

1. Reversible (or irreversible) attachment to solid surfaces;
2. Entrapment in porous matrices (such as gelatine, agar, calcium alginate, -carrageenan, chitosan, and polyacrylamide); and
3. Mechanical separation behind a barrier (such as microporous membrane filters or microcapsules).

Surface adsorption techniques for yeast cell immobilisation are often more effective than entrapment or mechanical separation techniques. Although some yeast cells may be washed out of the system, studies of yeast cell immobilisation by surface adsorption have indicated that yeast cell development is not greatly impacted (99). Similar bioethanol production efficiency was seen in yeast cells that flocculated on their own as well as yeast cells fixed on supports. Additionally, because the supporting material is not used, the bioprocess is less complex and more economically viable than the yeast cell immobilised on the supporting materials. To keep the yeast concentration within the bioreactor at a steady level, the yeast flocs may be flushed out of the bioreactor under carefully monitored circumstances. After washing away the yeast from the bioreactor, sedimentation or centrifugation may be employed to recover the yeast (99). Centrifugation uses more energy and demands more capital expenditures. Separated yeast cells may, however, be recycled and utilised in subsequent cycles of bioethanol synthesis, which lowers the cost of bioethanol production.

Both alcoholic fermentation and glycolysis

The muscles use glycolysis to generate energy when there is a lack of oxygen during lengthy and strenuous activity. Yeasts get their energy via a similar process known as alcoholic fermentation when they are living in anaerobic environments. Glycolysis is a chemical reaction that converts glucose into lactic acid and ATP, which is subsequently made accessible for cellular function. With the exception of the last step, glycolysis and alcoholic fermentation are similar processes. Pyruvic acid is converted into both carbon dioxide and ethanol during alcohol fermentation. While the alcoholic fermentation byproducts have long been used in baking or brewing, the lactic acid produced by the glycolysis process causes weariness.

Glucose is the starting material for both the anaerobic glycolysis and alcoholic fermentation processes. The glycolysis process, which changes glucose into lactic acid, calls on eleven enzymes. Enzymes are used exclusively in the first 10 stages of alcohol production. The last enzyme of glycolysis, lactate dehydrogenase, is replaced by two other enzymes when ethanol is produced. The enzymes pyruvate decarboxylase and alcoholic dehydrogenase convert pyruvic acid into carbon dioxide and ethanol during the fermentation process of alcohol. Thus, until the eleventh enzymatic breakdown, neither the procedure of alcoholic fermentation nor that of glycolysis detects any increase in energy (ATP).

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Cassava as an ethanol source

Mineral oil and sugars or starches can both be used to generate ethanol. Starches are the cheapest. The starchy crop cassava, which grows best in tropical climates, has the highest energy content per acre of all starchy crops. In the 1990s, Thailand already had a substantial cassava industry that produced it for use in wheat flour and as cow fodder. In Nigeria and Ghana, cassava-to-ethanol facilities are already under construction. The commercial production of ethanol from cassava is now feasible when crude oil prices are over US$120 per barrel.

Due to the creation of new cassava varieties, the situation is still uncertain. With irrigation and fertilizer, cassava can currently produce between 25 and 40 tonnes per hectare. Assuming a 22% starch content, a ton of cassava roots may produce around 200 liters of ethanol. One liter of ethanol has around 21.46 Mj of energy. The overall energy efficiency of producing ethanol from cassava root is roughly 32%. Endomycopsis fibuligera, a yeast, and occasionally *Zymomonas mobilis*, a bacterium, are used to prepare cassava

Alcoholic fermentation inducer

It is common knowledge that *S. cerevisiae* is the organism most frequently utilized in the fermentation of alcoholic beverages. In several fermentation-related sectors, this yeast is frequently utilized as a microbial starter. Because of their strong selection environment low pH, high ethanol and sugar concentrations, or anaerobic conditions *S. cerevisiae* becomes a dominant species during the alcoholic fermentation of fruits and juices.

Alcohols are essential to commerce and industry. Methanol, isopropanol, ethanol, or ethylene glycol are all types of commercial alcohol. Ethanol is a solvent, a gasoline additive, a component of lotions and medications. Typically, ethanol is referred to as ordinary alcohol. 1 Sugars are fermented to make ethanol. A material undergoes fermentation when it is chemically broken down by yeast, bacteria, or other microorganisms. One of the earliest types of fermentation is the conversion of carbohydrates into alcohol. Starting with a mixture of sugar supply, yeast, and water, fermentation is then allowed to proceed in an atmosphere without oxygen. Because of the anaerobic conditions, the yeast must stop burning sugar so they can produce alcohol. During alcoholic fermentation, glucose and fructose (sugars) are anaerobically transformed into carbon dioxide and ethanol, respectively. A few number of bacteria and yeasts perform the process (*Zymomonas mobilis*). Through the metabolism of hexose, alcohol fermentation replenishes the NAD+ that was depleted during glycolysis and provides yeast with a boost of two ATP molecules.

While fermenting grape juice, the yeast *Saccharomyces cerevisiae* primarily uses the pyruvate to produce ethanol to replace the NAD + lost during the glycolysis process. This phenomenon is referred to as alcoholic fermentation. During alcoholic fermentation, both glucose and fructose (sugars) are anaerobically transformed into ethanol or carbon dioxide respectively. A few number of bacteria and yeasts perform the process (*Zymomonas mobilis*).Through to the metabolism of hexose, alcohol fermentation replenishes the NAD+ that was depleted during glycolysis and provides yeast with a boost of two ATP molecules. While fermenting grape juice, the yeast *Saccharomyces cerevisiae* primarily uses the pyruvate to produce ethanol to replace the NAD + lost during the glycolysis process. This phenomenon is referred to as alcoholic fermentation.

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Media planning

The inoculum is made under aseptic circumstances once the required yeast (Saccharomyces cerevisae) has been chosen and isolated in pure form. To make the inoculums larger so they may be used for inoculation, the yeast is first cultivated in a flask. Fermentation Fermentation is carried out continuously. Continuous fermentation calls for maintaining bacterial growth in the fermenter for an extended period of time while introducing medium at regular intervals. 7 Between the ranges of 21 and 26 oC, the temperature and pH are both maintained. The product from the fermenter is frequently collected to prevent overflow, but the fermentation never stops and continues for a very long time with the supply of nutrients and the regular harvesting of the metabolite. Ethanol evaporates whenever the temperature is beyond 27 °C. Healthy organism growth initially requires aeration; thereafter, anaerobic conditions occur from the loss of oxygen and the production of carbon dioxide. When fermentation is finished, the fermentation broth includes ethanol in the volume range of 6-9%, which corresponds to a conversion of the substrate to ethanol of around 90–95%.

Yeast + Glucose = Carbon dioxide + Ethanol

Removal of ethanol

The mass is separated by centrifugation in a centrifuge. Ethanol may be extracted from the fermentation broth by distillations. The distillation procedure is utilized when the concentration is more than 95%. 100% alcohol is created by a specific type of distillation called azeotropic distillation. 8 To do this, benzene, water, and alcohol are first combined to form an azeotrope, and then the mixture is progressively heated to distill it. By employing this technique, only 100% alcohol is left after the first release of benzene, ethanol, and water.

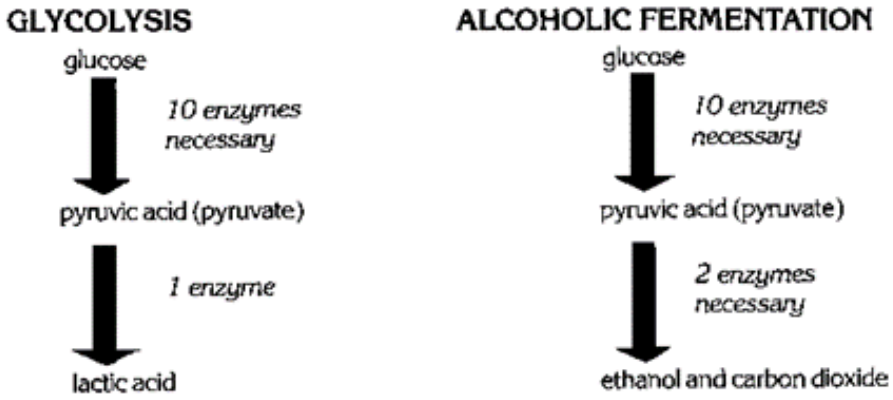

Figure 6.1: Comparative features of Glycolysis and alcoholic fermentation.

The muscles use glycolysis to generate energy when there is a lack of oxygen during lengthy and strenuous activity. Yeasts get their energy via a similar process known as alcoholic fermentation when they are living in anaerobic environments. During glycolysis, glucose is chemically broken down into lactic acid, releasing ATP as a source of energy for cellular function. Alcoholic fermentation resembles the glycolysis process exactly, with the exception of the last stage. Alcoholic fermentation causes the pyruvic acid to break down into ethanol and carbon dioxide.

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While the byproducts of alcoholic fermentation have been employed in brewing or baking for a long time, the lactic acid generated through the glycolysis process produces a feeling of weariness. Glucose is the starting material for both the anaerobic glycolysis and alcoholic fermentation processes. The glycolysis process, which changes glucose into lactic acid, calls on eleven enzymes. Enzymes are used exclusively in the first 10 stages of alcohol production. The last enzyme of glycolysis, lactate dehydrogenase, is replaced by two other enzymes when ethanol is produced. The enzymes pyruvate decarboxylase and alcoholic dehydrogenase convert pyruvic acid into carbon dioxide and ethanol during the fermentation process of alcohol. Whether wine, beer, or spirits are more your style, all of these alcoholic drinks have one thing in common: They all contain alcohol, which indicates that fermentation has taken place in each and every one of them. Although the general idea of fermentation is rather easy to understand, many drinkers aren't entirely aware of the nuances of this crucial step in the production of alcoholic beverages. A biological process called alcoholic fermentation, often known as ethanol fermentation, turns sugar into carbon dioxide and alcohol. Alcoholic fermentation is an anaerobic process since yeasts carry out this function and oxygen is not required. The fermentation process produces heat, carbon dioxide, water, and alcohol as byproducts. In this instance, our attention is on the latter.

Cells use aerobic fermentation, also known as aerobic glycolysis, to digest carbohydrates in the presence of oxygen while suppressing the body's regular respiratory mechanism. In yeast, it is known as the Crabtree effect. , which contributes to the Warburg effect in cancer cells. While aerobic fermentation does not yield large amounts of adenosine triphosphate (ATP), it does enable proliferating cells to convert nutrients like glucose and glutamine into biomass more effectively by preventing their unneeded catabolic oxidation into carbon dioxide, maintaining carbon-carbon bonds, and encouraging anabolism. At least three yeast lineages have independently developed aerobic fermentation (Saccharomyces, Dekkera, Schizosaccharomyces). Additionally, it has been seen in tumour cells, mutant E. coli, trypanosomatids, and plant pollen. When fed with extremely low glucose concentrations or when grown on the majority of alternative carbohydrate sources, Crabtree-positive yeasts will respire. The Crabtree effect is a regulatory mechanism wherein fermentation, with the exception of low sugar circumstances, suppresses respiration. The fermentation route is still completely expressed when *Saccharomyces cerevisiae* is grown below the sugar threshold and goes through a respiration metabolism, however the respiration pathway is only expressed in relation to the sugar availability. The Pasteur effect, which is the suppression of fermentation in the oxygenated environment and is seen in the majority of organisms, contrasts with this.

The increase of hexose transporter genes, copy number variation (CNV), differential expression of metabolic genes, and regulatory reprogramming were among the molecular processes that likely led to the emergence of aerobic fermentation. To completely comprehend the chromosomal underpinnings of this complicated event, further study is still required. Many Crabtree-positive yeast species are utilised in industrial operations to produce wine, beer, sake, bread, and bioethanol because of their capacity for fermentation. These yeast species have developed via domestication, sometimes by artificial selection, to better suit their habitat. Interspecific hybridization, horizontal gene transfer (HGT), gene duplication, pseudogenization, and gene loss are a few of the ways by which strains have developed.

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Crabtree effect in yeast: its history

A full genome duplication occurred throughout the yeast lineage around 100 million years ago (mya) (WGD). Post-WGD yeasts make up the bulk of Crabtree-positive yeasts. Due to the duplication of the genes encoding for alcohol dehydrogenase (ADH) and hexose transporters, it was thought that the WGD was a mechanism for the formation of the Crabtree effect in these species. Recent research has shown that aerobic fermentation, which may have benefited from the WGD, began before the WGD and developed as a multi-step process. In Saccharomyces Crabtree-positive yeasts, the initial stage of aerobic fermentation likely developed during the time between the capacity to grow anaerobically and the horizontal transmission of anaerobic. The second stage, a stronger Crabtree effect, most likely took place close to the WGD occurrence. The section on the genetic basis of the Crabtree effect provides a clearer understanding of later evolutionary processes that contributed to the emergence of aerobic fermentation.

It is thought that the simultaneous genesis of aerobic fermentation and modern fruit (about 125 mya) was a significant factor in the process's evolution. These fruits offered microbial groups, including yeast and bacteria, a plentiful supply of simple sugar as a food source. At that time, bacteria were able to create biomass more quickly than yeast. Bacteria may grow more slowly when a harmful substance, such as ethanol, is produced, making yeast more competitive. But some of the sugar that the yeast uses to make ethanol still had to be used. Greater glucoselytic flux, or increased absorption of glucose and conversion to pyruvate, is another characteristic of crabtree-positive yeasts that makes up for the fact that some of the glucose is used to build ethanol rather than biomass. As a result, it is thought that the initial motivation was to eliminate rivals. Research that examined the kinetic behaviour of the ancestor ADH protein, which was discovered to be tuned to produce ethanol rather than consume it, lends credence to this.

The effectiveness of this mode of living was probably enhanced by further evolutionary processes that led to aerobic fermentation, such as greater ethanol tolerance and respiratory route suppression. Except for its closest cousin Saccharomyces paradoxus, *S. cerevisiae* outcompetes and dominates all other yeast species under high sugar conditions. In contrast to aerobic fermentation, the capacity of *S. cerevisiae* to dominate in high-sugar settings emerged more recently, and it depends on the kind of high-sugar environment. The pH and nutrients in the high-sugar environment affect how other yeasts develop.

For thousands of years, people have used the fermentation process to produce ethanol. Mead, a beverage made by fermenting honey and water, was a specialty of the ancient Greeks. But in the meanwhile, honey has lost ground to other foods, namely wheat and grapes (for wine). Other fruits, such as berries, apples, and so forth, rice (for sake), and more are other basis items.

This is a big problem among producers of alcohol, especially in the natural wine industry. Native yeasts, sometimes referred to as wild yeasts or ambient yeasts, are found in abundance in cellars and on fruit skins. When a distiller opts to use native yeasts for fermentation, they are only depending on the yeasts that are already present on the ingredients and in the cellar where the fermentation is taking place. Naturally occurring fermentation often takes a lot longer, although this isn't always a negative thing. When a producer decides to employ cultured yeasts, it entails

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seeking for, buying, and adding a particular strain of yeast to the raw ingredients to initiate fermentation. Like spices, yeasts have a wide range of tastes and chemical compositions. Despite the fact that the fermentation process will often take significantly less time and the outcome is frequently more predictable and consistent, purists would claim that employing cultured yeasts detracts from the authenticity of a raw ingredient. This is often the approach followed by persons who produce big amounts of alcohol due to these factors.

The Distinction between Distillation and Fermentation

The process of utilizing yeasts to turn carbohydrates into alcohol is known as alcoholic fermentation. Higher-ABV drinks may be made from already-fermented base items using the distillation process. (For instance, distilling beer wort yields whisky, whereas distilling wine yields brandy.) Although not all fermented drinks are distilled, fermentation occurs in all alcoholic beverages. Any process in which bacteria, yeast, or other microbes cause a food to undergo a desired transformation is referred to as fermentation. You've undoubtedly heard of a few more forms of fermentation in relation to food and beverages than alcoholic and ethanol, such as lacto-fermentation and acetic acid fermentation. The fermentation process used to make kombucha, kefir, and ginger beer uses acetic acid. In the process of lacto-fermentation, bacteria that produce lactic acid, especially those belonging to the lactobacillus genus, break down the sugars in food to produce lactic acid, carbon dioxide, and sometimes alcohol. Usually, the procedure entails mixing water, salt, and sugar in an anaerobic environment (typically in the form of a vegetable or fruit). It's how classic dill pickles, kimchi, and sauerkraut are created. More daring bartenders have started experimenting with this kind of fermentation recently in order to create complexly flavoured components (and brine) for their drinks.

Yeasts

Yeasts are eukaryotic microorganisms that primarily inhabit water, soil, air, and the surfaces of plants and fruits, among many other ecological niches. At this stage, the latter habitat may be the most fascinating since it directly affects how ripe fruit decomposes and how fermentation occurs. Yeasts may successfully carry out their metabolism or fermentation activities in this natural setting because they have access to the essential nutrients and substrates. In terms of nutrition, yeasts are less demanding than other microorganisms like lactic acid bacteria. However, the presence of fundamental substances like fermentable carbohydrates, amino acids, vitamins, minerals, and oxygen helps to encourage their development. From a morphological perspective, yeasts exhibit a significant degree of divergence; the most typical morphologies are round, ellipsoidal, and oval. Microscopical analysis really comes first in the identification procedures, followed by more discriminating tests like microbiological or biochemical ones. The traditional categorization goes on to include additional, labor-intensive tests including those for sugar fermentation or amino acid absorption. Other crucial instruments for separating species include the capacity for producing and tolerance to ethanol, organic acids, and SO2. Yeasts mostly reproduce through budding, which creates a brand-new cell with the exact same genetic makeup.

The most typical form of asexual reproduction is budding, while yeasts of the genus Schizosaccharomyces are known to exhibit cell fission. Lack of amino acids, for example, may

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cause food deprivation during growth, which triggers sporulation, a survival strategy utilised by yeasts. Genetic diversity occurs in yeast cells as a consequence of sporulation. To preserve the genotype and maintain stable fermentation behaviour that does not stem from it for as long as feasible throughout industrial fermentation operations, asexual reproduction of yeasts is advised. At the metabolic level, yeasts are distinguished by their ability to ferment a wide range of sugars, with glucose, fructose, sucrose, maltose, and maltotriose predominating. These sugars are present in both ripe fruit and processed grains. Additionally, yeasts may survive in settings with pH levels as low as 3.5. Yeasts are separated into Saccharomyces and non-Saccharomyces for the sake of technical convenience. Morphologically, Saccharomyces yeasts may be spherical or ellipsoidal in form depending on the growth phase and culture circumstances. Due to its good fermentative ability, quick growth, and ease of adaptation, *S. cerevisiae* is the species that has been researched the most and is also the one that is used the most in the fermentation of wines and beers. They can withstand SO2 levels at which the majority of non-Saccharomyces yeasts typically cannot. Despite these benefits, it is still feasible to encounter *S. cerevisiae* representatives in nature that do not necessarily share these traits.

Non-Saccharomyces Yeasts

Non-Saccharomyces yeasts are a class of microorganisms that are employed in many fermentation processes because of their substantial metabolic diversity, which enables the production of many end products. Eliminating or maintaining low levels of several of these yeasts that might alter the sensory quality of wines was a fundamental goal in the past since they are often regarded as pollutants. It is customary to use sulfite to disinfect the tanks and fermentation containers in order to get rid of their activity during wine production. This impression has changed year after year as a result of the work of these yeasts during spontaneous fermentation, which has a favourable impact on the wine's ultimate sensory quality. When spontaneous fermentation first begins, these yeasts predominate until the ethanol concentration reaches 4 to 5% v/v.

Their development is therefore hampered by the alcohol and the depletion of dissolved oxygen. The fermentation is finished when the most ethanol-resistant yeasts, Saccharomyces, prevail and take over. Some non-Saccharomyces yeasts have reportedly been shown to persist near the conclusion of spontaneous fermentation and exert their metabolic activity, favourably influencing the sensory quality of wines. Based on this information, several scholars have recently concentrated their study efforts on comprehending the characteristics and fermentative activity of the non-Saccharomyces yeasts. The results showed how highly effective these yeasts may be in the fermentation of both conventional and novel drinks. While *S. cerevisiae* has some technological advantages over most non-Saccharomyces yeasts, such as higher fermentative power and ethanol production, non-Saccharomyces yeasts exhibit traits that aren't present in *S. cerevisiae*, such as the production of high levels of aromatic compounds like esters, higher alcohols, and fatty acids.

A method that may be utilised to lower the ethanol content of wines produced in coculture with *S. cerevisiae* is the observation that the fermentative activity of these yeasts manifests in the presence of tiny levels of oxygen, which increases cell biomass and decreases ethanol output. Fermentations using mixed and sequential cultures of *S. cerevisiae* may be carried out to create fermented drinks

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with various sensory profiles while maximising the benefits of non-Saccharomyces yeasts and minimising their drawbacks. The ability to produce a wide range of sensory-important compounds, which are required to improve the organoleptic quality of wines and beers, is the most significant fact. The results that have been so far published in the literature have caused people to reevaluate the function of these yeasts in fermentative processes and the employment of these yeasts in the creation of new goods. Candida, Kloeckera, Hanseniaspora, Brettanomyces, Pichia, Lanchacea, and Kluyveromyces are some of the non-Saccharomyces yeasts that have received the greatest attention from researchers and have attained exceptional prominence.

Processes used in Yeast Fermentation

Alcoholic Fermentations

The oldest and most significant biotechnology is the use of yeast to produce alcoholic drinks from sources of fermentable carbon. All alcoholic drinks are produced with yeast, which is essential. All alcoholic drinks are produced using yeast, and choosing the right yeast strains is crucial to maintaining the sensory quality of the beverage as well as maximising alcohol output. Fermentation of Wine For example, strains that are strong ethanol producers are required for wine fermentation in order to attain the average ethanol concentrations of 11–13% v/v. Conversely, beers and ciders have lower ethanol content and each has a unique taste profile that is both balanced and recognisable. New consumer demands for novel and inventive items have evolved in recent years.

Due to this circumstance, fermented drinks have to be rethought in order to satisfy customer demand. The richness and sensory appeal of fermented drinks are primarily due to yeasts. On the basis of this, present research is mostly devoted to the hunt for novel yeast types with technological applications. Non-Saccharomyces yeasts have long been regarded as pollutants in wine and beer production. As a result, methods for getting rid of them, such pasteurising must, adding sulfite, and sanitising machinery and processing areas, are often used. Since various studies have shown that non-Saccharomyces yeasts are crucial in determining the sensory quality of the finished product during spontaneous fermentations of wine, the unfavourable opinion of these yeasts has begun to change. Based on these findings, researchers are thoroughly examining the fermentative behaviour of a few non-Saccharomyces yeasts in an effort to identify the ideal production environment and strain for fermented drinks.

Fermentation of beer

The most popular alcoholic beverage consumed globally is beer. Four essential ingredients malted grains (either barley or another kind), water, hops, and yeast are usually used to make it. Each of these components helps to produce the ultimate flavour and fragrance of beer. Yeast cells turn cereal-derived carbohydrates into ethanol and CO2 during the fermentation process. Numerous secondary metabolites that affect the flavour and fragrance of beer are created at the same time. What enables yeast to have such a distinctive impact on beer taste is variation in these metabolites among various yeast strains. Even while the majority of brewers employ pure yeast cultures, certain speciality beers increasingly use spontaneous or mixed fermentation. These fermentation processes use a variety of yeast species (and bacteria as well) that each add something different to

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the finished product in a different order, giving the beer a high level of complexity. Breweries often have their own supply of chosen yeasts for their particular brews. As is common knowledge, there are two varieties of yeast used in brewing: *S. cerevisiae*, which is used to create ales and S. pastorianus, which is used to make lagers.

Fermentation of cider

Another alcoholic beverage created from the apple fruit sector is cider, which is highly well-liked around the globe but is most prevalent in Europe, North America, and Australia. Traditional ciders are made by the spontaneous alcoholic fermentation of juice by indigenous yeasts, however some *S. cerevisiae* strains are also often used. This guarantees that the final items will be of a high standard. The creation of cider involves the spontaneous fermentation of apple juice using certain other non-Saccharomyces yeast species. However, compared to Saccharomyces, these yeasts contribute less and have the potential to generate bad flavours. Compared to wine, this kind of product has less research publications, particularly when it comes to microbial activity-related phenomena. There has been a lot of recent research on the dynamics of the wine fermentation microbiome, the creation of starters, and the organoleptic enhancement of tasty and healthful foods. Although the two drinks appear to have a similar microbiome and fermentation process (both alcoholic and malolactic fermentations), it is still worthwhile to conduct research on the particulars of apple fermentation due to the inherent qualities of the raw materials and various production and environmental factors. A good overview of the microbial effects related to the manufacture of cider, including ecological concerns, associated activities, and the impact of process factors.

