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DEPARTMENT OF PG MICROBIOLOGY

STUDY MATERIAL
SEMESTER-IV
MBY-401: FERMENTATION TECHNOLOGY AND INDUSTRIAL MICROBIOLOGY

MBY-401: FERMENTATION TECHNOLOGY AND INDUSTRIAL MICROBIOLOGY

TYPES OF FERMENTATION

FERMENTATION :

Fermentation is a metabolic process where microorganisms like yeasts, molds or bacteria produce chemical changes in an organic substrate. There are various types of fermentation including batch, continuous, fed-batch,anaerobic, aerobic, surface, submerged and solid state fermentation.

INTRODUCTION :

Fermentation is a biological process that converts biomolecules into alcohol, lactic acid and acetic acid with the help of various microorganisms under anaerobic conditions. Although "fermentation" refers to anaerobic metabolism.Bacteria, yeast cells, and animal muscles all undergo fermentation.

The word "ferment" originates in the Latin word fervere, which means "to boil,". Alchemists of the late 14th century spoke of fermentation, although not in the contemporary sense. The scientific study of fermentation's chemical process began around 1600. Louis Pasteur became the first zymurgist or scientist to research fermentation in the 1850s and 1860s after proving that livecells brought on fermentation.

Louis Pasteur, a French chemist and microbiologist, recognised that ethyl alcohol and carbon dioxide were not the only products of fermentation in the nineteenth century and used the term fermentation strictly to characterise the changes caused by yeasts and other microbes developing anaerobically (withoutoxygen).

EXAMPLES OF FERMENTATION :

A large number of industrial products are created from the fermentationprocess. These products include:
Wine
Beer
Cheese
Yogurt
Bread leavening by yeast
Sour foods that contain lactic acid, which include kimchi, pepperoni, andsauerkraut
Hydrogen gas Sewage
treatment
Industrial alcohol production for bio-fuels and similar solutions

Fermentation is an essential process. Without it, wine, beer, and other products would be difficult or impossible to make. In fact, the sole way to produce beer iswith the fermentation process since it's currently the only method that's able toconvert sugar into alcohol.

TYPES OF FERMENTATION PROCESS

There are three different process of fermentation

1.Batch Fermentation

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2.Feb-Batch Fermentation
3.Continuous Fermentation

1. Batch Fermentation

In batch fermentation, all the components are mixed at once then the reaction undergoes without any further intake from outside. During the whole process, no extra nutrients are added. It is a closed system because all the components are added at once and no other components are added in between the processof fermentation.

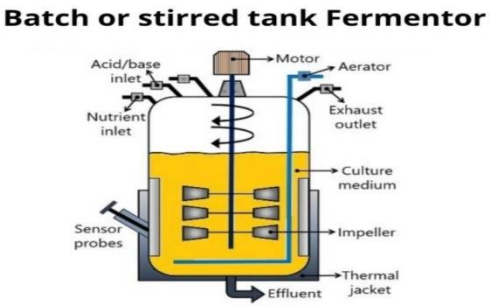
There are three phases in the batch fermentation process - lag phase, exponential phase, and stationary phase.

- **lag phase**

In the lag phase, microbes adapt to the environment of the culture

- **exponential phase**

In the exponential phase, the microbial cells grow rapidly and consume mostof the nutrients and the last phase is

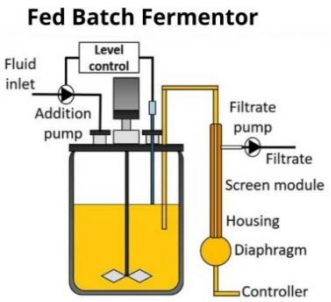


- **stationary phase**

The stationary phase is when the growth of microbes stops due to theconsumption of all nutrients. It is the simplest type of all industrial fermentation.

Fed-Batch Fermentation

- It is a modification of batch fermentation
- In this nutrition is added aseptically and the amount of liquid culture in the bioreactor increases as the culture is added systematically.
- It is a type of semi-open system.
- It yields a better result than batch fermentation.
- After consumption of early substrate continuous and constant nutrition isadded.



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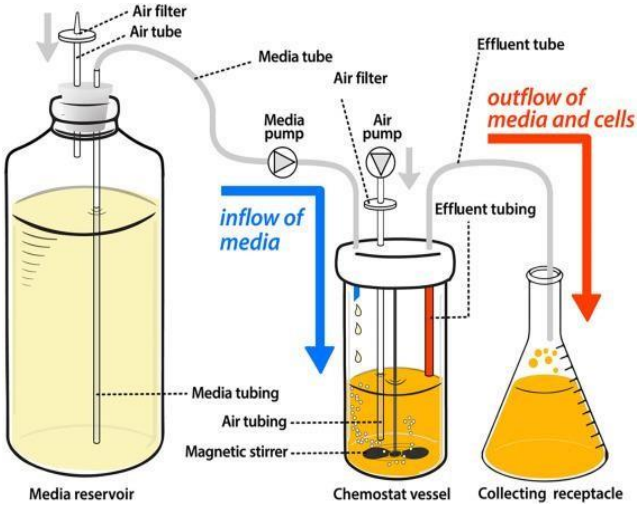
2. Continuous Fermentation

- It is a type of fermentation in which constant addition and flow of solutionoccur.
- Microorganisms and sterile nutrients are added continuously and thenutrient solutions and microbes are transformed simultaneously.
- It is a type of open fermentation system in which comments can be addedand removed in between the process.
- There are many methods of continuous fermentation.