There are other additional fermented drinks created from fruit in numerous nations across Africa, Asia, and Latin America in addition to these three internationally well-known ones. Drinks produced using fruits like grapes or bananas as the primary ingredient are particularly well-liked in certain nations, while only being consumed locally or regionally. Banana beer is the most popular alcoholic fruit beverage in Eastern Africa, and in addition to being delicious, it also has significant cultural significance. Banana beer is a mixed beverage prepared with cereal flour and bananas (often sorghum flour). Other of the most important goods are dates in North Africa, pineapples and cashews in Latin America, and jackfruit in Asia.

Fermentations without Alcohol

Additionally, yeast may play a role in the fermentation of non-alcoholic items such bread, cocoa, coffee, kefir, sodas, lemonades, and vinegar, as well as biofuels and other chemicals.

Bread Fermentation

The most important step in producing bread is the fermentation of the yeast-made dough. The amount of yeast cells that ferment during this fermentation is essential since it affects the bread's ultimate quality. In addition to producing CO2, yeasts also release additional metabolites that affect the final texture, volume, and flavour of the bread. The fundamental elements for process control are the yeast strain, pregrowth circumstances, its activity during dough fermentation, the fermentation conditions, as well as the dough components. The elements of the dough, especially

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the quantities of sugar and salt employed in its production, have an impact on how quickly it ferments. Currently, commercial bakeries create a variety of dough kinds, including frozen, sweet, and lean dough. It is advised to employ appropriate yeast strains with certain phenotypic characteristics depending on the kind of dough and to attain optimum fermentation rates.

Fermentation of coffee in the post-harvest stage of coffee manufacturing, yeasts are crucial. It may be performed in two stages. On the one hand, aerobically, where the freshly-gathered berries are placed in a tank and the yeasts are let to function. Basic factors like time and temperature are controlled throughout this procedure. An alternative is to place coffee berries in a container with water mixed in and let microbes function anaerobically (in the absence of oxygen). Compared to the aerobic process, the second one is easier to manage and more uniform. Coffee beans may sometimes undergo a mixed fermentation process in which they are first fermented aerobically and then anaerobically. Mucilage should be eliminated in order to develop these processes successfully and to maintain or enhance the organoleptic characteristics of coffee, increase its sweetness, manage its acidity, give it body, or add sensory notes (chocolate, caramel, or fruits). Even while the mixture's yeasts naturally carry out the process, it may be enhanced by adding the right enzymes (polygalacturonase, pectin methylesterase,pectin lyase,).

Fermentation of chocolate

Because they contain a significant amount of phenols, raw cacao beans have a bitter and astringent flavour. These polyphenols include anthocyanins, which contribute to astringency and give foods their reddish-purple colour. Proteins and carbohydrates within the bean may be broken down enzymatically during fermentation, which results in taste development. Microbial fermentation, which produces the ideal environment by fermenting the cacao pulp around the beans, helps with this. This stage of processing allows the taste of the cacao to be extracted and increases the final product's acidity. The cacao beans' juicy pulp is fermented by yeasts (and bacteria, too), typically after an anaerobic phase and an aerobic phase. Yeasts devour the pulp's sugars (sucrose, glucose, and fructose) during the anaerobic phase to produce carbon dioxide, ethanol, and small quantities of energy. Bacteria that produce lactic and acetic acids predominate in the aerobic stage.

Yeast Fermentation Special Issue

This issue of Microorganisms strives to further our understanding of yeasts from both a fundamental and practical standpoint. A paper on the brewing business and the recent isolation of the yeast Saccharomyces eubayanus is one of the outstanding contributions to this issue. The synthesis of volatile chemicals in wild strains and comparison to a commercial yeast have both benefited from the use of headspace solid-phase microextraction (HS-SPME-GC-MS) and gas chromatography-mass spectrometry. All of these discoveries demonstrate how this yeast has the potential to create new types of beers. Finding and choosing pectinolytic yeasts that could be used as a starting culture for coffee fermentation. Almost 30 isolates, eight of them with the potential to manufacture pectinase enzymes were found and verified by employing molecular biology methods. Additionally, phylogenetic trees that could be utilised to ascertain the evolutionary connection of yeasts based on the results of their tests were created using a useful bioinformatics tool. Biofuel generation by recombinant *Saccharomyces cerevisiae* strains containing critical

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genes and metabolic networks for xylose metabolism has been also described. The scientists have shown that PDE1 and PDE2 cAMP phosphodiesterase genes may be deleted to improve xylose uptake. Additionally, additional objectives for building different xylose-fermenting strains are made available. The use of xylose, the second-most prevalent sugar component in lignocellulosic material hydrolysates, is an important topic. When hexokinase 2 (Hxk2p) is implicated, understanding the connection between xylose and the metabolic regulatory systems in yeasts is essential. If polluted, all of these processes risk being affected. Contamination should always be taken into account since the majority of fermentation substrates are not sterile. An extremely creative method was employed to use a genetically altered strain of Komagataella phaffii yeast to produce lactate using glycerol as the basic material. In the pharmaceutical, automotive, packaging, and food sectors, polyactide, a bioplastic, is frequently employed. It was possible to accomplish the disruption of the gene encoding arabitol dehydrogenase (ArDH), which enhances lactic acid generation. Information on microbial contamination and its effects on yeast fermentations, as well as industrial applications of yeast fermentation, are included in this study.

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CHAPTER 7

Enzyme Fermentation

Upendra Sharma B. S.

Assistant professor, Department of Life Science, School of Sciences, B-II,

Jain (Deemed to be University), J C Road, Bangalore-560027.

Email Id- Upendra.sharma@jainuniversity.ac.in

Proteins that function as catalysts are called enzymes. Enzymes reduce the amount of energy needed for a reaction to take place without being consumed by the reaction itself. Enzymes are used by many different sectors to help in product production. These include things like cheese, beer, and bread. Enzymes can be produced by fermentation for industrial uses. Microorganisms like yeast and bacterium are used during fermentation to create the enzymes. There are two fermentation processes that are used to make enzymes. These include solid-state fermentation or submerged fermentation. Microorganisms in a liquid nutrient medium produce enzymes during submerged fermentation. The growing of microorganisms, and therefore enzymes, on such a solid substrate is known as solid-state fermentation. The bacteria, which generate the enzymes either intracellularly or extracellularly, degrade the carbon-containing molecules present in or on the substrate. The extracellularly generated enzymes are retrieved via centrifugation, while the intracellular enzymes are retrieved by lysing the cells. Enzymes are essential for the manufacture of several industries' products. Brewing, winemaking, baking, or cheesemaking are industries that utilise enzymes produced by fermentation.

Enzymes are already available commercially and cost about the same as chemical procedures. The commercialization of enzymatic depilation will thus benefit from any major reduction in the cost of enzyme production. Proteases make up one of the most important subcategories of industrial enzymes, making up over 60% of total enzyme sales. The production of dry cleaning, meat processing, detergents, digestive enzymes, cheesemaking, silver recovery from photographic film, but many medicinal treatments for the treatment of severe wounds or inflammation are the main uses of free proteases. They are among the many types of microorganisms that are commercially accessible, and during the starch processing business, they have nearly entirely replaced chemical hydrolysis of starch. Although numerous microbes generate this enzyme, Bacillus amyloliquifaciens, Bacillus licheniformis, or *Aspergillus niger* are the most often employed for their industrial applications.

Process of Enzyme Fermentation

Microorganisms may generate and secrete proteins with catalytic activity during industrial fermentation, which is utilized to create enzymes. The commercialization of several enzymes has been made possible by the advancement of fermentation techniques, microbial strain engineering,

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or recombinant gene technology. Enzymes are utilized in a variety of industrial sectors, including animal feed, personal care, textile, paper and pulp, food or beverage lactose removal, cheese flavoring, beverage juice treating, dough conditioning, baking bread softness, or animal feed.

Choosing the best organism or host is the initial step in the production of enzymes. The most typical strategy is to look into naturally occurring plants and microbes, where enzymes might already be performing functions that are needed for commercial applications. Life on earth is abundant and diverse, particularly in terms of microbes. For thousands of years, people have utilized enzymes to make foods and drinks including cheese, beer, yoghurt, or wine. A fungus called yeast produces enzymes that help glucose break down anaerobically into both carbon dioxide and ethanol. Yeast's enzymes convert sugar (glucose) into ethanol or carbon dioxide gas:

$$\text{Glucose} \xrightarrow{\text{Yeast}} \text{Ethanol} + \text{Carbon dioxide}$$

$$C_6H_{12}O_6\,(aq) \xrightarrow{\text{Yeast}} 2\,C_2H_5OH\,(aq) + 2\,CO_2\,(g)$$

To find novel, fascinating microorganisms that currently carry out desirable industrial activities, research is being done. Some of this research has focused on bacteria called extremophiles, which can survive in harsh conditions. For instance, thermophilic species flourish in settings with extremely high pressures and temperatures that are created by volcanic vents on ocean floors. Similar to this, alkaphilic species are abundant in high pH lakes in North Africa. These microorganisms already produce tough-as-nails enzymes that may be exploited to create novel and fascinating enzyme variations.

The goal of more recent methods is to create new, desirable enzyme characteristics in the lab by using existing microorganisms as well as genetics or molecular biology to insert new traits into the protein structure. Industrial enzymes are ultimately created by fermentation, much like beer or wine. Through either internal or extracellular expression, the microorganisms ferment under regulated circumstances and create the enzymes. The enzymes are then stabilized to ensure appropriate shelf life after being centrifuged or filtered to separate them.

Together, these techniques have generated a large variety of enzymes appropriate for commercial use, and the number is growing every day. EDT has built up a sizable enzyme library of prospective raw materials in order to develop tailored solutions for potential customers. This library is rapidly expanding as a result of the substantial research on cellulose breakdown for ethanol production that has been going on for the past few years. Exceptional performance flexibility and increased value for our clients are the ultimate results.

Alcohol is one of the numerous products that come from fermentation and the usage of enzymes; fermentation and enzyme use also create other compounds. Alcohol is created throughout the brewing and wine-making processes. Yoghurt, Cheese, and bread are among more goods. Lactic acid and carbon dioxide are released as a result of the enzymes and the microorganisms. By using the enzymes generated by bacteria, mold, or yeasts, which can happen in aerobic or anaerobic settings, fermentation modifies the features of the meal. Acetic acid, ethanol, lactate, or other basic compounds are produced during fermentation.

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Implementation Enzymes in Fermentation

The class of enzymes known as amylases is used in a variety of sectors, including textile, brewing, culinary, pharmaceutical, or detergent. They are the enzymes that digest starch or glycogen. Amylases come from a variety of sources, including bacteria, plants, and mammals. However, the main benefit of employing microorganisms to make amylase is their potential for affordable mass manufacturing. Additionally, it is simple to engineer microbes to produce enzymes with certain properties. Many microbial amylases are commercially accessible and in great demand in industry, notably for various fermentation processes. Lipases are among the most widely used enzymes in industry, playing a crucial part in the manufacturing of biodiesel, dairy and bread products, pharmaceuticals, detergents, oils, and fats, as well as the paper and pulp, pharmaceutical, or detergent industries. Because of their wide range of substrate specificity, stability, or selectivity, microbial lipases are promising in terms of availability or productivity. Fungi are known to produce more lipases than any other source, and they are also valuable in a number of commercial applications.

A significant amount of the biotechnology sector's overall output is produced by microbial enzymes thanks to the advancement of large-scale fermentation technologies. Large containers (fermenters) with capacities up to 1,000 cubic metres are used for fermentation.Nutrients based on renewable raw sources including soy, corn, and sugars are sterilised in the fermentation medium. To break down the carbon and nitrogen sources, microorganisms secrete the majority of industrial enzymes into the fermentation medium. Fermentation techniques that are batch-fed and continuous are frequent. In the batch-fed method, the fermenter receives sterile nutrients as the biomass grows. Sterilized liquid nutrients are continuously delivered into the fermenter at the same flow rate as the fermentation broth is expelled from the system.

The production will reach a stable state as a result. To optimise the fermentation process, variables like temperature, pH, oxygen consumption, and carbon dioxide generation are monitored and managed. First, in order to extract enzymes from the fermentation medium, insoluble products like microbial cells must be eliminated. Centrifugation is often used to do this. The majority of industrial enzymes are extracellular (secreted by cells into the external environment), thus even after the biomass has been taken out, they are still present in the fermented broth. The lime treatment is necessary to stabilise the biomass during storage and inactivate the microorganisms before it can be recycled as fertiliser. Depending on their intended use, the enzymes in the leftover broth are subsequently concentrated using evaporation, membrane filtering, or crystallisation. Pure enzyme preparations are often extracted by gel or ion exchange chromatography if they are needed.

Granulated enzymes are created from the raw powder enzymes to make them more usable for applications that need solid enzyme products. Because they are simpler to handle and dose when combined with other liquid medicines, liquid formulations are sometimes recommended. Enzymes are immobilised, usually on the surfaces of inert granules held in reaction, during the starch conversion process that turns glucose into fructose. Columns or towers used in the fermentation of enzymes. This is done in order to extend their working lives since these enzymes often continue to function for more than a year.

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Liquid-solid fermentation

The process of solid-state fermentation (SSF) is another one utilized to make enzymes. Microorganisms are grown on solid substrates like grains, rice, and wheat bran in solid-state fermentation. This process is an alternative to submerged fermentation for the manufacture of enzymes in liquid. SSF is far better than submerged fermentation in many ways. High volumetric productivity, relatively high product concentration, little waste produced, and simple fermentation equipment are a few of these. The SSF is capable of using a wide variety of substrates to produce enzymes. Wheat bran, rice bran, sugar beet pulp, and wheat and corn flour are a few of them. The cost and accessibility of the substrate are the two key variables that influence the choice of substrate.

The degree of moisture and particle size are further considerations. Smaller substrate particles provide more surface area for microbe development, but if they are too tiny, respiration efficiency will be hindered, resulting in poor growth and low enzyme synthesis. Larger particles provide more effective aeration and respiration, but the surface area is reduced. Regarding the particle size of the substrate for a certain procedure, a compromise must be achieved. Moisture on the substrate is necessary for SSF in order for the bacteria to generate enzymes. A greater or lower presence of water may negatively impact the microbial activity, hence the water content of the substrate must also be adjusted. Water exerts effects on the solid substrate's physicochemical characteristics as well. SSF has generated industrially significant enzymes. Proteases, glucoamylases, pectinases, and cellulase are a few examples.

Enzyme Utilization in Fermentation

The family of enzymes known as amylases is used in a variety of sectors, including textile, brewing, culinary, pharmaceutical, and detergent. They are the enzymes that digest starch or glycogen. Amylases come from a variety of sources, including bacteria, plants, and mammals. However, the main benefit of employing microorganisms to make amylase is their potential for affordable mass manufacturing. Additionally, it is simple to engineer microbes to produce enzymes with certain properties. There is a significant industrial need for microbial amylases, and there are several of them commercially accessible, particularly for various fermentation processes.

Lipases are among the most widely used enzymes in industry, playing a crucial part in the manufacturing of biodiesel, dairy and bread products, pharmaceuticals, detergents, oils, and fats, as well as the paper and pulp, pharmaceutical, and detergent industries. Because of their wide range of substrate specificity, selectivity, and stability, microbial lipases are promising in terms of availability and productivity. Fungi are among the several sources of lipases recognised to provide the finest enzymes and are also valuable in a number of industrial applications.

Do enzymes have a role in fermentation?

A protein that is generated as both intracellular and extracellular molecules is an enzyme. They assist to increase the pace of response by energising and catalysing biological processes with more selectivity. Today's commercially accessible enzymes are more affordable than chemical methods in comparison. Proteases are among the most significant industrial enzymes, making up around

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60% of all enzyme sales. Proteases are primarily used in the manufacturing of digestive enzymes, the processing of meat, dry cleaning, cheesemaking, recovering silver from photographic films, and detergents. Proteases are also specifically used in the treatment of infectious wounds and inflammatory conditions. In the starch processing sector, a large variety of microbes, including proteases, are commercially accessible and have almost completely replaced chemical starch hydrolysis. Enzymes are also utilised in contemporary wine processing methods for a variety of biotransformation processes, including pre-fermentation, post-fermentation, and wine ageing.

How does fermentation work?

Since ancient times, microbes have been used to ferment food, and the fermentation process is being used to prepare a variety of foods today. Since microbial enzymes are more stable than plant and animal enzymes, they are essential in the food industry. Because their process modification, optimization, and consistency are simple to accomplish, they are also manufactured via the fermentation process cost-effectively with little time and space requirements. They are used in the paper business to lower the viscosity of starch so that paper is properly coated, as well as in the detergent industry to remove starch-based stains. On the other side, amylases are used to warp the dimensions of fabrics. Enzymes with a wider variety of uses in the food business include lipases, proteases, and xylanases.

Techniques for processing starch

For thousands of years, enzymes have been utilised to create foods and drinks including cheese, yoghurt, wine, and beer. An organism known as yeast uses its enzymes to convert glucose into ethanol or carbon without the need for oxygen.

Fermentation is the term used to describe this kind of anaerobic process. When the glucose solution and yeast are maintained at a warm temperature, fermentation may function at its best. If the temperature is maintained too high, the enzymes lose their effectiveness. The process of fermentation is used to make alcohol and alcoholic beverages. To boost the ethanol content in the fermented mixture, stronger alcohols like vodka and whisky must be distilled following the fermentation process. This is due to the fact that ethanol tends to poison yeast and cause it to cease functioning if the concentration increases by more than 18% by volume. Bread dough is produced via the fermentation process in the baking business. After the dough is ready, it is placed in a warmer area to rise before going into the oven. This makes it possible for the yeast's enzymes to break down sugar and produce carbon dioxide.

When it comes to the pharmaceutical, food, paper, and detergent sectors, enzymes have a variety of uses. Nowadays, enzymatic hydrolysis and enzyme-based procedures are chosen over chemical processes because to the environmental friendliness, better yield, processing safety, and reduced refining costs of enzymes. Microbial enzymes are generated more successfully than plant and animal enzymes using fermentation processes including submerged and solid-state fermentation. Additionally, it is considerably simpler to generate microbial enzymes in greater quantities. It is also simple to alter microbial enzymes using various biochemical and molecular techniques. These enzymes may be produced in excess with a greater specific activity when their genes are overexpressed. There are several potentials to discover a broad variety of microbial enzymes in

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industrial applications, particularly in the food industry and fermentation of foods and drinks. However, many of these enzymes with a microbial origin are currently underexplored.

Because there are so many sources available, microbial enzymes are extensively employed in a variety of sectors. Microbial enzymes are thought to be more cost-effective than plant and animal enzymes and are capable of genetic modification. Application of fermentation techniques for the production of microbial enzymes entails the growth of bacteria, mould, and yeast to produce the required product. Based on a set of criteria, the fermentation process is divided into categories. Utilizing downstream processing techniques aiming at enzyme recovery and purification, several approaches are used to manufacture microbial enzymes.

Based on established principles, including the use of microbial sources, improved strains, and membrane-augmented downstream processing techniques, it is possible to enhance the concentration, purity, or percentage of recovery of enzymes. There are two fermentation processes that are used to make enzymes. These include solid-state fermentation and submerged fermentation. Microorganisms in a liquid nutrient medium produce enzymes during submerged fermentation. The growing of microorganisms, and therefore enzymes, on a solid substrate is known as solid-state fermentation. The bacteria, which generate the enzymes either intracellularly or extracellularly, degrade the carbon-containing molecules present in or on the substrate. Numerous industries use microbial enzymes in a broad range of ways. Brewing, winemaking, baking, cheesemaking, dairy, milling, drinks, and cereals all utilise the enzymes produced by fermentation.

Enzymes are biological catalysts that are "green," and they have revolutionised how we cook food. Worldwide, enzymes are employed in a variety of feed and food sectors, including those that deal with dairy, brewing, meat, baking, juice and drinks, vegetable processing, nutritional supplements, oils and fats, and many more. It's been common practise to use enzymes and microbes while preparing food. Since ancient times, enzymes and microorganisms have been utilised in the production of cheese, beer, and wine. With the use of biotechnology, raw materials may be processed in a variety of new ways to create food items with high nutritional value.

Microbial biotechnology known as fermentation produces value-added products including enzymes, alcohols, polymers, organic acids, and more from naturally renewable substrates. Alcohol and lactic acid are proton sinks for NADH, which is recycled to NAD+, allowing the cell to continue generating energy via glycolysis through substrate-level phosphorylation. Thus, microbes create various endor by-products to maintain energy balance. Targeted genetic engineering approaches applied to known commercial microbial strains have improved production of economically significant fermentation products today. The microbial strain as well as the environmental conditions used determine whether end or byproducts are produced. A microbial strain should be chosen and cultivated depending on the target result for an optimal fermentation process. Technologies for strain development include recombinant DNA and mutation.

Temperature, pH, dissolved oxygen, and the makeup of the fermentation medium all have an impact on the growth of microbes and the production of products. Therefore, it is necessary to adjust the growing environment and fermentation medium. The metabolic pathways should also

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be identified. Producers may influence the production by altering strains and/or circumstances by being aware of the biochemical changes that occur in fermented foods. Fermentation modalities have an impact on productivity in addition to growing circumstances, strain types, and medium. For maximum productivity, fermentation processes may be run in batch, fed-batch, or continuous modes. Fermentation operations may get beyond the substrate constraint by using fed-batch and continuous modes. Cell immobilisation, which raises the biomass concentration in the bioreactor and subsequently the concentration of biocatalysts in the reactor, is another method for increasing productivity. Recovery techniques for the product from the fermentation medium must be assessed and adjusted after the fermentation process, and they are often a cost constraint. The final product's purification is often a costly phase in the process, in fact. Biomass itself, extracellular products, or intracellular products are all examples of microbial end products. Examples of recovery techniques include filtration, homogenization, and extraction (liquid and solid).

Due to their catalytic properties, enzymes, which are byproducts of living organisms, have been used in the industry for a long time. For each process, enzyme activity should be adjusted since it relies on factors including temperature, substrate, pH, and inhibitors. Because enzyme recovery is challenging, using enzyme immobilisation may lower process costs. Microorganisms or plants may create enzymes, and they can also be isolated from tissues of mammals and plants. However, owing to their accessibility and specificity, microbial enzymes are chosen. Over 500 commercial goods are produced using enzymes. They are used in a wide variety of products, including food and detergents. The majority of enzymes are marketed and used to improve the quality of goods such as food, detergents, leather, paper, cosmetics, and medications. Lipase, amylases, pectic enzymes,proteases, and milk clotting enzymes are examples of commercial enzymes (rennet).

Microbial Sources

Industry-produced microbial enzymes are chosen from a variety of microorganism species, including bacteria, fungus, and yeasts. Although many enzymes are manufactured in industrial settings, protease, -amylase, glucose isomerase, and glucamylase are the most frequently synthesised enzymes on a big scale. It was discovered that the microbes used to create industrial enzymes have excellent biological activity. For the generation of enzymes, microbial sources are favoured over those from plants and animals primarily for the following reasons. Enzymes are affordable and easily generated on a big scale. In compared to plant and animal sources, the extraction and purification of enzymes from microbial sources is simpler. Microbial sources have the capacity to produce a wide range of enzymes under various environmental circumstances in a constrained amount of time and space. To increase the output of microbially generated enzymes, genetic modification is used. Several industrially generated enzymes come from microbes and are synthesised on a big scale.

Strain Improvement Methods

In industries, microorganisms are employed as a source for the creation of enzymes, biomolecules, and proteins. *Saccharomyces cerevisiae* and *Aspergillus niger* are a couple of examples of sources of microorganisms that are often employed in businesses to produce alcohol and enzymes. In order to enhance strains and boost production, a wild type strain is isolated. By changing cellular

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genetics, it is possible to increase the fermentation process's desired behaviour and development rate. It is also crucial to comprehend the basics of an organism's physiology and structural makeup. Each microorganism source has various methods, therefore in the case of a fungal source, the focus is mainly on cell wall porosity, differentiation, secretion, and branching. While in the case of yeast, the fermentation process includes ploidy and gene control, via which carbon sources will mostly play a role in the generation of proteins linked to heterologous gene expression. The wild strains that are employed to make metabolic concentrations are not cost-effective. The production of secondary metabolites is essential for strain improvement, which is regarded as a cost-effective approach. Desirable strain separation relies on a system and has the characteristics listed below. Rapid growth, genetic stability, non-toxicity to humans, large cells, shorter fermentation times, and tolerance to carbon or nitrogen sources present in greater concentrations are all characteristics of these organisms. Recombinant DNA technology, Recombination Protoplast fusion, and Mutations-Site-Directed Mutagenesis are a few techniques connected to the process of strain improvement. By raising the dosage of gene concentration, which will raise the product activity that contains one or more genes, such as enzymes, the effective use of these approaches is improved.

Molecular Enzymes

Originally, enzymes were taken out of the stomachs of calves, lambs, and newborn goats; however, nowadays, microorganisms including bacteria, fungus, yeast, and actinomycetes are responsible for producing enzymes. Microorganism-produced enzymes are superior to those from plants and animals. Microorganisms may be genetically altered to increase commercial-scale output. Enzymes have the ability to hydrolyze complicated molecules into simpler monomer units, such as the conversion of carbohydrates into simple sugars, which are organic compounds used in a variety of biological reactions. Each enzyme has unique requirements for the substrate, pH, and temperature needed to catalyse the reaction that changes a reactant into a product. There are more than 55 distinct microbial enzymes used in the food processing sector.

Production of Industrial Enzymes

While the need for more and more extra biocatalysts is being driven by the rising importance of biotechnological achievements, markets for conventional industrial enzymes continue to expand. Genetic engineering has made it possible to generate proteins and enzymes on a huge scale that are normally only produced in very small amounts. Depending on the enzyme's intended use, several levels of downstream processing are used. Industrial enzymes are often prepared in a very basic manner and need minimal downstream processing. 60% of commercial enzymes are produced by fungi, with 24% produced by bacteria, 4% by yeast, 2% by Streptomyces, 6% by higher animals, and 4% by plants. Enzymes from animals and plants were extensively employed in past technologies and continue to be the primary sources for certain enzymes today. For enzymes like proteases, lipases, and esterases, animal tissues and organs are excellent sources. For instance, lysozyme is mostly obtained from hen eggs. Similar to this, other enzymes, such papain (from papayas) and bromelain, are exclusively found in plants and are excellent sources of these enzymes (pineapple). There are a number of drawbacks associated with the production of enzymes from both plant and animal sources.

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There is a significant difference in distribution as a result of restricted supplies. In spite of all the challenges, the isolation, purification, and cost of industrial enzymes are the most significant. Since bovine sources carry a high risk of contamination from the prion disease known as bovine spongiform encephalopathy (BSE), which is brought on by eating abnormal proteins, microbial production of enzymes is employed. There is a chance that mammalian cell cultures may directly produce commercial enzymes. The cost factor, which is really large, comes in addition to the most significant barrier. Microorganisms are the most notable and acceptable source of commercial enzymes, despite the fact that certain therapeutic enzymes, such as tissue plasminogen, may be produced by cell culture techniques. Under ideal development circumstances, they may be induced to produce significant quantities of enzymes. Microorganism development and cultivation utilising inexpensive medium may be completed in a little amount of time. Additionally, the required product is created utilising genetic engineering methods on microorganisms. Compared to plant and animal sources, microbial enzymes are simpler to isolate, purify, and recover. In actuality, microbes manufacture the vast majority of the enzymes needed in industrial operations. For this, a variety of fungus, bacteria, and yeast are created.

Antifoam additives may be utilised to limit froth formation, and batch fermentation—rather than continuous processing—is often employed to produce enzymes. The bioreactor system must be operated under sterile conditions throughout the fermentation process. The fermentation period varies between 2 and 7 days in the majority of manufacturing procedures. Along with the intended enzyme(s), many additional metabolites are also created, and the desired enzyme(s) must then be improved and purified. The desired enzyme generated may be an intracellular enzyme that is bound inside the cells, or it may be an extracellular enzyme that is released into the culture media. Depending on the needs, commercial enzymes may be either liquid or solid and can be either extremely crude or highly refined. The degree of purity and intended nature of the enzyme will determine the improvement and cleaning stages that are used throughout various downstream processing phases. Extracellular enzymes are easier to recover than intracellular enzymes since they are present in the broth. For the release of intracellular enzymes, microorganisms' cells must be disrupted by a variety of techniques. Physical techniques, such as sonication, high pressure, glass beads, etc., may lyse the membrane of microorganisms. Similar to yeast, bacteria may also have their cell walls dissolved using enzymes like lysozyme and -gluconate.

A significant amount of the biotechnology sector's overall output is produced by microbial enzymes thanks to the advancement of large-scale fermentation technologies. Large containers (fermenters) with capacities up to 1,000 cubic metres are used for fermentation. The nutrients derived from renewable raw materials, such as soy, corn, and sugars, are sterilised by the fermentation medium. To break down the carbon and nitrogen sources, microorganisms secrete the majority of industrial enzymes into the fermentation medium. Fermentation techniques that are batchfed and continuous are frequent. In the batch-fed method, nutrients that have been sterilised are introduced to the fermenter as the biomass grows. Sterilized liquid nutrients are continuously delivered into the fermenter at the same flow rate as the fermentation broth is expelled from the system. The production will reach a stable state as a result. To optimise the fermentation process, variables like temperature, pH, oxygen consumption, and carbon dioxide generation are monitored and managed. First, in order to extract enzymes from the fermentation medium, insoluble products

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like microbial cells must be eliminated. Centrifugation is often used to do this. The majority of industrial enzymes are extracellular (secreted by cells into the external environment), thus even after the biomass has been taken out, they are still present in the fermented broth. The lime treatment is necessary to stabilise the biomass during storage and inactivate the microorganisms before it can be recycled as fertiliser.

Depending on their intended use, the enzymes in the leftover broth are subsequently concentrated using evaporation, membrane filtering, or crystallisation. Pure enzyme preparations are often extracted by gel or ion exchange chromatography if they are needed. Granulated enzymes are created from the raw powder enzymes to make them more usable for applications that need solid enzyme products. Because they are simpler to handle and dose when combined with other liquid medicines, liquid formulations are sometimes recommended. Enzymes are usually immobilised on the surfaces of inert granules contained in reaction columns or towers when they are utilised in starch conversion to convert glucose into fructose. This is done in order to extend their working lives since these enzymes often continue to function for more than a year.

Fermentation in Solid State

The process of solid-state fermentation (SSF) is another one utilised to make enzymes. Microorganisms are grown on solid substrates such grains, rice and wheat bran, bagasse, and paper pulp in solid-state fermentation. This process is an alternative to submerged fermentation for the manufacture of enzymes in liquid. SSF is far better than submerged fermentation in many ways. High volumetric productivity, relatively high product concentration, little waste produced, and simple fermentation equipment are a few of these. The SSF is capable of using a wide variety of substrates to produce enzymes. Wheat bran, rice bran, sugar beet pulp, and wheat and corn flour are a few of them. The cost and accessibility of the substrate are the two key variables that influence the choice of substrate. The degree of moisture and particle size are further considerations. Smaller substrate particles provide more surface area for microbe development, but if they are too tiny, respiration efficiency will be hindered, resulting in poor growth and low enzyme synthesis. Larger particles provide more effective aeration and respiration, but the surface area is reduced. Regarding the particle size of the substrate for a certain procedure, a compromise must be achieved. Moisture on the substrate is necessary for SSF in order for the bacteria to generate enzymes. As a result, the water content of the substrate must also be adjusted since too much or too little water might have a negative impact on microbial activity. Water exerts effects on the solid substrate's physicochemical characteristics as well. SSF has generated industrially significant enzymes. Proteases, pectinases, glucoamylases, and cellulases are a few examples.

Different Fermentation Methods

Depending on the manner of operation, there are two kinds of fermentation in liquid media:

Fermentation in Batch

The simplest method of reactor operation is using batch reactors. This option allows the fermentation to continue while the reactor is loaded with medium. The contents are evacuated for further processing when the fermentation is complete. The fermentation process is then restarted when the reactor has been cleaned, refilled, and infected.

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Permanent Fermentation

Permanent reactors: Fluid from the bioreactor is continually evacuated while new medium is continuously supplied. As a consequence, cells are continually removed for processing while also receiving new medium, products, and waste products. As a result, the reactor may run continuously for extended periods of time without needing to be turned off. Compared to batch reactors, continuous reactors may produce much more. This is partially because the reactor does not need to be shut down as often, and it is also possible to more easily regulate and optimise the pace at which the bacteria in the reactor develop. Additionally, cells may be trapped in continuous reactors to stop them from being removed, boosting the productivity of these reactors even more. Although not yet commonly employed in industry, continuous reactors have significant applications in the treatment of wastewater. The most popular kind of reactor used in industry is the fed batch reactor. Fresh medium is continuously or sporadically given to the bioreactor in this reactor; however, unlike a continuous reactor, there is no continual removal. When the reactor is full or fermentation is complete, the fermenter is completely or partly emptied. Due to the fact that the development rate of the cells may be controlled by adjusting the flow rate of the feed into the reactor, high productivities are achievable, just as with the continuous reactor.

The by fermentation produced enzymes

Nearly all living cells release enzymes to catalyse their own unique biochemical processes throughout the metabolic process. The nutritious content and taste of processed foods are being improved through the use of enzymes in food processing procedures. Enzymes are effectively used in the food processing sector for the production of cheese, leavened bread, wine and beer, yoghurt, and syrup.

α-Amylase

Complex starch molecules are hydrolyzed by amylase enzymes into straightforward glucose monomers. Amylase may be found in plants, animals, and microbes, but only microorganisms—particularly bacterial and fungal species—produce economically viable amylases. Some possible bacterial species, including Pseudomonas, the Clostridium family, Bacillus licheniformis, and Bacillus stearothermophillus, generate thermostable amylase. The food, beverage, and sugar sectors are heavily reliant on the starch-converting abilities of -amylases. The quality of breads with smaller crusts and worse crust colour is improved by amylase, which also makes up for the grain's nutritional deficits. -Amylase also breaks down the starch in wheat flour into tiny dextrins, which enables yeast to continue to function during dough fermentation, proofing, and the first stages of baking. Additionally, amylases are used in a variety of other food-related processes, such as the clarifying of beer, the juicing of fruits, and the preparation of animal feed to increase fibre digestibility.

Lactase

The milk sugar lactose is broken down into simple sugar monomers like glucose and galactose by lactase enzymes. Lactases are derived from bacteria, fungi, yeasts, moulds, plants, and animals. Aspergillus niger, Aspergillus oryzae, and *Kluyveromyces lactis* are used in the development of lactase enzymes for commercial production. Lactases of fungal and bacterial origin function best

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at acidic pH levels, whereas yeast and bacterial lactases function best at pH levels close to neutral. The lactase enzyme, also known as a brush boundary enzyme, is mostly abundant in infancy. Some individuals cannot adequately digest milk because they do not create enough lactase. This condition is known as lactose intolerance, and those who have it need more lactase enzyme to help with milk sugar digestion. The lactase enzyme also contributes to the production of ice cream and yoghurt preparation by making milk that has been treated with it sweeter.

Protease

Protein molecules' peptide bonds may be hydrolyzed by proteolytic enzymes, which are also known as peptidases, proteases, and proteinases. Endopeptidases and exopeptidases are the two main categories of proteases. Endopeptidases cut peptide bonds that are far from the amino or carboxy termini of the protein substrate, while exopeptidases cut peptide bonds close to those termini. Proteases may be found in a variety of creatures, including plants, animals, and microbes, although the most economically useful proteases are found in bacteria and fungi. The extracellular and intracellular proteases are secreted by microorganisms during the submerged and solid-state fermentation processes.

Pectinase

Components of pectin, which are present in the central lamella of plant cell walls, are broken down by pectinase. Pectin is a complex colloidal acid polysaccharide with a galacturonic acid residue backbone and a -1-4 linkage as its main structural component. Therefore, pectinase aids in dissolving plant cell walls so that cell sap may be extracted. Commercial pectinase may be obtained from potential microbial strains such Moniliella SB9, Penicillium spp., and Aspergillus spp. In addition to having several biotechnological uses in the fermentation of coffee and tea, the oil extraction procedures, and the treatment of pectic waste water from the fruit juice business, pectinases are now a crucial component of the fruit juice industry. Pectinase reduces the viscosity of fruit juice during the clarifying process by breaking down pectin in the juice and improving pulp pressing ability. At the same time, jelly structure is broken down and fruit juice yields are increased. The refining of vegetable fibres during the production of starch, such as the curing of coffee, cocoa, and tobacco, canning of orange segments, and extracting sugar from date fruits, is another notable use of pectinase enzymes in industrial processes.

Lipase

Lipases are essential for digestion, dietary lipid substrate transport, and processing because they catalyse the hydrolysis of ester bonds in lipid substrates. When triglycerides are hydrolyzed into diglycerides, monoglycerides, fatty acids, and glycerol in nonaqueous conditions, lipases often catalyse biochemical reactions such esterification, interesterification, and transesterification. The finest sources of lipase enzymes include microorganisms including Pseudomonas aeruginosa, Serratia marcescens, Staphylococcus aureus, and Bacillus subtilis. In the medicinal, chemical, and food sectors, lipases are often utilised. The breakdown of milk lipids, prominent cheese taste, reduced bitterness, and rancidity avoidance are lipases' commercial uses in the food business. In order to produce cheese with a pleasant taste and no bitterness, lipases may mix with a variety of other enzymes, such as proteases or peptidases.

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Laccase

The Japanese lacquer tree's cell sap was the original source of laccase enzymes. Insects, bacteria, fungus, and plants all produce laccase enzymes. In food processing, laccase is in charge of colouring, haze, stability of wine, baking, and seasoning. Laccase has an oxidising action that enhances the baking process and adds to the strength of the dough and baked goods, improving crumb structure and boosting softness and volume. The environmental sector uses laccase in a variety of ways to digest different types of xenobiotic compounds. The isomerization of D-xylose into xylulose is catalysed by xylose isomerase (d-xylose ketol-isomerase). In the physiologies of microbial cells, this is the first stage in xylose metabolism. Because they can convert d-glucose into d-fructose, xylose isomerases are also known as glucose isomerases.

The best sources of xylose isomerase are microorganisms; some possible microbial species include the well-known xylose isomerase producers Streptomyces olivochromogenes, Actinoplanes missouriensis, Bacillus stearothermophilus, Thermotoga maritime, or Thermotoga neapolitana. Under acidic circumstances, xylose isomerase loses up to 50% of its catalytic activity. The food processing sector is where glucose isomerase finds its greatest use; it primarily catalyses two important processes, namely the reversible isomerization of d-glucose to d-fructose and d-xylose to d-xylulose.

Cells are shielded from oxidative damage by reactive oxygen species by catalase enzymes, which convert hydrogen peroxide (H2O2) to water and oxygen molecules. Through a solid-state fermentation technique, *Aspergillus niger* is used to make commercial catalases. The main uses of catalase in the food processing industry include collaborating with other enzymes like glucose oxidase, which is helpful in food preservation and egg processing, and sulphydryl oxidase, which, when used in aseptic conditions, can eliminate the effect of volatile sulphydryl groups, which are responsible for the cooked/off-flavor in ultra-pasteurized milk and are produced from thermal induction.

Sugar Oxidase

A solid-state fermentation process is used to commercially generate the enzyme glucose oxidase from *Aspergillus niger* or Penicillium glaucum. The catalyzation of glucose oxidase and the conversion of glucose into gluconic acid during the presence of dissolved oxygen were originally noted by Muller. *Aspergillus niger* fungi have the capacity to synthesise significant quantities of glucose oxidase. Small quantities of oxygen are removed from food items or glucose from diabetic beverages using glucose oxidase enzymes. The development of food items' colours, flavours, textures, and shelf lives all depend on glucose oxidase.

Transglutaminase

Enzymes called transglutaminases combine amine, crosslinking, and deamination to initiate protein-altering processes. Acyl transfer, deamidation, and the inter- and intra-molecular crosslinking of the amino acid residues of glutamine and lysine are all processes carried out by transglutaminase. Transglutaminase enzymes are used commercially in the food processing industry to increase the creation of different kinds of protein components and their ability to

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emulsify, gelate, and produce viscous materials, all of which improve the quality of food products. Food items' water-holding capacity, suppleness, ability to produce foam, and stability are all improved by transglutaminase. Strepto verticillium spp., Strepto verticillium mobarens, Strepto verticillium ladakanum, or Strepto verticillium lydicus culture filtrate is used to isolate extracellular transglutaminase. Bacillus subtilis and spherules, two common microbial species, produce intracellular transglutaminase.

Enzyme Application in Food Processing

Since they come from natural sources, enzymes may easily be deactivated when a desired change has occurred. Enzymes, as opposed to inorganic catalysts, are extremely specialised, accelerating the transformation of only one substrate, the dissociation of a constrained number of chemically related substances, or the breaking of a particular bond. Byproduct generation in high-volume processes is reduced as a result. Energy expenses are decreased by the enzymes' ability to respond at low temperatures and pH levels (up to 100°C and pH 3 to 10 respectively). Enzymes are affordable and useful for commercial applications because of their low utilisation rates. Consumers prefer enzymes over chemical aids when it comes to food processing because they are seen as natural, benign food components that are sourced from plants, animals, or microorganisms. Enzymes have a wide range of industrial uses because of these characteristics.

Dairy Sector

Exogenous enzyme extract known as rennet has a long history in the dairy sector. The proteolytic enzymes (chymosin and pepsin), which were originally obtained from the stomachs of calves, swiftly coagulate milk's casein micelles by removing kappa-casein molecules. Micelles combine form the particle gel that produces cheese via a series of further processing processes as a result of the subsequent lack of steric stability. 3L of milk will typically yield cheese with the addition of 1mL of a commercial liquid animal rennet. Since fermentation-derived chymosin is mostly utilised for the industrial manufacturing of cheese in North America and Europe, little little rennet from calf stomachs is used nowadays. There are many different kinds of cheese, each with its own taste, scent, look, and texture. This variety is produced from a single primary material thanks in large part to enzymatic transformations. Historically, these enzymes were derived from bacterial and fungal sources; however, exogenous industrial enzymes are currently the preferred method for achieving a unique character in a shorter amount of time than via conventional ageing techniques.

Fish and meat industries

Exogenous enzymes are used in the meat and fish sectors for a variety of purposes. Enzymes may change animal feeding systems' results even before the animals are slaughtered since changing the feed's characteristics influences how muscles are transformed into meat. Phytase and xylanases are two examples. Arabinoxylans in grains are broken down by xylanases so that the animal can digest the reaction products, or the viscosity of the digesta from the animal's diet is sufficiently reduced so that nutrients may be absorbed more quickly. This improves the efficiency of feed conversion (the quantity of animal produced from a unit mass of feed). Phosphorus is released from phytic acid by phytase, allowing the developing animal to use this essential mineral to strengthen its bones. It is not necessary to supplement the animal's diet with as much extra

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phosphorus thanks to the in-situ enzymatic phosphorus release. Since some phosphorus is constantly expelled, the phosphorus-liberating ability provided by phytase lowers the environmental burden of the animal operation by restricting the entry of phosphorus into the system. The employment of proteases to increase the value of processed animal byproducts is one example from the actual meat business. One important textural element for meat products is tenderness. The connective tissues in the meat may be broken down and the meat products are made soft by injecting or infusing proteases into naturally tougher meat portions. Another example is increasing the value of byproducts in the fish sector. In this instance, protease is further introduced along with catalase and glucose oxidase. A crucial result for high-quality fish products is the elimination of oxidising agents (including free oxygen) in the system, since even a few hundred parts per billion of oxidised fatty acids may result in off-flavors.

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CHAPTER 8

Fermented Products

Dr. Divya Shrivastava

Professor, School of Life and Basic Sciences, Jaipur National University, Jaipur, India

Email Id- dr.divyashrivastava@jnujaipur.ac.in

Utilizing microorganisms' growth or metabolic processes to preserve and change food ingredients is a process known as fermentation. The metabolites produced by the fermenting organisms during food fermentation limit the growth of spoilage and pathogenic organisms, increasing the shelf life of perishable goods. For example, during the fermentation of lactic acid, lactic acid bacteria produce metabolites like lactic acid, carbon dioxide, ethanol, acetic acid, hydrogen peroxide, and antimicrobial peptides that work in concert to inhibit the growth and survival of pathogenic or spoilage microorganisms.

Because they offer and maintain large quantities of nutritious and healthful foods in a broad variety of tastes, smells, and textures that improve human diets, fermented foods are of utmost importance. They provide alcoholic drinks, vinegar, sausages, cheese, yogurt, sauces, or pastes made from vegetable protein amino acids and peptides that taste like meat, as well as leavened or sourdough pieces of bread. Specifically, proteases, amylases, and proteins, lipases hydrolyze polysaccharides, and lipids to produce harmless products with tastes, smells, as well as textures that are pleasing to human consumers. Fermented foods are food substrates that have been invaded or overtaken by edible microorganisms. Additionally, fermentation guarantees the food's microbiological safety. Some foods may become more digestible as a result of fermentation, such as in the case of cassava, which lessens the substrate's toxicity. The primary raw materials utilized in the commercial manufacture of fermented foods are milk, pork, cucumber, and cabbage. 20 different forms of cheese may be produced using these substrates, in addition to a wide variety of yogurt or fermented milk products, fermented sausages or salamis, sauerkraut, and pickles.

Fermentation Techniques for Food

Lactic acid fermentation, fungal fermentation, and alkaline fermentation are three general categories for traditional food fermentation methods. Yogurt, sausages, sauerkraut, cheese, and kimchi are a few examples of lactic acid-fermented foods, or foods largely fermented by lactic acid bacteria. Several lactic acid-fermented items, such as kefir, a somewhat alcoholic dairy beverage from the Caucasus, or kombucha, a fermenting sweetened tea from China, are also produced using yeast spp. Except for natto, which is generated by alkaline fermentation, the majority of the popular soy-based fermented foods from Asia, including tempeh or soy sauce, are made by fungal fermentation. Industrial fermentation processes employ submerged or solid-state bioreactors that are operated in batch, semi-batch, or continuous modes. Most food fermentation

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processes, including those used to manufacture tempeh, kimchi, miso, and sauerkraut, are batch-operated solid-state processes in which microbes are grown on the surface of a substrate that is insoluble in water. Submerged fermentation processes are used to create alcoholic beverages, yogurt, other dairy-based beverages, and culinary condiments like vinegar.

Modern Trends

In poor countries and the Far East, fermented foods make up a sizable portion of the diet. With the introduction of contemporary technology like refrigeration, food products have generally fallen out of favor in the West, except for cheese, bread, and sausages. The putative health advantages of fermented foods as carriers of probiotic microbes and health-promoting metabolites, however, have recently sparked a resurgence in interest in traditional fermented foods. The use of fermented foods to prevent or treat a variety of illnesses, from cancer to obesity, is widely encouraged. In contrast, kefir is said to lessen the symptoms of lactose intolerance, boost the immune system, and lower cholesterol, but have ant mutagenic as well as anti-carcinogenic characteristics. For instance, kimchi is said to have antiobesity, antiaging, anticancer, and anti-constipation benefits. Even though the majority of the health benefits associated with fermented foods are based on superstitions without any supporting evidence from science, some of these benefits have recently been confirmed by research using in vitro and animal models as well as human intervention trials. For instance, a recent study by a Korean team found that eating kimchi for two weeks reduced body mass index, waist-hip ratio, body fat, fasting blood sugar, blood pressure, or total cholesterol in overweight and obese people.

Figure 8.1: Examples of both conventional and experimental fermented foods and drinks.

One of the top ten food trends for 2016 is aged food sources, continuing the trend from previous years. Food businesses are responding to this trend by either commercializing traditional aged food sources or inventing brand-new matured food sources based on traditional ones. Examples include Rythem, a drink made with coconut milk and natural product juice that has been aged using kefir grains, and Bionade, improved malt-based beverages that have been matured using the starting

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culture of fermented tea. There are also a few soy- or cereal-based probiotic products available due to the increasing prevalence of dairy protein sensitivity, lactose and gluten intolerances, and lifestyle choices including veganism.