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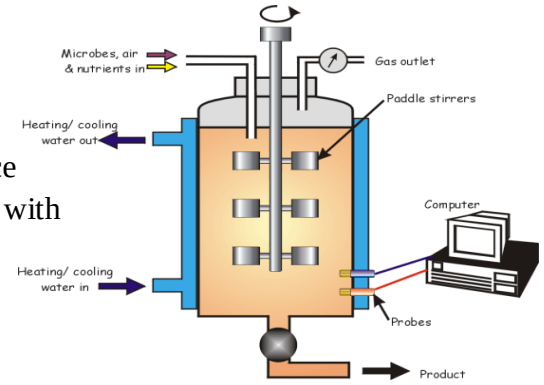
Fermenter:



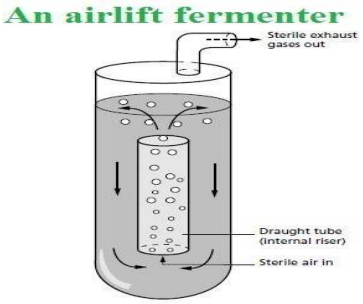
Fermenters, also known as bioreactors, are sterilized and enclosed vessels that areused for the growth of microorganisms under optimal conditions. The microorganisms can be grown in large quantities to produce metabolites for commercial uses. Fermenters are equipped with special components for heating, mixing, and aeration. Its volume can be as big as 500,000 litres for an industrial scale, or as small as 1 litre for laboratory uses.

Different Types of Fermenters

Continuous Stirred-Tank Fermenter

The continuous stirred-tank reactor (CSTR) is composed of a vessel with pipes, pumps, valves, agitator, motor, shaft, and impeller(s). The shaft is situated at thebottom of the tank, and the number of impellers depends on the size of the bioreactor.

In this type of fermenter, a structure called sparger is found that keeps adding air tothe culture medium. It is a ring-like structure with many holes. The sparger, along with the impellers, distribute gas in the entire vessel. The impellers break down thebubbles into smaller ones that are homogeneously distributed in the bioreactor.



Airlift Fermenter

The airlift bioreactors contain a baffle or a draft tube in the middle through whichair is pumped into the vessel. There are two types of airlift fermenters:

- **Internal loop airlift bioreactor:** It has a single central draft tube thatprovides inner circulation channels.
- **External loop airlift bioreactor:** It contains external loops that separate theliquids flowing into independent channels.

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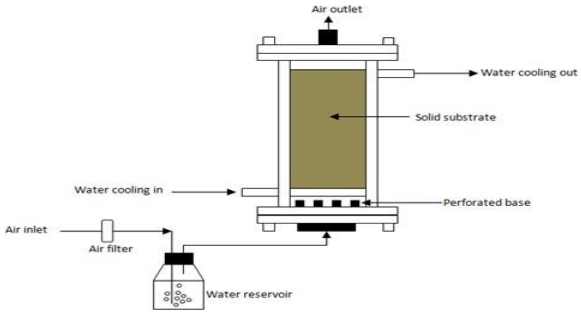
Packed Bed Fermenter

In a packed bed fermenter, a hollow tube or pipe is packed with a biocatalyst. Thebed is immobile in nature. The culture medium flows through the biocatalyst, which produces the metabolites continuously in the broth. These bioreactors are easy to operate but are often blocked due to poor oxygen circulation.

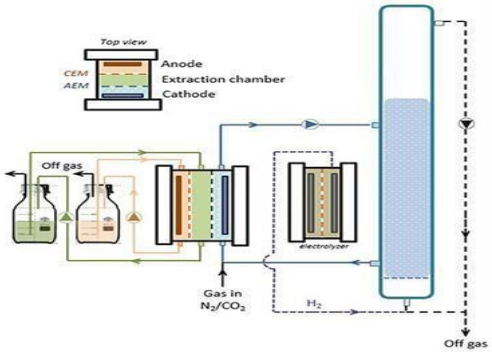


Fluidised Bed Fermenter

In this type of reactor, a solid granular bed that is usually made up of a biocatalyst is present. The fluid, that is, liquid or gas, is passed through the solid bed at high speeds, such that the suspended solid behaves like a fluid. This type of fermenter isused for microbial flocs, immobilized cells, and enzymes.

Membrane Fermenter

Membrane bioreactors work in conjugation with ultrafiltration and microfiltration. This type of fermenter is used for the biological treatment of wastewater. There are two types of membrane bioreactors:



- **Submerged membrane bioreactor:** In this type of fermenter, themembrane is found inside the vessel submerged in the wastewater.

- **Side-stream membrane bioreactor:** In this type of fermenter, the membrane is found outside the reactor and filtration by the membrane is anadditional step in the whole process.