By all accounts, the fate of mature food sources appears to be excellent, filled by the growing income by consumers in everything perceived as normal and to increase well-being and life duration. As shown by the various products currently on the market, this trend is expected to continue. Food manufacturers are also rediscovering the opportunities for creating remarkable as well as trademark flavors, dietary profiles, surface, or medical advantages while maintaining a 100 percent regular name through maturation. Flavouring agents are key food additives with hundreds of varieties like fruit, nut, seafood, spice blends, vegetables and wine which are natural flavouring agents. Besides natural flavours there are chemical flavours that imitate natural flavours. Some examples of chemical flavouring agents are alcohols that have a bitter and medicinal taste, esters are fruity, ketones and pyrazines provide flavours to caramel, phenolics have a smokey flavour and terpenoids have citrus or pine flavour.

"Flavorings or flavouring compounds are added to food to provide scent or taste," according to Codex Alimentarius. Similar to other food additives, their usage shouldn't endanger human health and shouldn't deceive customers. The amount of flavouring applied to meals should be as little as required to provide the desired flavouring effect. Additionally, flavours and flavouring agents must be of a food-grade quality and handled and processed in the same manner as food ingredients. Flavors are added to natural food items as additives to improve, alter, and replace flavours and aromas that may have been lost during food preparation. Foods like sweets and snacks that lack palatable flavours are given flavour by the addition of flavours. Natural flavourings, artificial flavourings, and flavourings that are similar to those found in nature are the three categories into which flavours are often divided.

Natural flavouring ingredients are derived from plants, animals, microbial fermentations, herbs, spices, and plants. Herbs, spices, sweetness, and oleoresins made from solvent extract with the solvent removed are all examples of natural flavourings. Natural flavourings cannot include any artificial or flavourings that are similar to those found in nature. They may be utilised either in their natural state or in processed form for human consumption. Natural flavourings and artificial flavourings are chemically similar, but artificial flavourings are more widely accessible and less costly. The fact that they may not be a perfect replica of the natural flavourings they are emulating, such as ethyl butyrate for pineapple or amyl acetate for bananas, is a negative.

The flavouring molecules that are created via synthesis or are extracted through chemical methods are known as "nature-identical flavouring agents." Artificial flavourings are chemically similar to their natural counterparts. No artificial flavourings are allowed in these flavouring agents. In addition to this group, there are also naturally occurring flavour enhancers like monosodium glutamate (MSG), which improve food flavours. They have a flavour that is distinct and cannot be categorised as sweet, sour, salty, or bitter. In reality, the flavour of MSG is referred to as "umami" and is the fifth taste, which is also present in meals heavy in protein, such as meat. Originally made from seaweed, monosodium glutamate is now commercially produced by the fermentation of starch, molasses, or sugar.

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Cuisine comes in a variety of forms, flavours, and textures, and everyone of us has preferences depending on the flavours we find appealing in a certain food. Flavors are the various impressions we get of food products as a result of chemical interactions between the food and our sensory systems. The flavour of the meal is influenced by both the senses of taste and smell, despite the fact that the majority of us identify food with its taste. Flavoring agents, also known as flavorants, are compounds that are added to food to either create a new flavour or change an existing one by changing the meal's natural aroma and flavour. They may be created either naturally or intentionally. Natural flavouring agents are oils and chemicals derived from plants that may be used to flavour food without altering their chemical structure.

The most popular sources of natural flavouring compounds include fruits, vegetables, buds, leaves, meat, spices, barks, fish, poultry, and dairy products. Flavoring agents that are similar to natural flavouring agents may also be created artificially in laboratories due to the dearth of natural sources and high processing costs. These substances are referred to as flavouring agents with Natural Identicality. Artificial flavours are artificial food additives that are used in the food business to mimic a natural taste without actually employing the flavor's actual natural source material. For instance, artificial flavouring chemicals are used to create strawberry-flavored ice cream rather than actual strawberries. Let's look at a few flavouring agent instances.

Glutamates

One of the most popular flavouring ingredients, glutamates provide a range of foods a savoury taste. When salts made of glutamic acid are dissolved in water, negatively charged zwitterions known as glutamates are created. Monosodium Glutamate is one of the most often used glutamic acid salts in the food and beverage sector (MSG). Ajinomoto, Chinese salt, and other trade names are only a few of the commercial names for MSG. While MSG may be obtained naturally in certain foods, such as tomatoes and cheese, it is created artificially by the fermentation of starch, sugar beets, sugar cane, or molasses. Glutamates' savoury taste may be found in many Asian dishes, including soups, Ramen, Manchurian, etc. Generally speaking, there are no safety issues with glutamate flavouring compounds; nonetheless, they have a powerful aftertaste that may lead some individuals to react negatively to them.

1. Food additives are substances that are added to food to preserve or enhance its freshness, safety, flavour, texture, or appearance. Some food additives, like salt (found in meats like bacon or dried fish) or sugar (found in marmalade), have been used for food preservation for generations (in wine).
2. Food manufacturing requires a wide variety of additives since preparing meals on a big scale vs preparing them on a small scale at home is extremely different. To keep processed food safe and in excellent condition from factories or industrial kitchens, through transit to warehouses and stores, and ultimately to customers, additives are required.
3. Only when there is a technical requirement, no consumer misinformation, and a clearly stated technological purpose such as preserving the food's nutritional value or improving its stability—can the use of food additives be justified.
4. Food additives may be made synthetically or from plants, animals, minerals, or a combination of these. They are purposefully introduced to food to carry out certain

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technical functions that consumers often take for granted. There are thousands of food additives in use, and each one is intended to serve a particular purpose in enhancing the safety or attractiveness of food. Based on their purpose, the WHO and FAO divide food additives into three general categories.

Flavouring agents

Flavouring agents – which are added to food to improve aroma or taste – make up the greatest number of additives used in foods. There are hundreds of varieties of flavourings used in a wide variety of foods, from confectionery and soft drinks to cereal, cake, and yoghurt. Natural flavouring agents include nut, fruit and spice blends, as well as those derived from vegetables and wine. In addition, there are flavourings that imitate natural flavours.

Enzyme preparations

Enzyme preparations are a type of additive that may or may not end up in the final food product. Enzymes are naturally-occurring proteins that boost biochemical reactions by breaking down larger molecules into their smaller building blocks. They can be obtained by extraction from plants or animal products or from micro-organisms such as bacteria and are used as alternatives to chemical-based technology. They are mainly used in baking (to improve the dough), for manufacturing fruit juices (to increase yields), in wine making and brewing (to improve fermentation), as well as in cheese manufacturing (to improve curd formation).

Natural colors are made in different ways depending on the source material and desired end format – oil vs. water soluble. These can include juicing, crushing, and selective extraction. But one of the ways we can make colors that is not commonly known is through fermentation. In this article we'll walk you through how fermentation colors are made and the types of colors that typically use this process. An organism turns a carbohydrate, such as starch or sugar, into an alcohol or an acid during fermentation, which is a metabolic process. In most cases, the term "fermentation" refers to an anaerobic process, which means it doesn't need oxygen. However, the term "fermentation" has been broadened to include both aerobic and anaerobic culture development in a fermenter. For instance, bacteria do fermentation when they convert carbohydrates into lactic acid, while yeast performs fermentation to acquire energy by turning sugar into alcohol. Long before the biochemical process was recognised, this method has been utilised for generations to produce food and beverage goods like wine, cheese, and beer.

The extent of fermentation

However, now that we are aware of the procedure, we may apply it to other facets of the food and beverage sector, such as cultivating organisms that can generate natural colours. The two primary sources of the yellow-orange hue beta-carotene, which is now utilised in foods and drinks, are the mould *Blakeslea trispora* and the microalgae *Galdieria sulphuraria*, which are both used to produce brilliant blue phycocyanins, which are produced similarly to spirulina (a cyanobacteria). The microorganism (i.e., the microalgae) is put into a fermentation tank, a bioreactor that offers a closed, sterile environment for development, to begin the process. To optimise output, the fermentation process involves careful monitoring and control of the temperature and pH

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conditions. The source material is "fed" a substrate like glucose to help it grow as it cannot photosynthesize in this sort of bioreactor since sunlight cannot enter. There are no more sugars in the final hue since the microbe just needs the sugar to proliferate.

Because the bacterium feeds on diverse substrates in the dark rather than photosynthesizing, this process is known as heterotrophic fermentation. The broth is taken from the fermentation tank and placed into the extractor after the batch has reached the appropriate size. While beta-carotene is extracted with alcohol, galdieria blue is delicately extracted with water (the alcohol is then boiled off). Then, it may be allowed to remain in liquid form or dried to become a powder.

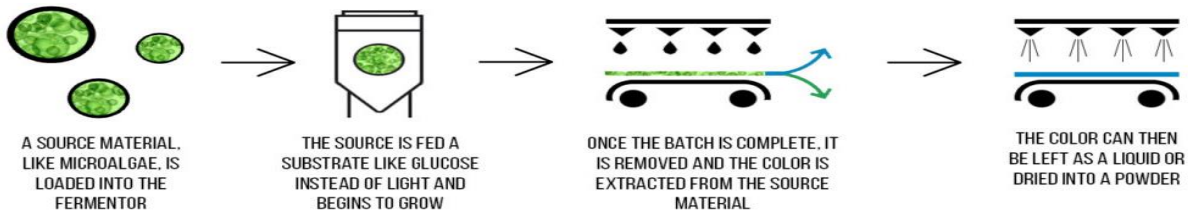

Figure 8.2: Fermentative operations.

Making natural colours is a breeze with bioreactors because of their closed design. It is simpler to preserve the target strain's purity, and it has advantages over conventional growth techniques like:

We can get productivities and densities 10 times higher than usual growth techniques in the same amount of time by growing sources like microalgae on sugars instead of sunlight.

A. **Manage:** Because heterotrophic fermentation is a highly regulated production process, it is simpler to control factors such production temperature, duration, purity, etc. to produce a consistent, high-quality product all year long.
B. **Quality:** Because bioreactors are closed systems, they shield the source from the outside environment, which means that certain sources are less likely to be contaminated than those in open pond systems.
C. **Consistency & Supply:** Since the microalgae are produced inside, they are not impacted by seasonal or weather changes like open ponds are.
D. **Sustainability:** Heterotrophic fermentation enables scalability to high quantities without requiring a lot of land. Additionally, it often consumes less water and electricity, reducing our environmental effect.

You can see that fermentation offers advantages despite not being the most popular method of developing natural colour supplies. It enables us to get certain pigments that would not otherwise be accessible and makes it possible to produce other colours in a more traceable and sustainable manner. To make food products more enticing and appealing, colours are added. The demands of the food industry have led to an amazing market for food colourants. There are several synthetic colouring compounds that have been authorised for use as food additives, and they are utilised in a range of foods that are made or produced all over the globe. However, there is increasing worry that, over time, the usage of synthetic colours may have detrimental effects on both human health and the environment. Synthetic food colourants may be replaced with natural pigments made from plants, animals, and microbes. Microorganisms have several benefits over animal and plant

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sources, including no seasonal effects on the quality and quantity of the pigment, simplicity of handling and genetic modification, suitability for large-scale production with little to no impact on biodiversity, etc. Algae, fungus, and bacteria are among the microorganisms that are utilised to make pigments for food colouring. The many kinds of microbial food pigments in use, as well as their advantages, methods of manufacturing, and related difficulties, are discussed in this review.

Various Microbiological Pigments for Food Coloring

Among the microorganisms that are well-known for creating a range of naturally coloured compounds with markedly different chemical compositions, functions, stabilities, and solubilities are fungi, bacteria, and microalgae. These naturally occurring hues are a result of secondary metabolites that are highly prized in the food and dairy, cosmetics, pharmaceutical, textile, and dyeing sectors for their economic value. They may be categorised into many categories based on their chemical composition, useful qualities, and natural occurrence, such as derivatives of flavonoids, pyrroles, carotenoids, etc. Riboflavin, beta-carotene, canthaxanthin, prodigiosin, phycocyanin, melanin, violacein, astaxanthin, and lycopene are the principal microbial pigments utilised as food colourants.

Conditions for Microbial Pigment Production During Fermentation

In order to be used as food additives or colourants, commercially accessible microbial pigments are being derived from bacteria, fungi, and algae. To be employed in the commercial production of pigments, the appropriate microorganism must be able to withstand a wide range of carbon and nitrogen sources, as well as process pH and temperature. The yield must also be sufficient for it to be cost-effective. To find and test the microorganisms that have the potential to produce interesting pigments, bioprospecting programmes may be applied in a variety of situations. Alternately, known pigment-producing bacteria may be subjected to strain boosting processes to get the required yield and characteristics. Both of the aforementioned techniques may be used alone or in tandem. A variety of factors, such as the kind of fermentation, the elements of the medium (carbon, nitrogen sources, and minerals), pH, temperature, incubation time, moisture content, and aeration rate, affect the growth and synthesis of pigments generated by microbial fermentation. Solid state and submerged fermentation techniques are used to create microbial pigments. Increased productivity and production, as well as the ability to utilise the fermented product directly as a colourant without separating it, are all advantages of solid-state fermentation. SSF is ideal for fungal growth, and using this technique lowers wastewater use and boosts metabolite production. The ideal values for a number of elements that affect the fermentation conditions vary depending on the microorganism used to create the pigment. For instance, although Pseudomonas species prefer temperatures between 35 and 36 degrees Celsius, Monascus species need temperatures between 25 and 28 degrees Celsius to manufacture pigment. Over the same incubation period, Rhodototrula and Micrococcus both generate pigment most effectively.

Technological Advances in Microbial Pigment Production

Recent scientific advancements have effectively created microbial pigments for a number of industrial applications. The advancement in several areas related to the production of pigments by fermentation is discussed below.

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Lessening of Strain

Microbial pigments with desired characteristics and increased output may be made by using proven strain boosting techniques. A wild microbial strain is often associated with constrained pigment production, which has a negative effect on the economics of the process. Pigment production has grown as a result of the typical process of random mutagenesis and selection. The production of microbial pigment may be increased by a factor of three by exposure to UV light and other mutagens, including 1-methyl-3-nitro-1-nitrosoguanidine and ethyl methyl sulfonate. Ethidium bromide treatment caused Serratia marcescens (GBB151) to develop greater pigment. By applying genetic engineering approaches, the microbial pigment production has been efficiently enhanced, and its molecular makeup and colour have been changed. The whole design of the biochemical production routes and the intermediates is often required for all of these genetic modifications that seek to boost output by eliminating the rate-limiting step. The author claims that Streptomyces coelicolor's Actinorhodin, a blue pigment, has undergone such genetic change. There have been reports of the development of cell factories using heterologous expression for the manufacture of microbial pigments. There are 8 genes involved in the production of the blue pigment and antioxidant defence that were found by using the transposon mutagenesis approach on Pseudomonas fluorescens.

Optimization of downstream processing and fermentation conditions

Microbial pigments may be produced inexpensively via the development of effective downstream processing and modification of fermentation conditions. Media optimization entails adjusting fermentation factors including temperature, pH, incubation time, nutritional sources, aeration, and agitation rate, among others, in order to determine the conditions that provide the maximum output. Response Surface Methodology (RSM), as compared to conventional methods for media optimization, has significant advantages. Fewer experiments are required to identify the ideal mix of all the variable components under investigation. As a result, optimising fermentation conditions requires less time and effort overall. The optimal ranges for temperature, pH, and saline concentration as well as the effect of light on total carotenoid production were determined using the response surface technique. The culture medium are modified by the Monascus anka mutant to produce yellow pigments via response surface techniques. The artificial neural network is yet another technique for investigating the impacts of fermentation conditions and how to best optimise them for microbial pigment production (ANN). The application of Artificial Neural Networks (ANN) in the modelling of a Liquid State Fermentation (LSF) for the production of red pigment by *Monascus purpureus* MTCC 369 and discovered that ANN models may be used to effectively predict the effects of fermentation parameters on the production of red pigment.

Due to the public's growing awareness of and concern about the use of safe food additives, it is projected that the industrial demand for natural pigments will increase dramatically in the future years. Not only do microbial pigments originate from natural sources, but they also offer a variety of health benefits that have been scientifically shown to exist, making them an enticing alternative to artificial food colours. Despite the fact that numerous bacteria can produce food-grade colours in the lab, mass producing and purifying products made from many of them are still challenging challenges. To optimise the medium and fermentation conditions for sufficient synthesis and

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straightforward recovery of microbial pigments, further study is required. Additionally, sophisticated genetic or metabolic engineering techniques as well as traditional strain augmentation techniques may be used for the long-term synthesis of extremely valuable microbial pigments. In order to screen and locate novel pigment-producing microbial strains from varied locations, bioprospecting programmes may also be utilised as a prologue to the strain enhancement procedures in line with the Nagoya protocol and other relevant state legislation. Research into the traditional fermentative foods found in isolated or tribal places may also lead to the discovery of possible isolates that create colour. Despite the fact that only non-pathogenic bacteria are allowed in food grade pigments, it is still possible for supposedly "safe" organisms to co-produce harmful or undesirable substances, therefore sufficient, cost-effective purification techniques must be created.

Benefits of using microbial pigments as food-safe colouring additives

In nature, microorganisms perform a wide range of roles and may be found in almost every environmental niche. They are in responsible of the fermentation of food products, which is another connection to food. Microbial pigments are a better alternative to synthetic food colours than plants because they are more readily available, non-seasonal, scalable, produce more per hectare, and need less complex downstream processing. Monascus, Arpink Red (a naturally occurring red-industrial name) from Penicillium oxalicum, -carotene from Blakeslea trispora, and Astaxanthin from various bacteria are only a few examples of the microbial pigments already used by the food industry to colour food. Many studies have been conducted to lower the costs involved with the production and processing of natural colours, as well as to increase stability and shelf life, in order to compete with the use of synthetic colours. Many of these pigments not only serve as colouring agents but also have positive health effects. (Bioactivity of several microbial pigments. Microorganisms produce a large array of physiologically and pharmacologically active compounds, including antioxidants, antibacterial, anticancer, immuno-regulatory, and anti-inflammatory molecules.

Antioxidant activity

Microbial pigments such violacein, carotenoids, anthocyanins, and naphthoquinone have been shown to be potent antioxidants. Strong antioxidant violacein supports the mucosal defence mechanisms to protect against oxidative damage in stomach ulcers. It is a purple pigment that is mostly produced by Chromobacter violaceum and Pseudoalteromonas. The yellow pigment staphyloxanthin, which Staphylococcus aureus produces, shields Swiss albino mice from the oxidative harm caused by carbon tetrachloride. Many pigments, including astaxanthin, granadaene, canthaxanthin, lycopene, riboflavin, -carotene, torulaenin, and many more, may have antioxidant properties.

Anticancer Characteristics

Numerous studies have revealed that microbial pigments have anticancer effects. These pigments have the power to initiate apoptosis, which kills cancerous cells. By inhibiting the activity of the protein kinase that regulates the cell cycle, the greenish-yellow pigment scytonemin, produced by aquatic cyanobacteria, has an antiproliferative effect. Prodigiosin is a red pigment that Serratia

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marcescens and Pseudomoalteromonas rubra produce that has potent anticancer properties. Its apoptotic effect is anti-human cervical carcinoma. Prodigiosin analogues and synthetic indole derivatives show anticancer activity in vitro. Violacein activated Caspase-8 and p38 MAPK through the TNF signalling cascade, which led to lethal effects on HL60 leukaemia cells. Numerous pigments have been demonstrated to have anticancer characteristics, including -carotene, Canthaxanthin, Monascorubramin, Riboflavin, Rubropunctatin, and others.

Biochemical Activity

Many of the antimicrobial compounds generated by various bacteria are used as antibiotics. An endophytic fungal pigment was shown to be more efficient than the common antibiotic Streptomycin. It was effective against germs including Klebsiella pneumoniae, Staphylococcus aureus, Salmonella typhi, and Vibrio cholera. It is generally known that violacein both prevents and eliminates bacterial development. It also possesses antiprotozoal, antifungal, and antiviral effects. Researchers are searching for novel and unique chemicals that may be used as antibiotics in light of the recent rise in microbial strains that are resistant to various medications and antibiotics. Finding novel microbial pigments that produce colour and are both antimicrobial is tremendously advantageous.

Problems with Natural Food Colors

The commercial development of natural pigments as food colourants is difficult despite the fact that there are several varieties of them from diverse microbiological sources. The discovery of novel chemicals for food application, especially as a colourant, faces significant regulatory obstacles. Natural colours are five times more costly than synthetic ones, particularly when employed in confectionery products where they may cost up to twenty times more. Equal amounts of natural and synthetic colours must be produced using significant amounts of raw materials. Usually, higher concentrations of a natural colour are required to get the desired hue, which raises the price.

Regarding cost, application, technique, and quality, natural pigments face several product problems. Microbial pigments may react with various food matrices and have a lesser tinctorial strength, which can result in unfavourable smells and aromas. Over the last 50–60 years, the food industry has come to depend on synthetic food colourants, which are generally well-behaved and reliable in their performance. In the food sector, switching to natural colours may be difficult, especially given the limited number of natural hues that have been given the go-ahead for usage in food. Another problem with natural pigment goods is deodorization since many of the available natural pigments have an unpleasant odour in food preparations. Additionally, natural colours often have a higher sensitivity to light, pH, UV, temperature, oxygen, and heat, which results in colour fading and a shorter shelf life. Some organic pigments, metal ions, proteins, and other environmental factors may make them susceptible. It is commonly known that vitamin C can increase the stability of beverages that include carotenoids like beta-carotene and paprika oleoresin, yet the same vitamin will also cause anthocyanins to degrade.

These restrictions also apply to important microbial pigments including carotenoids, chlorophyll, anthocyanins, and others. Strongly pigmented isoprenoid plant chemicals known as carotenoids,

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which are highly conjugated, become unstable when exposed to oxygen or light. Chlorophyll rapidly degrades as a result of enzyme processes or elements like light, oxygen, heat, or acid, giving rise to derivatives of chlorophyll. These natural hues may be difficult to formulate, and techniques like micro-encapsulation can be used to increase stability and, in certain circumstances, solubility. Due to the presence of mycotoxins, several fungal pigments are forbidden as natural colourants. Therefore, it's crucial to utilise non-toxic and non-pathogenic strains while extracting natural pigment. Metabolic engineering may be utilised to produce toxin and pigment under controlled conditions when a potential pigment-producing microorganism is found. Technologies to Improve the Production of Pigments

The goal is to commercialise microbial pigments by removing them from petri dishes. Alternative colourants that are reasonably priced, entirely natural, safe, and don't create any recalcitrant intermediates are needed. The investment taken to acquire the finished product, its regulatory clearance, and its market impact all have a role in how successful a natural pigment will be commercially. Natural pigments are produced industrially via the following three crucial processes: Improved application, cost-effective manufacturing with consistent quality, and the discovery of newer and more innovative alternative sources. To stabilise natural pigments in various food matrices, extend shelf life, prevent the influence of various environmental parameters on the pigment, find inexpensive organic substrates for the growth of the microorganisms that produce pigment, and improve fermentation process efficiency, rigorous trials are needed.

Newly Created Intelligent Screening Techniques

The rapid and simple identification of microbial pigments has undergone several recent advancements. The condensed portable Raman spectrometer, which uses a 532 nm excitation laser to identify pigments, is among the finest examples. This is capable of detecting both common and rare carotenoids, bacterioruberin, and other recognised pigment molecules. This portable tool has been used to locate microbial pigments in diverse ecological niches, including those inhabited by halophilic microbes.

In order to rule out or modify hazardous and pathogenic pigment makers for food colouring, intelligent screening also entails having previous knowledge of the pigment producer's harmful metabolite route. QuornTM, a mycelial food item produced by Fusarium venenatum, is also known to create the cytotoxic substance 4, 15-diacetoxyscirpenol.

Fungal strains that produce pigment may also be more quickly identified and categorised using mass spectrometry with electrospray ionisation. Since more than 15,000 microbial metabolites are already known, it is crucial to quickly replicate and identify existing molecules. Known compounds may be quickly identified even within very complicated mixtures using HPLC, mass spectrometry, LCMS, nuclear magnetic resonance (NMR), and UV-VIS spectra, without the necessity for individual constituent purification.

Development and Fermentation of Strains

Scaling up the production of microbial pigments presents a number of hurdles; however recent technological advancements have helped to partly overcome these obstacles. A cost-effective and

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industrially viable production process for pigments and other natural compounds has been developed with the aid of the use of fermentation tanks for large-scale pigment production, strain-improving techniques, strain development through random mutagenesis, and multiple selection rounds. Because the pigments generated by wild type strains are sometimes too few in number and need longer fermentation durations, the procedure is typically unprofitable and strain development is crucial. Common mutagens that may boost pigment production by a factor of many, such as 1-methyl-3-nitro-1-nitrosoguanidine (NTG), EMS, and UV, are used to improve strains.

The technique of medium adjustment is crucial for increasing the yield of the fermented product. Controlling operational factors including temperature, pH, aeration, agitation, and media components is part of optimising the medium. An efficient way for improving the manufacturing of pigments is response surface methodology (RSM). This reduces the quantity of experimental trials required to assess several variables by solving the multivariate data acquired to solve multivariate equations. For the cultivation of Serratia marcescens to produce prodigiosin, an ideal medium composition. The addition of glycine and sucrose as an energy and carbohydrate source boosted the synthesis of prodigiosin. Inorganic KH2PO4 supplementation stimulated cell growth and boosted prodigiosin synthesis. The standardisation of the medium and effective fermentation design are key components of developing an affordable manufacturing process. The use of statistical approaches may increase output responsiveness, lower variability, and lower overall costs.

Efficacious Downstreaming

It is also necessary to create more affordable recovery and separation methods for microbial pigments. Using standard techniques to separate and recover pigments on a large scale is expensive. While significant volumes of organic solvents are used up throughout the lengthy and difficult process of organic solvent extraction, the yield of the high purity product may be very low. Additionally, because the majority of organic solvents are synthetic, utilising solvents other than water and ethanol might undermine the goal of creating a natural pigment for regulatory reasons. Numerous nucleic acids, organic acids, peptides, and other compounds have been effectively separated and purified utilising the approach of employing non-ionic adsorption resins. Due to their great loading capacity, these resins aid in the recovery of several chemicals. Additionally, these resins may be utilised straight away to absorb substances from the culture broth. By using fewer extraction solvents and enhancing its reusability, it aids in reducing the cost of separation. a successful process for the isolation and purification of prodigiosin. They utilised non-ionic resins straight from the culture broth, skipping the stage of cell separation and producing a concentrated, partially purified product.

Molecular engineering

The cloning of pigment biosynthesis genes and the ability to manipulate genes to produce more of these pigments as a result of recent advances in molecular biology and metabolic engineering. It has been widely researched and designed to modify the molecular makeup and hue of a pigment as well as to overproduce it. Streptomyces coelicolor's Actinorhodin, a blue pigment, has been genetically altered to provide kalafungin, a similar bright yellow polyketide that is utilised to create

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antraquinone, a reddish-yellow pigment. By expressing biosynthetic pathways from unknown or well-known pigment manufacturers, heterologous expression has been exploited to create cell factories that generate pigments quickly and effectively.

Understanding the biosynthetic processes by which microbial pigments are produced is a crucial first step. Next, the pigment-producing genes and gene cascades must be found, and then these genes must be engineered to produce too much colour. Cloning the pigment biosynthetic genes onto microbial vectors, such as yeast or bacterial cells, has emerged as a more affordable and efficient method of commercial manufacturing. E. coli, Bacillus subtilis, Pseudomonas putida, *Corynebacterium glutamicum*, and Pichia pastoris are industrially dependable microorganisms that may be utilised to create custom recombinants and genetically design the synthesis of colours.

To create strains that produce more than usual, procedures like random and chosen mutagenesis are utilised. To achieve this, chemicals and physical techniques like antymicin A, 1-methyl-3-nitroguanidine, or ethyl methane sulfonate, as well as gamma radiation and UV light, are used. Genetically modified yeasts like *Candida utilis* and *Saccharomyces cerevisiae* and bacteria like *Xanthophyllomyces dendrorhous* or *Erwinia uredovora* or *Agrobacterium aurantiacum* are used to manufacture carotenoids like lycopene, -carotene, or astaxanthin. At the moment, only non-carotenogenic microorganisms like *C. utilis* or *S. cerevisiae* have been used to alter the genes responsible for carotenoid pigments. We used metabolic engineering and mutagenesis to enhance carotenoid production in *R. mucilaginosa* KC8, which produces carotenoids, primarily -carotene and torularhodin. There is almost no published data on the metabolic engineering of wild type carotenoid producers like *B. trispora, Dunaliella salina*, and *R. mucilaginosa*. production in *Saccharomyces cerevisiae*, a heterologous microbial host, utilising glucose as a substrate. They also demonstrated that new betalain derivatives might be produced by feeding the culture various amines.

Natural ingredients and additives are necessary for the significant and expanding food category known as "natural foods." As a result, there is a huge need for natural pigments to replace synthetic colours in foods and drinks. Because they may be scaled up and are easier to handle than plants or insects, microbial sources are especially helpful. The development and integration of innovations like as fermentation strain development, systems biology, metabolic engineering, and protein engineering may significantly impact the amount and quality of natural food colours. Fermentations that are efficient have consistent yields and are unaffected by the weather or environment outside. However, further investigation is needed to determine the most optimal growth settings, employ genetically modified organisms to increase production, as well as the presence of different elicitors for pigment formation in order to maximise pigment features, such as composition and yield.

Although valuable, metabolic engineering has its own regulatory difficulties. Technology-wise, metabolic engineering may increase output, allow the transfer of pathways from slowly growing species to rapidly growing ones, and permit guided manufacture of analogues of a pigment to change hue or other features. CRISPER-Cas9 may be used to establish cell factories, and heterologous expression of biosynthetic pathways from well-known or unknown pigment manufacturers can be a helpful tactic. Techniques like micro-encapsulations and nano-

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formulations may be used to overcome the poor stability or low solubility of natural food colourants, allowing a broader application of microbial pigments to different food matrices. Encapsulated pigments have a longer shelf life because they are more manageable, more soluble, and more stable under ambient circumstances. When colouring transparent and semi-clear liquids, nano-emulsions may be employed to increase solubility and give invisible particles that are helpful.

In contrast to the wide variety of synthetic colours, the present selection of natural colours that may be added to meals is somewhat limited. However, there is a growing desire for organic foods and dyes. Therefore, finding new and unique natural hues is crucial, as is developing methods to make the manufacture and formulation of natural pigments more efficient and less expensive. In order to make these strains more cost-competitive with synthetic pigments, new natural sources of pigment-producing microorganisms are needed. The essential technology involves the creation of low-cost organic substrates for the growth of bacteria that produce pigments, innovative techniques to boost pigment production, and stabilising techniques to enhance pigment application. The pursuit of a larger range of colours, the use of pigments with health advantages, extending the shelf life of pigments, and reducing manufacturing costs should be the main goals of natural pigment research.

By 2050, the world's population is expected to expand to 9.7 billion, necessitating the production of more food than is feasible under the existing production methods. According to its high consumption, meat products are the favoured source of protein for the majority of consumers. The amount of meat consumed per person nowadays is more than what the World Cancer Research Fund recommends. High meat intake has been linked to a number of negative health outcomes, such as type 2 diabetes, cancer, and even an increased death risk. According to the World Health Organization (WHO), consuming 50 g of processed beef daily raises colorectal cancer risk by 18%. In order to avoid certain chronic non-communicable illnesses that lower quality of life and place a financial strain on healthcare systems, it may be beneficial to consume protein from sources other than animal sources. Despite the cognitive dissonance that exists between eating meat and protecting animals, the majority of consumers do not seem to be moving toward cutting down on their meat intake. However, there is at least some acceptance of non-meat sources of protein, for which new commercially viable substitutes are required to meet the anticipated demand. Insects that can be eaten, plant-based protein substitutes, cultured meat, and single-cell protein (SCP) are some of the alternative protein sources that are now available. SCP may be isolated from a variety of microorganisms, including fungus, yeasts, bacteria, and algae, and is also known as bioprotein, microbial protein, or protein biomass.

In various regions of the globe, food shortages in the 1950s, 1960s, and now have contributed to an increase in the production of unicellular protein, and SCP has been proposed as a potential solution. The fact that complex carbohydrates (both soluble and insoluble fibre), fats, minerals, and vitamins may also be found in protein-producing microbes is vital to take into account since this further supports their nutritional importance. The use of microorganisms to obtain protein for food purposes has some benefits over conventional sources, such as the rapid growth rate of microorganisms and, therefore, rapid protein production, as bacteria and yeasts double their

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population in 5 to 15 minutes, while algae and moulds require less space than plant and animal sources of protein, which require large areas of land; reduced water requirements compared to plant and animal protein production; and; SCP production is not reliant on the weather or the seasons, but it does need a growing medium in a controlled environment with constant mixing, oxygen, light, and temperature. Insecticides, herbicides, fungicides, and fertilisers, among other possible toxins, are not necessary when using SCP, reducing soil wear and environmental contamination.

Different microbes may be used to produce SCP. In addition to producing alcohol, recombinant vaccines, medicines, and antibiotics, fungi have also been reported to help with bio-control and land restoration. These are followed by its usage as a protein-producing organism. Molds and yeasts are both types of fungi, with yeasts being the most common kind of microbe used to make SCP. *Saccharomyces cerevisiae* and Kluyveromyces marxianus are the two yeast species that are most well-known and often employed. The utilisation of these two species to ferment various substrates and generate SCP with distinct sensory and nutritional properties has been supported by multiple scientific investigations, which has led to a considerable increase in research interest.

Producing food biomass needs a lot of resources (land, water, fertiliser, labour, and others), which has a big financial impact. By making use of biomass that would otherwise go to waste, such as bruised but otherwise healthy fruits and vegetables, we may avoid squandering the resources necessary to produce them. Utilizing leftover food for SCP manufacturing would reduce the cost of storing, managing, processing, and correctly discarding it, which would further benefit the economy.

A food-grade substrate is required for SCP manufacture for human consumption; yet, tonnes of food are wasted each year and may be utilised as an excellent supply of unneeded nutrients. In a circular economy system, at least some of this can be used to create SCP, which might have a beneficial effect on food security and even provide financial rewards for farmers and agro-industrial processors. To meet the steadily rising demand for this macronutrient and reduce food waste, food that would otherwise go to waste may be utilised as an input for a process that produces protein. The current study examines the production, dietary, and safety implications of using food leftovers from the agroindustry to create SCP.

SCP may be derived from a large variety of microbes, however its availability is limited when it is intended for use as food. The yield, growth rate, substrate, and ideal growing circumstances, such as temperature, pH, and nutritional needs, all have an impact on choosing the best species (particularly carbohydrates, vitamins and minerals). The chosen bacterium must also be non-pathogenic, have a low nucleic acid content, and be harmless [14]. The choice is also greatly impacted by the protein production of the organism; for microalgae, bacteria, and yeasts, respectively, these values fall between 60 and 70, 30 and 80, and 30 and 50% w/w. However, depending on the substrate employed in each instance under ideal growth circumstances, these percentages may change.

Food residues are a source of lipids, proteins, carbohydrates, vitamins, minerals, and bioactive substances when it comes to substrate. Thus, it is possible to think of the harmless waste products

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produced by the food industry as substrates for this process, which would reduce the quantity of wasted food. This strategy may increase the value of waste material by enabling it to reenter the food production chain as a crucial step in the application of the so-called circular economy. It can also help ensure the safety of the world's food supply. If the input material (substrate) utilised in the primary SCP manufacturing process is of low financial cost, then production expenses will be reduced. The goal of SCP production thus is on the utilisation of food industry by-products, which are naturally rich in several substrates that are often used for growing microorganisms and whose cost may be almost negligible.

The substrate must contain nutrients (especially carbon and nitrogen) in a way that the bacterium can access them, such as monosaccharides and disaccharides. In this regard, it has been noted that a higher percentage of fermentable carbohydrates increases SCP yield. For instance, *S. cerevisiae* grown in a substrate made of potato peels had a yield that was higher than that of carrot pulp, banana peels, and orange peels, whose carbohydrate contents were 61.86, 59.00, and 54.17%, respectively. Additionally, it has been shown that adding volatile fatty acids to the waste fermentation liquid may control the carbon supply and its ability to convert, leading to an increase in biomass output by photosynthetic bacteria. This is because metabolic pathways that convert volatile fatty acids into tricarboxylic acids and/or acetyl-CoA, both of which are crucial for the formation of SCP, are stimulated.

Antimicrobial substances should also be taken into account since they may prevent microbial growth and, therefore, protein synthesis. For instance, limonene, terpenes, and camphene found in citrus peels have been observed to cause a drop in yield. If the substrate has a high concentration of substances that have comparable antibacterial actions, pretreatments must be applied before it may be utilised. It has been suggested that autoclaving is a straightforward procedure for this goal since it may reduce the concentration of limonene in orange peel by 62%, improving the development of *S. cerevisiae*. However, this depends on the individual components.

Other crucial elements that must be taken into account during the formation of SCPs are temperature and the sources of nitrogen. They grows more quickly even at temperatures above 40 °C, and most of its strains do not engage in alcoholic fermentation, which is preferable in the production of SCPs where ethanol is undesirable. *K. marxianus* cultivated on cheese whey lactose responded to higher fermentation temperatures by producing more protein, and this protein content may still be boosted in various ways. For instance, introducing ammonium salts caused a 45% (dw) increase in protein, with a significant association between protein content and ammonium sulphate concentration (up to 2 g/L), even though it only slightly decreased biomass.

Nitrogen must be properly added to the growth medium; if done at a 10:1 carbon:nitrogen ratio, it nearly resembles what is often found in microorganisms, however each specific species may need optimization [10]. According to reports, when SCP is produced by industrial fermentation of food waste, adding ammonium sulphate > ammonium nitrate > (sodium nitrate, corn steep, or liquor-urea) to the *S. cerevisiae* culture medium enhances SCP output. However, it's also crucial to manage the source and amount of nitrogen since *K. marxianus's* development and ability to withstand high temperatures are severely impacted by high concentrations of ammonium ion ($NH4^+$), which may necessitate switching between organic nitrogen sources.

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SCP must be separated after the bacteria have grown a large enough biomass. These separation procedures include a number of extraction and purification stages that may extend production time, require more energy, raise costs, and compromise sustainability. After SCP has been separated, it may be converted into ingredients, a process whose difficulty and expense depend on the required level of purity or uses. Concentrating the protein-producing organism and removing the aqueous medium it was enclosed in via dehydration or drying are required in order to extract the protein from the biomass. For instance, coagulation/flocculation, filtration, and centrifugation are some techniques that may be used to collect microalgae. It is important to damage the cell membranes and split apart the cells after the biomass has dried and the protein within has been released. Cell disruption may be accomplished via physical, chemical, or biological techniques, including, but not limited to, pulsed electric fields, hydrodynamic cavitation, pressure changes, enzymes, or alkalis. Protein purification next mostly involves (ultra) centrifugation and filtration, followed by protein fractionation based on its solubility. After this step, the product consists of various-sized particles with all their constituents mixed, including lipids, proteins, nucleic acids, etc. Protein purification then proceeds primarily by (ultra) centrifugation and filtration. Proteins are concentrated after they have been fractionated using a variety of techniques, including dehydration, precipitation, ultrafiltration, spray drying, and lyophilization. Proteins may also be isolated based on their size, density, or dispersibility (other writers have discussed these procedures in depth). Although required, the procedures might be expensive, which raises the overall cost of the finished items.

Protein from *Arthrospira platensis* microalgae by carrying out the following series of procedures and resulting in the generation of effluents: they caused cell rupture with a microfluidizer, then centrifuged, precipitated by modifying pH (isoelectric point), mixed, centrifuged, and purified by tangential-flow diafiltration. These procedures need a lot of energy, and they also point out that making a meat replacement based on mycoprotein requires a lot of energy. Additionally, they note that the mycoprotein-based product outperforms chicken meat, dairy- and gluten-based meat replacements, and laboratory-grown meat in terms of environmental effect, which is evidence that more study is needed to improve SCP manufacturing methods.

Safety

SCP may include elements that might be dangerous to human health, including heavy metals, allergies, poisons, nucleic acids (RNA), and infections. Since excessive purine intake may raise uric acid concentrations (hyperuricemia), which is a precursor to disorders like gout or kidney stones, the nucleic acid content of SCP may pose a threat to human health. Choosing a suitable microorganism that typically has low quantities of nucleic acids, such as algae over fungus and bacteria, is a preventative method for nucleic acids. Ribonucleases, heat treatments, adding salts, acids, or hydroxides, as well as other strategies for reducing it, have all been considered. The nucleic acid concentration of *S. cerevisiae* produced when cultured on potato peels was reduced by a number of methods, including heat shock (70 °C for 80 s), a base (NaOH, 1 N), an acid (HCl, 2%) and salt (NaCl, 2%) that each had a different effect, with the heat shock treatment reducing the concentration by 43%, 36%, 20%, or 17%, respectively.

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It's important to pay attention to the existence of microorganism-produced toxins. This may be avoided by choosing species, substrates, and medium conditions carefully. For instance, choose algae over fungi since certain fungus species have the potential to develop mycotoxins that are potentially carcinogenic or allergic. Toxins (exotoxins and endotoxins) produced by certain microbes, such as mycotoxins, ochratoxins, and aflatoxins, among others, may have serious negative consequences on human health. Selecting the best microorganism is crucial since 50 species of Fusarium in particular create mycotoxins (fumonisins), which may cause harm to the central nervous system. This strengthens the case for this statement. The ingestion of supplements or meals containing microorganisms has also been linked to allergic reactions or other comparable conditions in people. For instance, a teenager had anaphylaxis after ingesting Spirulina (*Arthrospira platensis*) pills, and phycocyanin was shown to be the cause. A instance of a youngster developing acute tubulointerstitial nephritis after using Chlorella pills for three months was also reported. Thus, it is necessary to take into account any allergies or sensitivities to the microorganisms.

Because there are so many sources available, microbial enzymes are extensively employed in a variety of sectors. Microbial enzymes are thought to be more cost-effective than plant and animal enzymes and are capable of genetic modification. Microorganisms must be multiplied in order to produce the desired result while producing microbial enzymes using fermentation techniques. Based on a set of criteria, the fermentation process is divided into categories. Numerous businesses, including those that deal with food, wine, dairy, baking, milling, drinks, and cereals, use microbial enzymes. Utilizing downstream processing techniques aiming at enzyme recovery and purification, several approaches are used to manufacture microbial enzymes. Based on established principles, including the use of microbial sources, improved strains, and membrane-augmented downstream processing techniques, it is possible to enhance the concentration, purity, and percentage of recovery of enzymes. The essay addresses the fundamentals of microbial sources, strain enhancement strategies, and contemporary approaches for enhancing enzyme activity and the recovery process. The relevance of microbial enzymes in biotechnology and their use in diverse sectors are emphasised.

A thorough screening procedure might eliminate or significantly decrease other specific allergies, pollutants, or dangerous compounds from the substrate, especially if it originates from food waste or another business where meticulous treatment is not taken into account. Single substrates like glucose, dextrose, or sucrose may thus also be thought of as potential additives to the process. These would reduce potentially dangerous compounds that could be present in the substrate, ensuring a high degree of safety for goods intended for consumption by people or animals. If prior measures are taken into account, such as properly choosing the microbe and using harmless substrates, treatments to remove hazardous compounds or potential contaminants should preferably be undertaken as little as possible. Otherwise, extra stages in the processing might lower yield and/or raise costs. Applications and Challenges of Single-Cell Protein SCP has been presented as a potential protein source that may be employed in a variety of formulations (such as protein supplements) for human and animal nutrition. SCP is produced by the fermentation of dietary wastes. This section shows a few of the potential uses that different writers have thought about.

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Date molasses, a byproduct of their production, was employed by the author because it may enable the formation of a significant yeast biomass and, thus, SCP. The high concentration of reducing sugars (73.12%), according to the scientists, made it appropriate to add to yeast growth medium, where it enhanced fermentation characteristics. It was stated that these date by-products may serve as a substrate that delivers carbon and minerals for the manufacture of SCP since they could successfully transition into SCP. The fact that the SCP wasn't particularly evaluated in an animal model to support this use, which emphasises the need for further research, they suggest that it might be utilised industrially as an animal feed additive. In addition to receiving clearance from the appropriate authorities (such as the FDA in the USA), further research is required to indicate its potential usage as an ingredient in human diets and to assess its economic viability in contrast to other protein sources of animal or plant origin.

Large volumes of wastewater are produced during the industrial processing of coffee, and this wastewater has been utilised as a biotechnological substitute to create a nutritious extract that is rich in sugars, proteins, and salts. An effective alternative protein source for animal feed additives, yeast (*Candida sorboxylosa*) was able to efficiently absorb glucose, mannose, and fructose (reducing sugars) from this substrate, producing high SCP yields of 37.4% to 39%. Similar to this, researchers found that Candida utilis was able to convert wastewater from potato peels (supplemented with 5% glycerol) into SCP, with a yield of 30 g dw/L and an efficiency in protein production of 12.2 g/L. They contend that since the FDA has deemed this yeast safe for eating, both the food sector and the manufacture of animal feed may use its biomass and metabolites. This data suggests that wastewater from many businesses might be converted into SCP; however, the authors did not investigate the applications suggested for utilising it as animal feed or a supplement in any animal model, thus further research is still needed to confirm this possible use.

One of the most promising methods is the manufacture of SCP from food waste since it is both a cost-effective source of biomass and ecologically benign. potato peel, carrot pomace, banana peel, and citric residues) for the manufacturing of SCP, emphasising the greater output of potato peel. The authors subsequently added their newly acquired SCP to breads made with wheat flour, noting that doing so up to 4% did not impair the loaves' organoleptic qualities. This finding suggests that it is feasible to turn these byproducts into SCP that could be added to other culinary goods as a supplement. They also claimed that the SCP they got was abundant in vital amino acids, which suggests that it might be utilised to enrich goods made from wheat flour and enhance their nutritional qualities. Before focusing on human nutrition, further research is necessary to identify its potential health bioactivities in vitro or in animal models in addition to the described sensory qualities. Employed orange peel as a substrate for Candida to use in the submerged fermentation process to create SCP. The authors mention it as a great source of minerals, especially calcium and potassium, and carbs. However, they largely concentrated on the creation of the bioprocess to produce it, while its use in an edible product was not precisely explored, highlighting the necessity for more testing. They claim that the SCP developed might be employed in the food sector.

Wastewater and different food wastes may be used as inexpensive substrates to create SCP, which can lessen their environmental effect. Although the data is encouraging, this field of research is still in its early stages and has not yet been thoroughly examined. Authors who have created SCP

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from diverse sources have suggested a large number of possible uses, although the most of these have not been in-depthly examined. They have not been well studied in terms of their influence on the physicochemical and organoleptic features of a product to which they are added or their effects on health. Additionally, there aren't many in vitro or in vivo investigations of its potential bioactivities since the majority of writers are now concentrating on improving the method for obtaining SCP. To fully investigate its real-world applications, which will remain a problem for the future years/decades, a variety of complementary experiments within this large area are required. According to reports, the need for protein will rise dramatically over the next decades. The demand for alternative protein sources and innovation in this area necessitates the performance of these studies into the applications and bioactivities of SCP. This calls for coordinated action from several players, especially from diverse enterprises and investors, whose economic and/or technical capabilities may be focused on resolving the myriad problems that this emerging sector is experiencing. Government bodies must regulate quickly and effectively if they are to encourage the use of healthy protein substitutes (SCP in this case, whose composition is suitable to be included in foods for human consumption). Toxicological tests must be carried out, nevertheless, to rule out any potential health risks for customers, including those from heavy metals, minerals, and RNA, among others. How SCP may be included into the typical diet is still another significant problem. Various SCP-based items are already available on the market; however they are still a niche item that is not commonly used or eaten. This may be partly resolved by creating and providing consumers with alternatives manufactured from or containing SCP, by serving SCP-based meals in restaurants to make them more well-known among a larger audience, or by creating functional foods with improved nutritional qualities. Regarding this, adding *Arthrospira platensis* (4%) to a white chocolate formulation enhanced its protein, amino acid, fat, and mineral contents by 23.1%, 45%, 10.3%, and 13.5%, respectively, without changing its sensory acceptability. This implies that SCP might be added to a variety of meals to boost their nutritional value and perhaps provide health advantages owing to its inclusion of vital amino acids, peptides, and minerals, among other things, among other foods.

To optimise potential commercial adoption, considerable consumer acceptability also demands effort, maybe even more than that observed for cultured meat and plant-based meat substitutes. The possible health advantages of SCP may be highlighted in order to increase consumer acceptability. In this context, *Arthrospira plantensis* has provided peptides with antioxidant capacity and inhibitory ability against angiotensin-converting enzyme (ACE), a bioactivity that may help to maintain healthy blood pressure. Others claim that an astaxanthin-rich SCP with a strong antioxidant potential was generated from *Haematococcus pluvialis* when it was subjected to red LED light. The nutritional value and health-promoting properties of SCP have been the subject of numerous reports, but more study is still needed, especially to confirm the characteristics of products derived from large-scale industrial sources because the majority of the information is based on samples that were collected in laboratories. Finally, a thorough, systematic analysis of the effectiveness of C and N utilisation from food waste is required in order to compare it to other substrates and to conventional sources of these elements. Such information would also allow for a more precise assessment of the economic advantages of reintroducing this biomass into the food production chain, increasing its allure for investors, producers, and consumers while also giving policymakers hard data about the benefits of this practise, furthering this developing field.

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SCP derived from food residues (including food waste and by-products) may include critical amino acids and have a greater protein level than traditional meals such meat, milk, and fish. SCP has shown higher sensory acceptance to lab-grown meat and plant-based protein substitutes when added to meals. Some SCP ingredients have shown potential health advantages, including as antioxidant activity, angiotensin-converting enzyme inhibition potential, and gut health advantages. SCP is a promising alternative protein source, but its production still faces some difficulties, including industrial scaling with profitable yields, purification optimization of isolation processes, and elimination of toxic substances, in addition to the additional tests needed to validate its use in animal or human nutrition (or other purposes). Alternative protein source research and innovation still need to be supported by a variety of parties, including as corporations, investors, academics, and governments, whose financial, scientific, technical, or governmental backing may result in substantial improvements. In order to effectively address the technical issues discussed throughout the text, several research groups must collaborate and all need ongoing financing from public and commercial sources. As a relatively young area, SCP manufacturing lacks industry-accepted gold standards, but as research on the topic intensifies, emphasis should be put on standardising them in order to make development that is both quicker and more significant. All contemporary cultures are interested in the development of this sector as food security becomes a more pressing concern. In a circular production system, the bioengineering of food waste and agricultural by-products from the food industry might be a viable option to acquire protein. This method has the potential to have positive effects on health, the environment, and the economy.

Production of Protein

Proteins are essential to life since they regulate all biochemical processes, provide organisms structure, operate as transport molecules for essential substances, and even act as antibodies to protect the body. According to the core tenet of molecular biology, messenger RNA (mRNA) serves as an intermediate template that allows DNA to transmit genetic information to proteins via the transcriptional and translational processes.

Protein Isolation

The next stage, protein isolation, involves separating the protein from samples like cell lysates or medium. Protein purification processes include cleavage of fusion moieties, protein refolding, and chromatography methods. Protein chromatographic purification at research scale is the main topic of the book Protein Purification. A set of procedures designed to isolate one or a few proteins from a complex mixture must typically be used to separate and purify the protein before it can be identified and its features can be examined. The method of purification may first separate the mixture's protein and non-protein components before separating the target protein from all other proteins. To finish this process in the fewest stages possible, a protein's purification strategy must be adjusted.

Production and Purification of Proteins

By choosing the proper lysis reagents and purification resin, protein output and activity may be optimised. In order to enable the selective purification of the desired protein, the majority of

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recombinant proteins are produced as fusion proteins with brief affinity tags, such as polyhistidine or glutathione S-transferase.

A complex system of biotechnologies called "protein production" impacts each phase of the process in a different way. The features of the recombinant protein as well as the expression system used determine the recombinant protein purification process in large part. With many years of expertise in protein expression, numerous protein purification and refolding methods, and one-stop service from gene to pure protein, Sino Biological delivers. To satisfy high-throughput or large-scale protein expression and production, we have a variety of protein expression systems, including bacterial, yeast, baculovirus-insect, and mammalian expression systems, as well as 30+ purification systems to handle volumes ranging from 2 to 1000 L.

Platform for Producing Protein

The biotechnological process of producing a particular protein is known as protein production. Systems for producing proteins from bacteria, baculovirus/insect cells, mammalian cells, and yeast are often employed. The secret to success is choosing the appropriate expression system for your unique application. When selecting an expression system, characteristics including protein solubility, functionality, purification speed, and yield are often important to take into account. Each system also has its own advantages and disadvantages, which are significant factors to consider when selecting an expression system.

Methods for Protein Purification

Protein fractionation, also known as downstream processing, is what protein purification entails. Pumping and ultrafiltration, two procedures that entail large shear settings, are used in the purification of proteins. More significantly, protein tags are a practical and helpful method for enhancing recombinant protein solubility, accelerating protein purification, and providing a simple means of monitoring proteins throughout protein production and purification. There are several protein purification techniques that may be combined to create an effective purification strategy. Rarely can proteins be purified in a single step; typically, a sequence of procedures must be taken. At earlier phases of the purification system, low-resolution and high-capacity technologies are combined. Methods like fractional precipitation and two-phase partitioning are often used for low-resolution protein purification. Chromatography may be used to selectively purify the target protein for applications needing the greatest purity and relatively tiny quantities of protein. Without a question, recombinant protein synthesis in microbial systems has transformed biochemistry. Small quantities of a specific protein no longer need the enormous volumes of biological fluids or kilogrammes of animal and plant tissues that were formerly required for its purification. Every scientist who starts a new study that calls for a pure protein instantly considers how to produce it using recombinant technology. The creation of commercial products, biochemical analysis of the desired recombinant protein, and usage of the protein in industrial processes are all made possible by the capacity to produce and purify the protein in high quantities.

The procedures required to produce a recombinant protein are theoretically rather simple. The protein is ready for purification and characterisation once you take your gene of interest, clone it in whatever expression vector you have available, convert it into the host of your choice, and

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induce. But there are a lot of things that may go wrong in real life. Down the road, issues including poor host development, inclusion body (IB) formation, inactive proteins, and even no protein at all are often seen. This subject has been extensively explored in a number of reviews in the past. These articles get more than 2000 citations when taken as a whole. However, advancements are constantly being made in the area of recombinant protein expression and purification. For this reason, we discuss the most current developments in the field in this review. We also address the questions that needed to be answered at the outset of the project and describe the numerous options and methods that have been successful for expressing a large number of proteins over the last couple of decades for those with less experience in the production of heterologous proteins. Finally, we provide a troubleshooting manual that will be helpful when dealing with proteins that are challenging to express.

Nucleoside and nucleotide production

Although nature has perfected the synthesis and polymerization of nucleotides and amino acids, it is difficult to replicate these processes in the lab under mild, prebiotically plausible, and straightforward circumstances, without activation. In this regard, much research has been done on the uncatalyzed synthesis of nucleosides and nucleotides from their precursors. By dehydrating ribose with the appropriate purine base, adenine or guanine, in the presence of inorganic polyphosphate salts, Orgel and colleagues were able to synthesise tiny quantities of adenosine and guanosine nucleosides in independent reactions5. They also demonstrated that the addition of salts to the reaction solution, which may promote dehydration processes, increased nucleoside yields. Canonical pyrimidine cytosine, thymine, and uracil nucleosides cannot be made directly from the base and ribose since doing so is difficult. The intrinsic reactivity of the various amine groups makes non-canonical nucleosides the favoured result in the absence of strict control.

The most recent synthesis needs a final photoanomerization as well as the step-by-step addition of the components reacting under certain circumstances. It has been successful to generate non-canonical nucleoside/nucleotides using nucleobase analogues and (5′-phosphorylated) ribose9,10. This is intriguing since the synthesis suggests that in a "pre-RNA world," there may be alternatives to genetic polymers. However, it would be intriguing to think about how amino acids and peptides would figure in this scenario. This is due to the discovery that under moderate circumstances, simple hydration-dehydration cycles caused by heating amino acid solutions may result in the production of peptides. Recent studies have demonstrated that a mixture of amino acids and ribonucleotides can form mixed polymers of nucleotides and amino acids as well as oligo-dipeptide backbones using thioester derivatives as a mediator in the presence of an activating agent (such as carbodiimide, ethylimidazole, or magnesium chloride).

Here, we investigate how easily dehydrated amino acids and nucleotide building blocks interact. The simultaneous formation of nucleotide and nucleoside isomer structures from both purines and pyrimidines is achieved through a straightforward one-pot dehydration reaction of an aqueous mixture (pH 2.5) containing nucleotide building blocks (ribose, phosphate, and nucleobase) without the use of additional activated or catalytic agents. Additionally, we see nucleobase exchanges inside and between nucleoside and nucleotide molecules, which shows that early-forming nucleic acid monomers are in a dynamic environment. When we include amino acids in

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our reactions, we also get a clear isomeric selection on the glycosylation products. This provides more evidence in favour of the nucleic acid/amino acid coevolution theory that amino acids may have had the ability to control the chemistry of primordial nucleoside/nucleotide production. The sum of our findings point to intricate, perhaps indirect routes leading to a stable pool of nucleic acid monomers, which were almost probably subject to dynamic isomeric exchange and interaction with nearby small molecules.

The Importance of Fermented Dairy Products for Human Health

Probiotic bacteria and fermented dairy products decrease cholesterol absorption. Dairy products may benefit body mass and body fat, in part because of whey proteins, calcium, medium-chain fatty acids, and other minerals. Fermentation is the process involved in the oxidation of an organic substrate like glucose when an organic molecule acts as the electron acceptor. So the process by which bacteria transform raw food into fermented food might be a tasty definition of fermented cuisine.

This is due, in part, to the unique odors and sensations that foods like aged cheese, hot kimchi, and fresh yogurt have. Of course, there is also the seductive allure of a quality wine, artisan brew, or single malt scotch. The various health advantages of many of these items have contributed to the popularity of fermented meals. The Fermented Product's main component. Natural antioxidants that support heart health and combat illness are abundant in foods like wine, chocolate, and coffee, for instance. The living bacteria, though, are what matter most too many consumers. This is due to the fact that many of the bacteria present in fermented foods have positive effects on gut health. Below figure shows the major content of Fermented Product

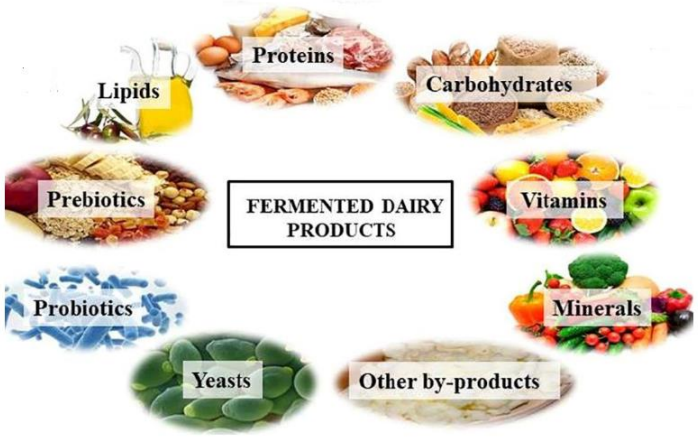

Figure 8.3: Fermented dairy products.

The Advantages of Fermented Foods for Health

Hutkins outlined a variety of justifications for include them in your diet. For instance, epidemiological studies have demonstrated that yogurt intake is often linked to improved weight management, lowered risks of heart disease, metabolic syndrome, and type 2 diabetes (T2D), among other outcomes. Although these products are rich providers of calcium, protein, and other minerals, many of these health advantages are now thought to be derived from the living microbes

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that are present in these products. Streptococcus thermophilus or Lactobacillus delbrueckii subsp. bulgaricus from the yogurt starting culture are also present, along with Bifidobacterium or Lactobacillus strains added expressly for their probiotic characteristics. According to one study, consuming fermented milk reduced the symptoms of irritable bowel syndrome, presumably because it alters the gut flora in a way that is healthy. The diarrhea that people frequently experience after taking antibiotics seems to be relieved by other fermented foods. Hutkins also discussed the most recent findings in the microbiota-gut-brain axis field, making the case that probiotics or prebiotics may have behavioral effects.

Effects of Fermented Foods on Health

Hutkins contends that the effects of fermented meals may be explained by the transformation or alteration in the food caused by microbes. More precisely, the microbes that enter the stomach and create healthy byproducts are likely responsible for these positive health impacts. Actually, there is growing proof that bacteria found in fermented foods are active in the colon physiologically. And these actions could be a component of the processes that underlie advantageous outcomes. Numerous research back up the idea that fermentation can be used to re-engineer the gut micro biota and treat disorders caused by symbiosis. The well-known phenomena of colonization resistance is one of the main areas of research for determining a function for these organisms. A varied, complex, or stable micro biota that is able to withstand colonization by foreign microbes may be found in the healthy human gastrointestinal tract. In addition to lactobacillus and bifid bacterium that are added to ferment dairy products as probiotics, they may also include harmful organisms. Recent studies, however, indicated that frequent yogurt eating appears to boost the quantity of Lactobacilli in the stomach. Additionally, some participants' gut microbial diversity appears to marginally rise in response to yogurt ingestion. These findings imply that functional genes and bacterial species missing from a person's gut micro biome may be restored, opening the door to precise and customized micro biome reconstruction.

Among these, fermented dairy products have been linked to illnesses connected to the immune system, metabolic disorders, and the prevention of obesity. By giving the customer nutrients that are simple to metabolize and advantageous microbes, fermented meals may result in these health advantages. In his final remarks, Hutkins cautions us that not all fermented foods contain live creatures. For instance, steps are done to get rid of the microbes in beer and wine like yeast that allows fermentation. To render the bacteria inactive, heat is used to other fermented foods. Bread is cooked, and sauerkraut is typically canned. These meals may be nutrient-dense, but unlike yogurt, kefir, or any other fermented dairy product with probiotics added, they lack probiotic activity. Additionally, we must be mindful that many fermented foods do not necessarily serve probiotic functions. By definition, probiotics must confer a health advantage. It follows that the probiotic must have been identified and have scientific proof that it has health benefits. Cultures must fulfill this criterion in order to be considered probiotic. As a result, not all meals that have been fermented may be considered probiotic, however not all probiotics come in the form of fermented foods.

Major Fermented Milk Products

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Fermented or cultured milks are milk products made by lactic acid fermentation (such as yogurt) or a mix of this and yeast fermentation (such as kefir). In this chapter, the word "fermented" will be used. Yogurt, Scandinavian sour milk, cultured buttermilk, cultured cream, and koumiss, a product made from mares' milk, are all examples of goods that fall under the category of fermented milk. The product's milk is injected with a starter culture, which causes portion of the lactose to be converted to lactic acid, giving fermented milk its general name. These molecules give the goods their distinctive fresh flavor and scent and include carbon dioxide, diacetyl, acetic acid, acetaldehyde, and numerous more, depending on the kind of lactic acid bacteria utilized. Ethyl alcohol is also created by the same microbes that generate kefir and koumiss.

Lactic acid is produced when lactose is converted, which preserves milk. Cultured milk's low pH prevents putrefactive bacteria and other harmful organisms from growing, extending the product's shelf life. On the other side, acidified milk creates a particularly favorable habitat for yeasts and moulds, which if allowed to infect the products, result in bad flavors, blown packaging, etc. Some people's digestive tracts are deficient in the lactase enzyme. As a result, lactose is not converted during digestion into less complex carbohydrates. These folks are only able to eat extremely little amounts of regular milk. However, they are able to ingest fermented milk, in which bacterial enzymes have already partially broken down the lactose. The starting culture has to have the ideal growing conditions in order to produce fermented milk. Along with flavor and scent, accuracy of appearance and consistency are crucial characteristics. The pre-processing factors used affect them. The "foundation stones" for the creation of the coagulum throughout this incubation phase are adequate heat treatment as well as homogenization of the milk, sometimes in combination with techniques to raise the MSNF concentration, as for milk intended for yoghurt. The following is a description of some of the most significant fermented milk products. Numerous other fermented goods are produced using similar methods; milk is pre-treated similarly, for instance. Therefore, the process explanations for other products focus mostly on the stages of manufacturing that are different from those in yoghurt manufacture.

Yoghurt

A milk product called yogurt is made by fermenting milk-specific bacteria with lactic acid. Streptococcus thermophilus but also Lactobacillus delbrueckii subsp. bulgaricus, two particular microorganisms for yogurt, produce lactic ferments, which are responsible for its production. Lactose, the milk sugar, is first transformed into both glucose and galactose during the fermentation process, and then these simple sugars are transformed into lactic acid. It contributes some acidity, which aids in the caseins' coagulation and creates the unique flavor and texture of yogurt, as well as compounds.

Curd

Curd, also known as dahi, is a classic yogurt or fermented dairy product that is native to the Indian subcontinent. It is often made from cow's milk, however occasionally it can also be made from buffalo or goat's milk. It is well-liked everywhere in the Indian subcontinent. The phrase yogurt refers to the commercially produced, pasteurized type known as heat treated fermented milk, whereas the term curd refers to the (naturally probiotic) handmade variety.

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Bacterial fermentation of milk produces curd. Several probiotic microbes turn milk's lactose into lactic acid during this process. Species engaged in the fermentation can include Lactococcus lactis, bulgaricus, Lactobacillus delbrueckii subsp. Streptococcus cremoris, Streptococcus diacetylactis, and Streptococcus thermophilus, depending on the temperature or humidity of the environment.

Milk is boiled and chilled once the starter is prepared, or preserved from a previous production of curd. The curd and its whey are combined in a separate dish before being combined with the milk. After that, it is allowed to sit quietly for five to ten hours. Add 3% skim milk powder for the best results. Add the remaining ingredients after blending 3% excellent curd in milk. Keep at 40–45°C with a cloth covering to prevent cooling. Only place in refrigerator after it begins to taste a little sour. Avoid unpleasant flavors that might hurt. Never use bitter curd again. This method may also be used to make curd from milk alternatives like soy milk. In the absence of oxygen, microbes convert sugar to acids as well as alcohol through the process of fermentation. Milk is fermented by bacteria like the streptococcus and lactobacillus species to produce yogurt and curd. Alcoholic drinks including beer, brandy, wine, and whiskey are created by the yeast fermentation of grains, grapes, rice, and other ingredients. Dough may also be fermented with yeast to produce bread. Gasohol is a fuel that is created by fermenting both gasoline and alcohol. In addition to these, fermentation is employed in the creation of several medications.

Cider Fermentation

While contemporary commercial containers are typically temperature-controlled vessels with a capacity of 2000–9000L and made of lined concrete, lined mild steel, or lined stainless steel, traditional wooden fermentation barrels are still used today. Fresh juice or reconstituted concentrate may be supplied with fermentable sugars before fermentation. It has been shown that cider yeast function is impaired in fermenters deeper than 14.5 m, which generate hydrostatic pressures of 1.5 atm. Alcohol levels seldom rise over 6.0% (v/v) without additional fermentables, which are typically obtained from cane sugar. Traditional fermentations are carried out by native yeasts that come from the fruit's skins, the environment, and the processing machinery. The majority of the yeasts detected in sound ripe apples are *Kloeckera apiculata, Aureobasidium pullulans,* or species of *Rhodotorula, Candida, Torulopsis*, and Metschniko*wia; Saccharomyces species* as well as other sporulating yeasts are uncommon. Lactic acid bacteria are uncommon although acid-tolerant bacteria, such as *Gluconobacter species,* are often found. Most fermentations of the classic kind take place at room temperature. They often begin slowly, within 1-2 days, and last for many weeks. In contemporary commercial operations, pasteurisation, sterile filtration, or high-speed centrifugation are used to treat juice to eliminate microbiological contamination, which is shown in below figure.

The traditional condiment known as vinegar is made from raw materials such as rice, malt, apples, alcoholic liquid, and several other agricultural resources using two separate fermentation processes known as acetous and alcoholic fermentation. Cider vinegar, which is prepared from apple juice or juice concentrate, is widely used in a number of nations, including India, Austria, the United Kingdom, the United States, and Switzerland. Despite being a common substance, vinegar is receiving more attention as a result of its alleged medicinal benefits. For instance, as compared to rats on a high cholesterol diet without cider vinegar, the rats in all groups had lower triglyceride

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and produced VLDL levels. In the atherogenic prediabetic condition, it has also been thought to be helpful for reducing insulin resistance and metabolic abnormalities. Recently, it was discovered that acetic acid is a potent anticancer agent and that using it to treat cancer is a viable option based on research using rat stomach epithelial cell lines.

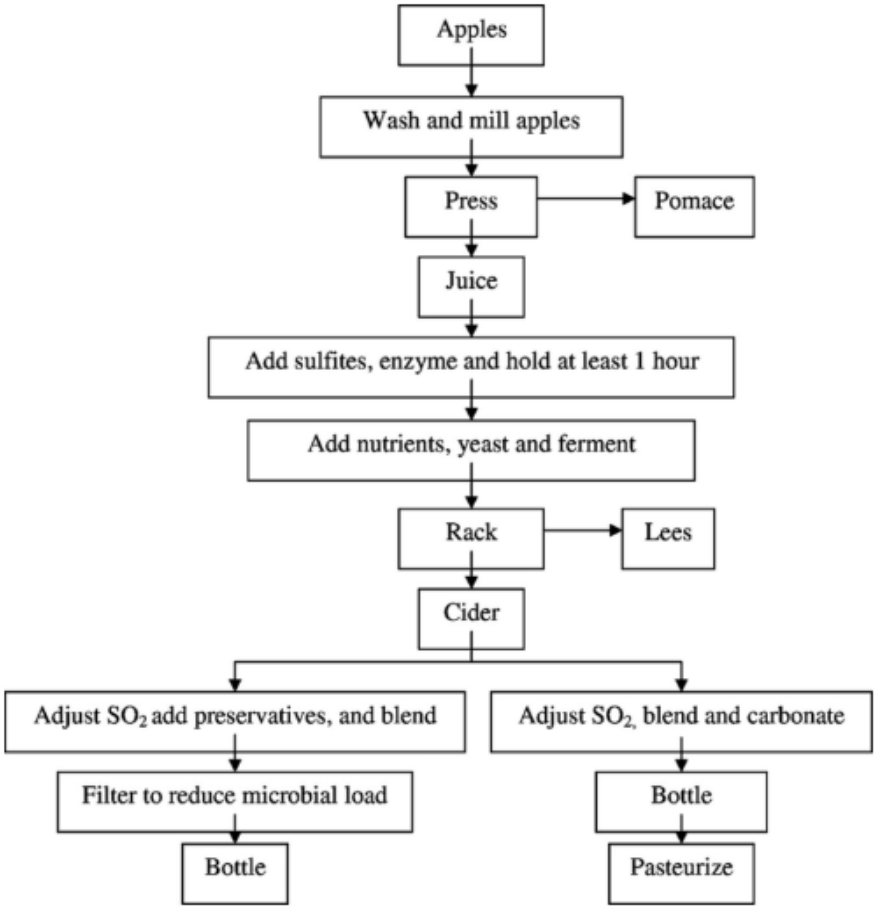

Figure 8.4: Fermented production of apple cider vinegar.

The main component of vinegar, acetic acid, has a minimum legal strength that varies from nation to country for cider vinegar. Acetic acid (w/v) should not be less than 4%. Depending on the chemical makeup of the utilised cider, it has been divided into low-strength and high-strength cider. Low-strength cider is defined as having acetic acid concentrations of no more than 4% and high-strength cider as having acetic acid concentrations of up to 9%. After alcoholic and subsequent acetic fermentation, fresh, smashed apples are used to make cider vinegar. It is then left to age in oak barrels, mostly by spontaneous and uncontrolled fermentation, which results in low yields. The technique for making vinegar spans from the classic process of submerged fermentation in sophisticated acetators to using oak barrels and surface culture. According to the conventional approach, naturally occurring yeast and acetic acid bacteria, which carry out the alcoholic and acid fermentations, respectively, ferment apple juice in the same barrel. The alcoholic and acetous fermentation required to turn apple juice into cider vinegar is finished in approximately 5 to 6 months after the barrel is put in a damp but warm environment and its bung

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hole is closed with a piece of cloth to keep out dust and flies. The first fermentation is called ethanolic fermentation, in which *Saccharomyces cerevisiae* var. ellipsoideus, a naturally occurring microflora in the environment, is mostly responsible for turning the juice's sugar into alcohol. The primary disadvantage of this method is that the alcoholic fermentation carried out by the natural microflora is sometimes delayed and incomplete. Additionally, the ensuing acetic fermentation produces modest amounts of acetic acid, leading to subpar cider vinegar. By improving the fermentation process for the production of cider vinegar, these issues may be resolved.

Initial soluble solids, sulphur dioxide, and nitrogen supply are thought to be crucial for any alcoholic fermentation. What should be the concentration of sulphur dioxide or a nitrogen source like diammonium hydrogen phosphate (DAHP) in the must to prepare the base wine by ethanolic fermentation by the yeast? These questions are related to the initial total soluble solids (TSS) that should be kept in the must prior to alcoholic fermentation. For the manufacturing of alcoholic drinks like cider and wine from apples and other fruits, such characteristics have been standardised. As acetic acid bacteria are very sensitive to high ethanol level and high temperature (greater than 30 C) is inhibitory, it is crucial that the alcoholic substance being converted into vinegar has an alcohol content of 6-7%. If not, the acetic acid fermentation for vinegar is terminated.

The juices often lack a sufficient nitrogen supply, and this deficiency causes fermentation to proceed slowly. Both organic and inorganic sources of nitrogen are regarded as important, although exogenous additions of inorganic nitrogen, such as DAHP (diammonium hydrogen phosphate), are often required. Sulfur dioxide is similarly provided to suppress the natural microflora and enable the additional inoculum to carry out fermentation as intended; however, its larger concentration may inhibit the development of the desired bacteria as well. In order to ensure effective ethanolic fermentation of the cider vinegar, these parameters must be optimised. The current investigation was organised and carried out taking these criteria into account.

When the control variables have an independent and combined impact on the intended responses, response surface methodology (RSM) is a useful technique for improving the process conditions. It has been used a lot in the literature to enhance several fermentation processes. As a result, using RSM, the initial TSS (total soluble solids), diammonium hydrogen phosphate (DAHP), and sulphur dioxide (SO2) in the alcoholic fermentation of apple juice were taken into consideration when optimising the process of base wine production as part of studies on standardizing the technology of making cider vinegar. This message has included a discussion of the findings.

Probiotic

Probiotics are live microorganisms that, when administered in adequate amounts, confer a health benefit on the host, mainly through the process of replacing or including beneficial bacteria in the gastrointestinal tract. Fermented dairy foods such as yogurt, fermented milk and cheese are the major vehicle in delivering probiotics, and probiotic delivery have been traditionally associated with these fermented dairy foods. Additionally, many other non-dairy probiotic products and non-food form such as capsules, pills and tablets are also available and some of these non-food forms are highly popular among the consumers. Certain non-dairy probiotic foods, especially beverages that are non-fermented products, can also play an important role in probiotic delivery. There is an

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increasing demand for non-dairy probiotic foods (both fermented and non-fermented) including fruit and vegetable juices, soy and certain cereal products due to vegetarianism, lactose intolerance and dairy allergies, as well as interest in low cholesterol foods. In this context, this review mainly focusses on the different types of probiotic food products including beverages with special reference to their viability followed by a brief account on the applicability of using fermented and non-fermented beverage products in probiotic delivery. As shown in below figure categories and varieties of probiotic drinks.

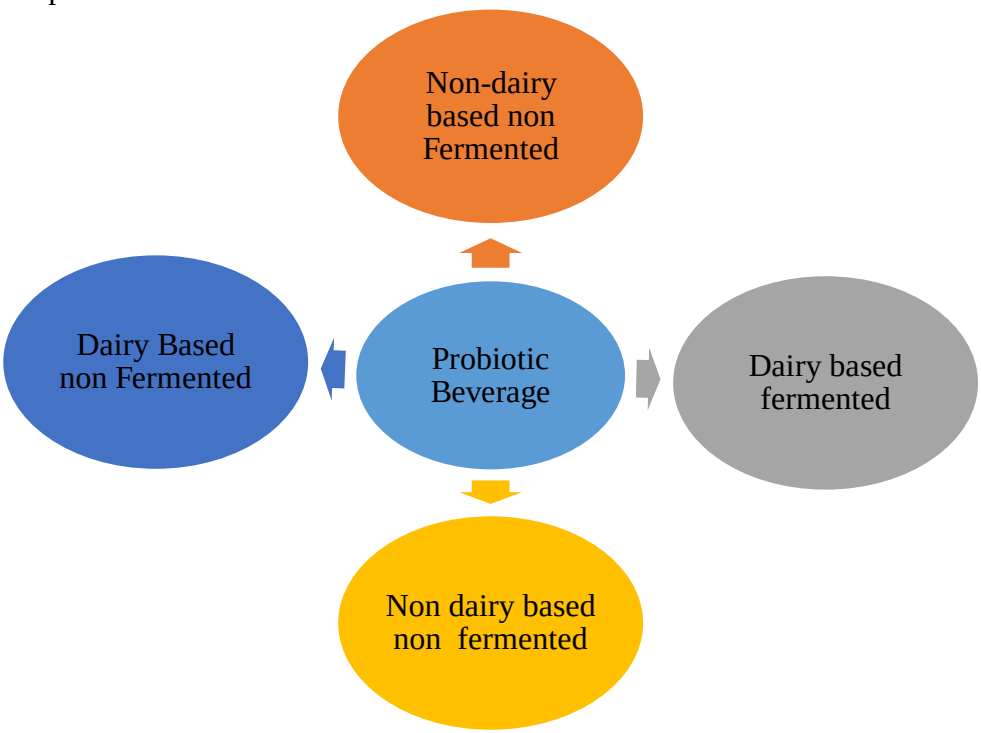

Figure 8.5: Categories and varieties of probiotic drinks.

Beverages: Fermented vs. Non-Fermented

One of the earliest uses of food technology is fermentation, and fermented products are the result of a complex microbiota's metabolic activity, which includes both naturally occurring native microorganisms and/or chosen microorganisms, such as bacteria and yeasts that are inoculated as starter cultures. The synthesis of organic acids during fermentation aids in food preservation. It also gives food items more nutritive value and pleasant sensory qualities. Fermentation is not required for the creation of probiotic beverages. On the international market, there are several varieties of probiotic fermented milk sold under different brand names.

The kind of probiotic microbe, type of milk, and inclusion of additional starting cultures in the product all have a significant impact on the major physicochemical characteristics of probiotic fermented milk products. Additionally, the textures of fermented probiotics dairy products vary, ranging from liquid beverages like kefir and acidophilus milk to semi-solid or hard foods like drinking yogurt or villi. Microorganisms employed in starter cultures have significant industrial importance since they are crucial to the development of the flavor and texture of fermented food items. These starting cultures might not always have probiotic qualities. Because yogurt starter

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cultures have a poor digestive tract survival rate, the name "probiotics" may not be appropriate for them. However, certain positive health-promoting benefits of yogurt starter cultures have also been noted, such as enhanced lactose utilization or immune system stimulation.

Selection of the right conditions, such as the ideal temperature during fermentation, is a crucial parameter that must be taken into account to prevent lethal but rather sub-lethal damages towards the probiotic cells even during processing but also subsequent storage. Fermentation is a complex process when it comes to achieving desirable food characteristics. The biomass output is also impacted by these circumstances. The length of the fermentation process may also have an impact on the end product's quality. Drinking dairy drinks might come from the partial fermentation of dairy meals, and these items are now in great demand. Additionally, probiotics in fermented foods may exhibit higher product stability since fermentation time might provide them a chance to proliferate and solidify effectively. However, today's health-conscious consumers have a preference for both matured and unmatured probiotic food products, notably probiotic beverages. This fact may help to increase sales of these refreshment products despite variations in the production process and any changes to the product's physical, chemical, tactile, nutritious, and useful features.

Dairy products that have been fermented continue to be the best probiotic carriers. However, there are many non-dairy foods that are accessible that are both fermented and unfermented in nature, and these goods also play a big part in helping people get probiotics. In general, non-fermented formulations make up the majority of probiotic drinks, especially those with fruit or vegetable origins. The effectiveness of probiotics when administered through fermented vs. non-fermented state of a specific carrier food matrix with a special reference to drinks has not been adequately explored, and further study in this area is required to reap the full advantages of probiotics. In order to improve formulations, sensory testing should also be used to gauge customer approval of these beverage items.

Since prehistoric times, humans have made fermented foods a mainstay of their diet. Because fermentation has historically been a successful method of food preservation, many various kinds of food, including dairy, vegetables, meat, grains, and fruits, have been fermented. Nutritionists, biologists, and consumers alike are now more interested than ever in understanding and creating innovative fermented foods because they include a variety of potent bioactive compounds that may be responsible for their superior health-promoting properties. Diverse cultures and communities eat a variety of native fermented foods that are produced naturally, and these foods are often fresh sources of probiotic strains that may be used in the production of additional fermented food items. As a result, it has lately become more important to read and comprehend fermented meals carefully, and they have been redefined as "foods generated by intended microbial growth and enzymatic transformations of food components." Foods that have undergone fermentation often exhibit a number of distinctive physicochemical and biological changes, including an extended shelf life, better organoleptic qualities, and a higher bioavailability of vitamins and minerals. Additionally, investigations in both preclinical and clinical settings have shown that fermented foods are useful in treating long-term human diseases like cancer.

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In the course of fermentation, bacteria break down the food's nutrients into simpler, often distinctively different components. For instance, the breakdown of proteins by microbes during fermentation often results in the production of bioactive peptides, which have a number of positive impacts on health. Exopolysaccharides, which contribute to the taste and pharmacological effects of fermented foods, are also produced by microbes during the fermentation of carbohydrates. The fact that main macronutrients are not the sole substrates for microbial fermentation in food must be taken into account. More and more data points to a deeper connection between secondary plant compounds like polyphenols and fermenting microorganisms, notably probiotic bacteria. In fact, a bidirectional interaction between polyphenols or probiotic bacteria is quickly garnering attention, notably in the context of fermented foods and health. The goal of this study is to inform the audience on recent advances in the fermentation of foods high in polyphenols by probiotics. We first provide a general introduction of the fermentation of macronutrients before reviewing the current research on meals containing polyphenols. The interaction between polyphenols and probiotic bacteria, along with the efficacy and application of fermented polyphenolic meals, are next discussed, with a focus on the creation of innovative functional food items.

Macronutrients in food are fermented by microbes

Depending on the kind of fermenter microorganisms, fermentation may be roughly divided into two types: spontaneous fermentation and starter culture-dependent fermentation. When bacteria are present in the raw food or the processing environment organically, spontaneous fermentation takes place. For more controlled and desirable changes in the fermented meal, starting culture fermentation uses direct inoculation of certain microbes into food ingredients. Starter cultures are widely utilised in the food sector because they often produce distinctive sensory, dietary, and health-improving modifications. Due to the metabolism of numerous macronutrients included in the meal, microbial fermentation significantly affects the chemical composition of the fermented food product regardless of the source. To do this, microbes have various distinctive enzymes that the host may lack, including proteases, peptidases, ureases, polysaccharide degrading enzymes, lipases, amylases, esterases, and phenol-oxidases. Probiotic bacteria utilise the various carbohydrates found in raw dietary material, including monosaccharides, disaccharides, and polysaccharides, as a substrate. Different probiotic bacterial species can metabolise various kinds of carbohydrates, making them useful for a wide range of uses in the food business. Numerous carbohydrates-metabolizing enzymes, including glycosyl hydrolases, sugar ABC transporters, and phosphoenolpyruvate and phosphotransferase systems that may operate on both plant- and animal-derived carbohydrates, are often expressed by probiotic bacteria. Probiotic bacteria mainly use carbohydrates to meet their energy needs. They then convert these carbohydrates into organic acids like short-chain fatty acids (SCFAs), while non-digestible oligosaccharides like hemicellulose and pectins also serve as prebiotics to promote the growth of probiotic bacteria. Due to their well-documented capacity to modify cell signalling pathways, enhance gut epithelial barrier integrity, and suppress pro-inflammatory immune responses in the gut, SCFAs are of particular importance and their high levels in fermented foods are frequently associated with improved health benefits. Additionally, during the fermentation of carbohydrates, bacteria create exopolysaccharides, which have a crucial role in determining the texture, taste, and shelf-life of fermented foods. Additionally,

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some exopolysaccharides may function as prebiotics and have positive impacts on the host's health, such as modifying the immune system and gut flora.

Protein degradation plays a significant role in defining the nutritional content and quality of fermented foods, particularly dairy products, in addition to the oxidation of carbohydrates. Large proteins are broken down into tiny pieces during fermentation, often producing bioactive peptides that have been shown to have anti-thrombogenic, anti-oxidant, anti-hypertensive, and anti-inflammatory properties in various in vitro and preclinical investigations. Several distinct kinds of bioactive peptides have been discovered and are now maintained in a newly created database, depending on the food supply and probiotic microbes employed. The fermented bioactive peptides in dairy proteins, animal proteins, and vegetable proteins have been extensively studied. Additionally, the potential allergenicity of certain dietary components, such as milk and its derivatives, may be reduced by the fermentation of particular proteins (for example, casein), which increases the acceptability of such foods in sensitive groups. Similar to this, probiotic microbes may enhance the overall digestibility and nutritional content of proteins in meals by breaking down complicated storage proteins into more soluble forms that are easier for the body to absorb. Additionally, probiotic bacteria have the ability to metabolise amino acids via procedures like deamination and decarboxylation, which help control the synthesis of biogenic amines in meals that are otherwise thought to be harmful. The co-ingestion of pea proteins with strains of the probiotic Lactobacillus paracasei for two weeks significantly increased the absorption of amino acids like methionine, leucine, histidine, isoleucine, valine, and tyrosine and thereby increased the nutritional value of consumed food in a randomised clinical trial on healthy subjects.

Probiotic fermentation alters the phenolic profile qualitatively and quantitatively. A varied group of secondary metabolites known as polyphenols is found in a wide variety of foods, including fruits, vegetables, spices, nuts, grains, and legumes. Chemically, these molecules have at least one aromatic ring coupled to one or more hydroxyl groups. Flavonoids, stilbenes, phenolic acids, and lignans are a few of the families of polyphenols that are further subdivided depending on this characteristic. Foods high in polyphenols, whether they are naturally abundant or fortified, are highly prized, and consuming them has long been linked to positive impacts on health. This is mostly because to polyphenols' potent antioxidant properties, which have been shown in both animal and human studies to improve cellular redox equilibrium. Natural polyphenols are often thought of as anti-microbial, but mounting research points to a deeper and more reciprocal relationship between probiotic bacteria and polyphenols that may have ramifications for the creation of innovative fermented functional foods. It is becoming clearer that probiotic fermentation of polyphenols May biotransform complicated polyphenols into simpler, free, but more soluble molecules, eventually leading to increased levels of total bioactive components as well as better overall polyphenol bioavailability in the fermented product. On the other hand, given that polyphenols tend to have distinct impacts on the microbial community as a whole, the presence of polyphenols in meals may have some benefit for the particular development of probiotic bacteria. Furthermore, polyphenols are not well absorbed in the small intestine and are mostly excreted into the colon, where the gut microbiota substantially breaks them down into low molecular weight phenolic acids, which are subsequently transported by the portal vein to the liver for further metabolism. Due to their slower rate of absorption, polyphenols remain in the stomach

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for a longer period of time, allowing the gut microbiota to undergo significant biotransformation. Numerous polyphenol-rich foods may undergo both qualitative and quantitative changes in their polyphenolic content and bioactivity when fermented with certain probiotic strains, according to studies, increasing their functional food value.

Numerous food kinds have been fermented and made available for purchase, but the fermented polyphenol-rich foods and their potential in the food sector have not yet received the full and worthy attention they need. This may be partially explained by the classical antibacterial properties of polyphenols, which are now being questioned in new investigations, as is also covered in this publication. The fundamental idea behind using polyphenolic fermented foods is linked to their dual benefits, namely, the power of probiotics to biotransform polyphenols throughout fermentation, resulting in increased bioavailability as well as content of the native phenolic profile, which has enormous potential for the creation of novel functional food products. Polyphenols have the ability to suppress a variety of pathogens while simultaneously promoting probiotic growth. However, care must be given since interactions between polyphenols as well as probiotic bacteria may not always be universal and may instead rely on the kind of polyphenol(s) utilised as well as the strain(s) of probiotic bacterial species. In order to determine the best probiotic bacteria and polyphenol combinations for fermentability and potential health benefits, in vitro and in vivo testing is advised. Studies examining combinations of medicinal plants high in polyphenols for their probiotic bacteria fermentability, possible alteration of native chemical profile, and health effects thereof are also scarce and need to be studied. In conclusion, it is advised that the combination of probiotic and polyphenolic compounds in foods be viewed as falling under the umbrella of synergy because it represents an unexplored research field that may serve as a significant source for the development of the following generation of functional foods.

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CHAPTER 9

Application of Fermentation in Medical Procedures

Dr. Indrani Jadhav

Professor, School of Life and Basic Sciences, Jaipur National University, Jaipur, India

Email Id- indranij4@jnujaipur.ac.in

The origins of traditional medicines produced with age innovation date back millennia. For instance, throughout mankind's history, knowledge of the numbing and antiseptic effects of ethanol and the preservation and enhancement of the pharmacological and nutritional qualities of matured food varieties and drinks was commonplace. Antimicrobials have been known for their effectiveness for a long time, much too how rotting soybean cards were used to cure bubbles or form contaminations in the foot. This has been evident for the entirety of Chinese medicine. With Sir Alexander Fleming's 1929 discovery of penicillin, the primary anti-infection, the era of anti-microbials began. This was followed by extensive research efforts to boost penicillin development from the research facility size to the present scale, which was thousands of times greater, providing a major example of contemporary anti-microbial drug invention [6]. Several anti-toxins entered clinical usage around 1940 and gained notoriety due to their ability to eradicate numerous types of germs and, when used methodically, their fairly non-poisonous nature. With more knowledge of the tools and the pathogenicity of many human illnesses today, a growing number of specified, therapeutically important products are being given via maturation innovation. The objects might be whole bacteria or microbial cells, necessary or optional metabolites, chemicals, products of biotransformation, as well as recombinant bio-atoms depending on their compound nature. In terms of clinical implications, fermentation byproducts are currently being utilized to treat a variety of medical illnesses, such as malignancies, metabolic disorders, malnutrition, cardiovascular diseases, infectious diseases, and metabolic disorders [7].

Antibacterial Medicines

Certain species create antimicrobial medications at specific stages of growth, and these substances either stop the growth of other organisms or are poisonous to them. One of the most significant and often utilized types of fermentation products is antimicrobial chemicals. These can be categorized as antifungal, antiviral, antibacterial, or antiparasitic substances and are used to treat microbial infections in humans and animals. The commercial manufacture of antimicrobial medications uses both solid-state and liquid-submerged fermentations, and the choice is made based on the kind of organism, the substrate, as well as the product yield.

Antiviral:

Viral infections are treated with antiviral medications. Antiviral medication preparation is difficult because the drug must be supplied to the host cells and disrupt viral protein synthesis or

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reproduction mechanisms, unlike antifungal, antibacterial, or antiparasitic treatments, which specifically attack the organism outside the host cells. Antiviral medications thus run the risk of harming the host cells as well. It takes a considerable understanding of the genetics of the virus and the host cells to prepare the proper antiviral medications, ensuring that they have a minimally harmful effect on the host cells [8]. To be effective against a variety of viruses from the same class or family, antiviral drugs should have a wide spectrum of action. They are made to target protein synthesis, viral attachment, or the replication process. Examples include interferon, acyclovir, amantadine, oseltamivir, and other well-known antiviral drugs.

Antibacterial

Bacteriostatic antibiotics are chemotherapeutic drugs generated by living organisms as secondary metabolites that either stop a particular bacterium from growing or kill it (bactericidal). Molds now produce the majority of commercially available antibiotics. However, gram-positive bacteria release several antibiotic substances that have a bacteriostatic impact on gram-negative bacteria. Penicillin is the first antibiotic to have been made available for purchase. The mold Penicillium chrysogenum is now employed for the commercial manufacturing of this antibiotic utilizing the SMF procedure, even though Penicillium notatum was first used for production, and both SSF, as well as SMF, were used to compare the product yield. There are other additional antibiotics produced economically employing feed batch fermentation in addition to penicillin [9].

Antifungal

Antifungal drugs are medications that selectively eliminate fungal development while causing little to no harm to the host cells. While molds, bacteria, and even blue-green algae release antifungal compounds, bacteria produce the bulk of antibacterial substances. Antifungal drugs like those in the azole family are used to treat mycotic infections caused by filamentous fungi or yeasts. Like antibacterial drugs, antifungal drugs are divided into groups based on how they function. These groups include:

- Destruction or inhibition of fungal cell membranes.
- Inhibition of the development of fungal cell walls, and
- Inhibition of DNA synthesis.

The commercial production of antifungal drugs employs both synthetic and fermentation methods. Following this, substantial research was conducted to advance penicillin development from the size of the first research facility to the current scale, which was thousands of times larger, serving as the primary example of modern anti-microbial medication creation. Around 1940, some anti-toxins were first utilized in clinical settings and became well-known for their capacity to kill a wide variety of germs and, when administered properly their largely non-poisonous nature. Today, a rising number of specific, therapeutically significant products are being provided by maturation innovation because of increased knowledge of the methods and the pathogenicity of many human disorders [10]. Depending on their complex nature, the objects may be complete bacteria or microbial cells, essential or optional metabolites, chemicals, byproducts of biotransformation, or recombinant bio-atoms. Our expanding knowledge of the human genome and the underlying

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causes of disease has led to the development of new biomaterials for use as target-specific pharmaceuticals and the identification of novel therapeutic targets. These biomaterials were originally developed and produced on a small scale at research facilities and were commonly produced on a big scale with the aid of fermentation technology. The fine-tuning of the culture environment, well-growing cell substrates, and high-yield recombinant system emerged, animal product-free media, efficient bioreactors, proper process control, as well as genetic modification of host bacteria or mammalian cells have all been used to address several inherent problems with fermentation technology's upstream processes, such as low yield. The assembly technology for commercial scale production of new-age aging products, like therapeutic recombinant proteins and monoclonal antibodies, has sufficiently advanced to deliver many kilograms of finished goods yearly in a financially viable manner with the use of large, worked tank bioreactors with a limit up to a large number of liters and cluster, looked after group, or consistent maturation.

The most of us will have had first-hand contact with the fermentation process via its most well-known and widespread usage, the brewing of beer. The original definition of fermentation is "the anaerobic conversion of sugar to carbon dioxide and alcohol by yeast. As time went on, this initial description was broadened to include "the processing of organic materials into relatively simple compounds by micro-organisms - basically efficient, adaptable bio factories." Microorganisms produce a vast variety of various chemicals during their development and lifetime that are necessary for their survival and capacity to reproduce as well as for their ability to adapt to stressful situations and aggressive, competing microbes.

Microbial fermentation

The creation of a large variety of pharmaceutical compounds, which may be used to treat almost any medical condition, is based on microbial fermentation. Examples include hormone imbalance treatment, anti-infectious disease medicines and vaccines, anti-cancer cytotoxic medications and vaccinations, and many more indications. There are particular, multi-step, complicated pathways that are used during the manufacture of endogenous compounds in nature, some of which may be modified to facilitate the creation of foreign molecules. By significantly changing their endogenous pathways, microorganisms may be metabolically engineered or genetically edited.

The major components of fermentation development include medium and process development, scale-up to increase productivity, and strain selection and optimization. Different methods are used in downstream processing to separate, concentrate, and purify the product from a diluted fermentation broth. Fermentation technology is a multi-disciplinary methodology that includes microbiology, organic chemistry, biochemistry, and molecular biology. Fermentation derived product diversity refers to the recovery and selective purification of the specific desired product out of the entire molecular repertoire.

The only method for producing chemical APIs that depends purely on microorganisms and lacks a counterpart in other biologic systems is fermentation (e.g. mammalian cells). Examples include adjuvants like lipid A produced by gram-negative bacteria or antibiotics/secondary metabolites produced by fungus that function as anticancer or anti-infectious agents. These organic compounds may be created from their constituent parts using a multi-step synthesis process. Organic

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compounds, on the other hand, may have features like chiral centres, sizable stereospecific rings, or distinctive conjugated double bond systems. The synthetic technique is more time-consuming, expensive, and needs more development than the fermentation alternative.

The semi-synthetic method makes use of fermentation's benefits in the creation of novel medications. The production of natural compounds involves fermentation, followed by synthetic modification that increases potency, selectivity, and reduces toxicity while also overcoming bacterial resistance to conventional antibiotics. Additionally, natural medicinal proteins that are only expressed in microbial systems may only be obtained by fermentation. Proteins are intricate, high- to mid-molecular-weight molecules. Their secondary and tertiary structure, as well as a number of post-translational changes, most notably glycosilation, are crucial to both their functioning and stability. The synthesised option is only available for very short peptides.

Recombinant technology makes it possible for microbial systems, including those derived from human sources, to express foreign genes that encode therapeutic proteins. Since microbial systems like E. coli lack post-translational mechanics, using microbial fermentation is useful for the production of proteins that do not need these modifications. Another strategy is to just express the protein's minimally effective region (in the case of antibodies, nanobodies or peptidebodies). According to the chart below, time and yield are the main benefits of fermentation over the mammalian system, which eventually affect cost.

Up until recently, therapeutic proteins that needed modification, such antibodies that needed to be glycosilated, were produced in mammalian cell cultures. Due to budgetary constraints, researchers sought to produce glycosilated therapeutic proteins in microbial systems. This led to the development of the unique technique known as glycoengineering, which included altering the native glycosilation pathway in high yield of recombinant yeast. As a result of the modified system's ability to mimic the human pathway, humanised antibody fragment expression was made possible.

As a secondary metabolite of Cannabis, annabinoids, also known as terpenophenolic in the meroterpene class, are created by a prenyltransferase enzyme joining an isoprenoid precursor with a second fatty acid derivative precursor. By prenylating geranyl diphosphate and olivetolic acid, this enzyme produces cannabigerolic acid (CBGA) (GPP). A variety of cyclized chemicals, including tetrahydrocannabinolic acid (THCA), cannabidiolic acid (CBDA), and cannabichromenic acid, are then created from CBGA (CBCA). The major cannabinoids found in Cannabis spp. are THC, CBD, and CBC, which are produced as a result of decarboxylation of these chemicals.

In recent years, numerous nations, including the USA, Canada, Israel, and many European nations, including the Netherlands, Germany, and the Czech Republic, have legalised the use of Cannabis spp. for medicinal purposes. However, because to legal concerns, Cannabis spp. cannot be grown in a number of nations. Cannabis cultivation as a means of producing cannabinoids for agricultural use has a number of drawbacks, including low yields caused by climatic changes and plant diseases, low cannabinoid concentrations in the plant, the need for extraction procedures, and sociopolitical factors for cannabis use, which has historically been viewed primarily as a source of

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narcotics. However, due to the intricacy of the chemical synthesis of cannabinoids, which often results in poor yields and high production costs, it does not provide an alternate method of manufacturing. These factors make the microbiological manufacture of these chemicals by modified yeast a viable, dependable, environmentally benign, and economically advantageous alternative strategy.

Other Vital Terpenoids in Medicine

There are additional therapeutically significant terpenoids with a variety of pharmacological actions and functions in addition to artemisinin, taxol, and cannabinoids. According to studies, the monoterpenes geraniol, D-limonene, and perillyl alcohol are useful in both the prevention and therapy of certain malignancies. Breast, lung, pancreatic, colon, prostate,and liver cancers may be prevented or treated with them. Their primary method of combating cancer activity involves blocking the post-translational isoprenylation of proteins, which is essential for the development of tumour cells. Some monoterpenes, such as menthol, limonene, or sabinene, exhibit antimicrobial actions on a variety of bacteria, including Bacillus subtilis, Streptococcus spp., Staphylococcus aureus, E. coli, or Candida albicans. These effects are in addition to their anticancer activity. Additionally, menthol and limonene enhance medication absorption via the skin through transdermal delivery. These terpenoids have low toxicity, strong activity, and minimal skin irritation. On Helicobacter pylori, the sesquiterpene patchouli alcohol also shown antibacterial action. Triterpene ginsenosides also has a number of pharmacological properties, including anti-oxidation, anti-inflammatory, hepatoprotective, anti-diabetic (hypoglycemic activity), and anticancer properties. Inhibition of cardiomyocyte hypertrophy and thrombosis are only a few of the therapeutic benefits they show in the prevention and treatment of different cardiovascular illnesses. They also have therapeutic effects on vascular function. Betulinic acid and its semi-synthetic derivatives, such PA-457, have also shown extraordinary pharmacological capabilities, including as inhibitory actions against the human immunodeficiency virus (HIV) and cytotoxicity activity on several types of cancer cells. Very intriguingly, betulinic acid's biotransformation has been regularly studied with the goal of finding new derivatives for pharmacological investigations.

Due to the important functions that pharmaceutical terpenoid manufacturing plays in the prevention and treatment of many illnesses, interest in this field has grown in recent years. The demand for these items develops as the mortality rate of the globe increases as a result of the prevalence of serious illnesses like cancer and malaria, necessitating the rapid development of sustainable alternatives to natural and chemical sources. In contrast to conventional approaches, microbial fermentation of these unique chemicals promises to be sustainable, affordable, and high yield. For the purpose of mass terpenoid synthesis, *S. cerevisiae* has been shown to be an effective cell factory. Success examples include the industrial manufacture of artemisinic acid and -farnesene.

As *S. cerevisiae* cannot natively manufacture the target terpenoids, process optimization via the screening of various fermentation techniques is crucial to improving TYP of medicinal terpenoids. Only with the effective integration of these two disciplines, metabolic engineering and fermentation process development, is commercial terpenoid use viable. Improvements to medium

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composition, physicochemical conditions, and the use of effective downstream processing may boost productivity and lower production costs. The majority of terpenoid titers measured, mostly in batch fermentations, are in mg/L ranges and have minimal economic significance. The most promising method for the commercial manufacture of pharmaceutical terpenoids is bioreactor fermentation with fed-batch operating mode, which may produce titers at g/L scale, high productivities, while reducing the toxicity of the end product. The pH and DO are important elements for certain production scenarios when considering factors impacting terpenoids fermentation. Although a pH range of 4 to 6 is ideal for yeast development and achieving high cell density, certain terpenes may not be soluble in this environment, which might make the subsequent processing more difficult. However, this may be resolved, for example, by adding base solution to harvesting tanks with high pH levels.

To lessen the hazardous effects of terpenes during cultivation, extraction agents may be used, however this adds to the process's expenses. By improving the feed strategy and product removal in fed-batch fermentation, this issue may be solved. For the purpose of controlling yeast growth and product generation in fed-batch processes, the DO is often utilised as a trigger. Due to the fact that low levels may reduce yeast productivity and excessive levels can cause oxidative stress, which can be hazardous to cells, optimal oxygen concentrations are necessary to maintain yeast health and productivity. Similar to how low temperatures lead to higher cell densities, high temperatures may reduce cell viability. The media components must be optimised as well since they might contribute significantly to the process expenses. Finding and using inexpensive carbon sources, like as agricultural byproducts, in *S. cerevisiae* metabolism, as widely studied in case of the synthesis of other biomolecules, which also adds to process sustainability, might be one option to reduce feedstock costs. In fact, there is a tendency toward more medicinal terpenoids being produced via improved fermentation. New potential to create complex terpenes, such as the in-demand product "cannabinoids," will be made possible by the strength of synthetic biology techniques and advancements in metabolic engineering methodologies for introducing novel terpenoid biosynthesis pathways in *S. cerevisiae*. When a rising industrial demand for these products is anticipated, the microbial fermentation of these very promising and uncommon chemicals at a low cost and high purity, independent from Cannabis plant growth, might unquestionably boost the pharmaceutical sector.

Opportunities and Emerging Trends in Food Fermentation

By using its primary recyclers, microorganisms, which have evolved a biological apparatus with a variety of enzymes like proteases, amylases, and lipases, amongst many others, having the capability of hydrolyzing organic matter and ultimately trying to convert it into its components, fermentation is one of nature's processes for recycling organic matter. As they saw their stored food change into items with distinct but desired organoleptic qualities, such fruits or grains turning into alcoholic beverages, ancient people may have accidently discovered fermentation. Unfortunately, it wasn't until the beginning of the nineteenth century that microbes were identified as the agents of fermentation. As a result, fermentation was extended outside the context of food processing and into a considerably larger range of biotechnological applications, resulting in scientific triumphs like the creation of a fermentation method for the synthesis of antibiotics.

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Today, fermentation is employed in many different processes, including those that create enzymes, industrial chemicals, medicines, cosmetic components, flavorings and artificial sweeteners for use in food.

Food processing and preservation using fermentation

The stability and modification of food ingredients are accomplished through the food preservation or processing technique known as fermentation. The environment produced by the metabolites produced during fermentation supports the development of some organisms while hindering that of others. Fermentation of food slows the growth of harmful and spoilage organisms, lengthening the life span of perishable agricultural goods. For example, during the fermentation of lactic acid, lactic acid bacteria produce a wide range of primary and secondary metabolites, such as organic acids like lactic acid and ethanol, ethanol, hydrogen peroxide, carbon dioxide, diacetyl or antifungal compounds like phenyllactic acid, antimicrobial peptides like bacteriocins, and antimicrobial agents like reutricyclin, which together create a milieu that suppresses pathogenic and spoilage microorganism. Figure below shows the as a crucial method for bio refining or adding value to food waste but also inferior products, fermentation.

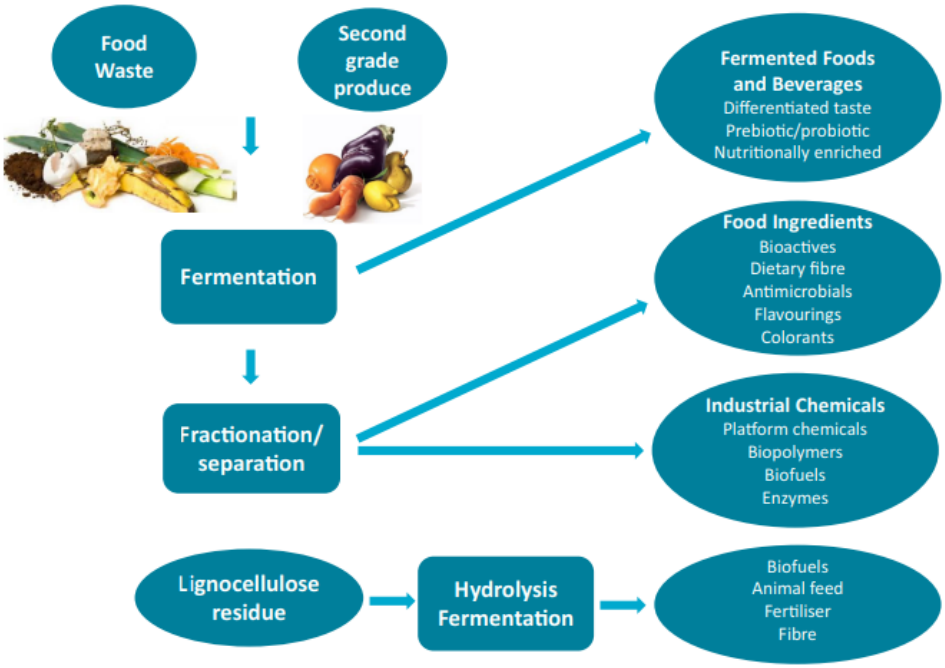

Figure 9.1: Applications of food fermentation.

Authentic Fermented Foods

Early human civilizations invented fermentation as a way to preserve perishable agricultural goods including milk, vegetables and meat. However, a wide range of fermented foods and drinks were developed all over the world as technology advanced throughout time as a method of imparting specific organoleptic and functional properties in meals. These include alcoholic beverages such as beer and wine, dairy products such as yogurt, cheese, or kefir, grain products such as bread or

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kvass (Russia), animal products such as sausages, but rather plant-based products such as kimchi, kombucha, and sauerkraut. A list of some traditional fermented meals.

Brand-new fermented foods

People in the poor countries in the Far East consume a sizable amount of their diet in fermented foods. This is no longer the case in Western industrialized countries, possibly as a result of the year-round availability and affordability of fresh produce thanks to the fast food industry's transportation and distribution network as well as access to contemporary food preservation technology. As a result, with the exception of a few items like bread, cheese, yogurt, and sausages, which are manufactured on an industrial scale in addition to by artisanal makers, the majority of traditional fermented goods in these nations have largely faded to the background with industrialization. Nevertheless, several ancient fermented foods have recently seen a kind of resurgence, mostly because of their alleged health advantages. Traditional fermented packaged foods are gaining popularity with consumers and the food industry alike due to their perceived naturalness as well as health benefits, as well as the rising prevalence of lactose but also vegetarianism, gluten intolerance, and veganism as well as the movement toward sustainable processing.

The resurgence of interest in fermented foods is expected to last into the future, propelled by rising rates of metabolic syndromes like obesity and high blood cholesterol, a variety of food intolerances and intolerances (gluten intolerance, lactose intolerance, etc.), lifestyle references like vegetarianism and veganism, and rising consumer interest in everything perceived to be healthy. The push for zero-waste utilization of biological resources will only intensify in the future due to global megatrends like the world's population growth, rising affluence and consumption in developing nations, depletion of fossil fuel reserves, global warming, and their potential effects on global food security. Growth will play a significant role in the transition from the petroleum-based economy of today to the bio-based economy of tomorrow because it takes into account both the reduction of food waste and the conversion of undeniable waste into value added food ingredients, biofuels, but also modern synthetics.

Application and Future Prospects of Fermentation Technology

Since ancient times, fermentation has been employed for a variety of purposes. People have made cheese and wine using the advantages of fermentation since 7000 BC. Luis Pasteur made the original discovery that fermentation is caused by living things in 1857. The majority of people are aware that fermentation produces alcohol. This suggests that alcoholic beverages like beer and wine are made from grains and fruits through the fermentation process. Fruits that have soured or spoiled are referred regarded as being "off" or fermented by humans. Fruits and milk are used to make alcoholic beverages and dairy goods. It comes from fermentation.

Fermentation of alcoholic beverages

The brewing industry is well aware of the significance of fermentation in the creation of alcohol. Alcohol or carbon dioxide is created during the fermentation of sugar by yeast enzymes. The fermentation of yeast or sugar from foods like grapes, rice, grains, and berries results in the

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production of several alcoholic drinks, including beer and wine. This fermentation is carried out by the yeast Saccharomyces cerevisiae. Two bacteria ferment milk to create milk in the dairy industry's yogurt manufacturing. Streptococcus lactis and Lactobacillus bulgaricus or Lactobacillus acidophilus are these microorganisms. These bacteria absorb lactose from the milk, condense it, and form the lactic acid and acetaldehyde compounds that give yogurt its distinctive flavor. When a tiny amount of sugar or yeast is added to the batter for the bread, the sugar ferments, releasing carbon dioxide gas. This process is carried out by yeast enzymes. The bread's texture and volume are both improved by the carbon dioxide. The fermentation process uses a variety of microbes to give the food items the required taste. Rye bread is produced by fermentation assisted by Lactobacillus delbruckii.

In producing Vitamin C

Vitamin C or Ascorbic acid is a very essential nutrient for humans. A human can't synthesize nutrients by himself. Vitamin C is produced in two steps by the fermentation process. D-sorbitol is converted to L- sorbose and L-sorbose are converted to L-ascorbic acid (Vitamin C). This fermentation process is mediated by Gluconobacter oxydans. This is the reason why is fermentation important in producing vitamin C.

In Fuel production

You know, fermentation mainly produces ethyl alcohol or ethanol. This ethanol is used to produce Gasohol. Gasohol is a combination of gasoline and alcohol. Gasohol is used as fuel for cars in the United States. It reduces more air pollution than gasoline. Methane is another fuel that is produced by fermentation through the action of Methonosarcina and Methanothrix. These strains convert acetate or formate to methane.

In wastewater treatment

When it comes to the treatment of wastewater, the fermentation process is crucial. Through the fermentation process, aerobic microorganisms in the activated sludge process break down the organic elements in wastewater. Solid wastes or organic materials are converted into carbon dioxide, water, and mineral salts during the fermentation process. Food preservation: When food is fermented, beneficial bacteria are encouraged to flourish while bad bacteria are suppressed. Food deterioration is therefore prevented by good microorganisms. Acid and alcohol both work well to stop spoiling by preventing the development of germs. For instance, the acids in vegetables and fruits prevent food from becoming bad.

When making biopolymers

Microorganisms use the fermentation process to make biopolymers. Gelatin, cellulose, starch, chitin, collagen, polyvinyl acetate, or other biopolymers are among them. Biopolymers are used in the food sector, water purification, packaging, production of nanomaterials, and biomedical applications. The microbes Rhodobacter sphaeroides, Bacillus subtilis, Gluconacetobacter xylinus, Xanthomonas, etc. mediate fermentation to generate biopolymers. In medicine, fermentation is used to create several antibiotics and other medications. For instance, a fermentation process turns the chemical diosgenin into the medicine cortisone (A plant steroid). An enzyme from the mold

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Rhizopus nigricans aided in the fermentation process. Glycerol is a pharmaceutical substance that is made from molasses utilizing fermentation and Saccharomyces cerevisiae.

Health Benefit

Fermentation enhances digestion by transforming nutrients into digestible forms. Soybean protein is plentiful and indigestible without fermentation. This bean becomes easily ingestible amino acids by fermentation by lactobacilli. Many people find lactose, the milk sugar, uncomfortable. Lactic acid is produced by the fermentation of lactose in milk by the bacteria Bifidobacterium lactis.

Future Prospects

Fermentation technology is frequently used in the food sector to process microbial enzymes. However, the development of this kind of enzyme is essential for progress in the future. Numerous innovative industrial or analytical applications have lately been created for the production of new commodities. The development and advancement of fermentation technology are necessary for the food and beverage industries. Its objective is to achieve larger yield or production quantities with the use of cutting-edge models, bacterial strains, construction procedures, and process monitoring. They have developed some cutting-edge ideas in these domains that might serve as examples of elegant, affordable solutions. In SSF, where advanced instrumentation and sensor development are lauded, process monitoring is essential. Modern technology has been described thus far using a variety of sensors, including respirometry, magnetic resonance imaging, x-rays, and infrared spectroscopy. This method is inappropriate for large-scale applications due to its main disadvantage and high cost. One of the greatest bioreactor designs for the growth of powerful photo-bioreactors or phytocultures is the production of algae and micro but also macroalgae-derived food. Another possible strategy to increase metabolic productivity in bioprocesses is the use of carefully regulated ultrasonication. It is possible to take advantage of the beneficial effects of ultrasound on sono-bioreactor performance (mass transfer enhancement), and their function, such as stimulated sterol synthesis, cross-membrane ion fluxes, altered cell morphology, as well as increased enzyme activity or biocatalysts cells and enzymes. Its potential for genetic engineering in the area of food fermentation is undeniable. Food fermentation has enhanced nutritional status by using a balanced selection of food-fermenting microorganisms based on knowledge of their diet but also human gastrointestinal microbiota. In this perspective, food fermentation has been viewed as a continuation of food digestion as well as being healthy.

Leaders in the biotechnology industry refer to fermentation. As consumers continue to search for dairy and protein alternatives, more biotech companies are using fermentation to produce alternative proteins. Lactic acid is produced when lactose is converted, which preserves milk. Cultured milk's low pH prevents putrefactive bacteria and other harmful organisms from growing, extending the product's shelf life. On the other side, acidified milk creates a particularly favourable habitat for yeasts and moulds, which if allowed to infect the goods, result in bad flavours, blown packaging, etc. Some people's digestive tracts are deficient in the lactase enzyme. As a consequence, lactose is not converted during digestion into less complex carbohydrates. These folks are only able to eat extremely little amounts of regular milk. However, they are able to ingest fermented milk, in which bacterial enzymes have already partially broken down the lactose.

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The starting culture has to have the ideal growing conditions in order to produce fermented milk. These are accomplished by heating the milk to kill any rival microbes. Additionally, the milk has to be maintained at the temperature that the appropriate starting culture prefers. To terminate the fermentation process, the cultured milk must be promptly chilled when the best flavour and fragrance have been established. The flavour will suffer and the consistency will be off if the fermentation period is too lengthy or too short. Along with flavour and scent, accuracy of appearance and consistency are crucial characteristics. The pre-processing factors used affect them. The "foundation stones" for the creation of the coagulum during the incubation phase are adequate heat treatment or homogenization of the milk, sometimes in combination with techniques to raise the MSNF concentration, as for milk intended for yoghurt. The following is a description of some of the most significant fermented milk products. Numerous other fermented goods are produced using similar methods; milk is pre-treated similarly, for instance. Therefore, the process explanations for other products focus mostly on the phases of manufacturing that are different from those in yoghurt manufacture. You probably already consume fermented foods, particularly if you prefer aged cheese, yoghurt, and wine, to mention a few. In essence, fermentation is a method of food preservation that dates back to ancient times. As a result, fermented foods retain all of their nutrients and don't expire quickly. These kinds of foods often have powerful, pungent aromas. Fermented foods do have certain health advantages, but they also have some drawbacks.

Intestinal bacterial equilibrium is improved as a result

Probiotics are mostly found in fermented milk, yoghurt, and other foods that have experienced the fermentation process. They are a kind of bacteria that create lactic acid. The Journal of Applied Microbiology's June 2006 edition reported that eating foods containing lactic acid bacteria may enhance the health of digestive tracts. Consuming such foods might help lessen the symptoms of lactose intolerance, increase the bioavailability of minerals, and decrease the prevalence of allergy in those who are vulnerable.

It makes the heart healthier

The risk of coronary heart disease is linked to dairy intake. However, certain milk products that have undergone fermentation are regarded as heart-healthy. According to the February 2006 edition of Current Opinion in Lipidology, there is proof that very high blood pressure (also known as hypertension) may be moderately decreased by consumption of fermented milk products.

The immune system is strengthened

Consuming foods that have undergone fermentation helps strengthen the gut and reduce the risk of intestinal diseases. The immune system may also be strengthened by consuming fermented foods, as is the case with the acidic beverage kefir, which is produced by fermenting milk with grains. Although kefir is simple to digest, it also helps to colonization the intestines with bacteria that support a strong immune system. According to the United Nations University, kefir has been used in the treatment of cancer and TB.

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Questionnaire

Important Questions for Practices:

1) Why do fermentative pathways exist in the first place?
2) What is fermentation's most significant byproduct?
3) What drives the production of fermentation products by bacteria?
4) What kind of technology does fermentation employ?
5) Describe aerobic and anaerobic fermentation.
6) How does fermentation generate wine?
7) What elements influence the fermentation of ethanol?
8) What purposes does ethanol serve in fermentation?
9) What takes place in ethanol after fermentation?
10) Which kind of fermentation takes place in ethanol?

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Recommended Books for Further Reading/Reference Books:

[1] Computer Applications in Fermentation Technology: Modelling and Control of Biotechnological Processes" 4th Edition by N M Fish.

[2] Fermentation Technologies: Industrial applications" 2nd Edition by P L Yu.

[3] Microorganisms and Fermentation of Traditional Foods (Food Biology Series)" 4th Edition by Ramesh C Ray and Montet Didier.

[4] Principles of Fermentation Technology" 7th Edition by P F Stanbury Dr A Whitaker.

[5] The Art of Fermentation: An In-depth Exploration of Essential Concepts and Processes from Around the World" 1st Edition by Sandor Ellix Katz.

[6] Practical Manual on Fermentation Technology" 1st Edition by S Kulandaivelu and S Janarthanan.

[7] Fermentation and Enzyme Technology" by 2nd Edition Wang D I C.

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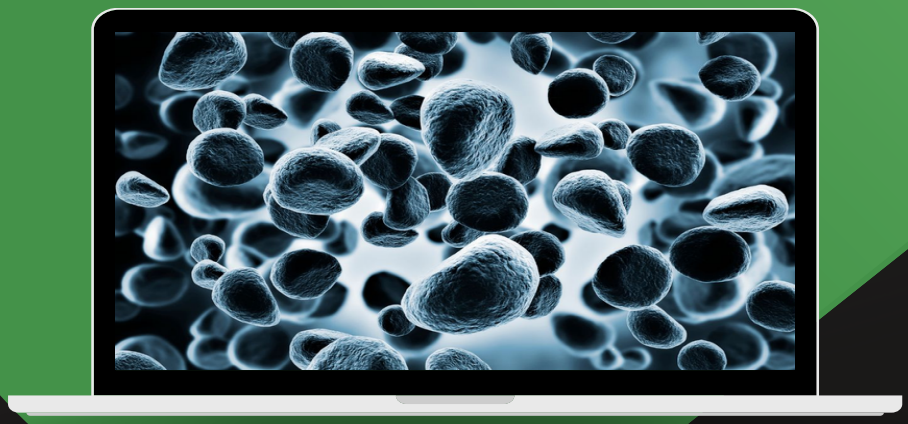

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