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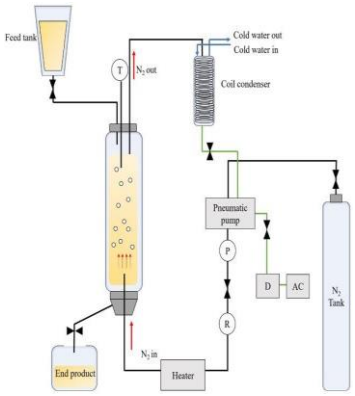
Bubble Column Fermenter

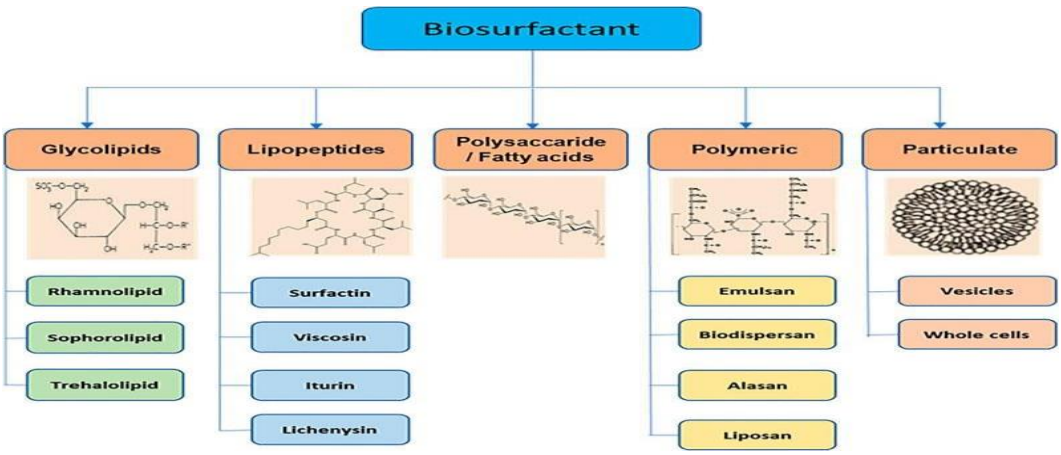
A bubble column fermenter is equipped with a cylindrical column that is filled with liquid, and gas is inserted into it from the bottom. It is vertically arranged, such that the introduction of gas from the bottom creates a turbulent stream and allows optimum gas exchange. The sparger mixes the contents of the vessel. Theliquid flows either in a parallel direction or in a counter-current direction.



Bio surfactants:

Bio surfactants are active compounds that are produced at the microbial cell surfaceor excreted, and reduce surface and interfacial tension. Microbial surfactants offer several advantages over synthetic ones, such as low toxicity and high biodegradability, and remain active at extreme pH and salinity.

Glycolipids are the most common type of biosurfactants. Some of the common glycolipid biosurfactants such as rhamnolipids, trehalolipids, sophorolipids, and mannosylerythritol lipids (MELs) contain mono and disaccharides combined withlong-chain aliphatic acids or hydroxy-aliphatic acids



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Media And Materials Required For Fermentation:

What is Fermentation?

The metabolic process that induces chemical changes in organic substrates by the action of enzymes is known as fermentation. It uses microorganisms such as bacteria, algae and fungi. The use of this process on a large scale to produce pharmaceuticals, enzymes and proteins is known as industrial fermentation.

Media Requirements:

To obtain a good product from fermentation, the medium in which the microorganisms are grown must be supplied with enough energy sources and nutrients. Several factors must be kept in mind before designing or choosing the growth medium for fermentation. To obtain primary metabolites such as citric acid and ethanol, the media should be rich in components that support good growth. Similarly, for secondary metabolites such as alkaloids and antibiotics, the substrate requirement for product formation must be kept in mind.

While doing fermentation on a small scale, such as in laboratories, pure graded chemicals are used that are expensive. However, in large scale industrial fermentation, cheaper and unrefined chemicals are used. Therefore, the choice of media for fermentation is a crucial step that requires a lot of thought processes.

Media Components:

The fermentation media can either be liquid, known as broth, or it can be a solid-state fermentation. The media should satisfy all the nutritional requirements of the microorganism and should also obtain the target molecule. A typical media requires a carbon source, a nitrogen source, salts, water and micronutrients**.**

1. Carbon Source:

- Typically sugars and carbohydrates are used as carbon sources, but alcohols may also be used in making products such as vinegar. For laboratory uses, refined and pure carbon sources such as glucose, sucrose and glycerol are used that give a uniform product. However, in the case of industrial fermentation, inexpensive sources such as whey, malt extract, molasses, corn steep liquor or sugar cane juice are used.

2. **Nitrogen Source:**

- The nitrogen source for microorganisms may be used in the form of organic or inorganic compounds. Inorganic sources include ammonium salts or the free form of ammonia. Inexpensive nitrogen sources are used for bulk production, such as tryptone, peptone, soy meal, corn steep liquor and yeast extract.

3. **Growth Factors:**

- Trace salts and growth factors are important components in the fermentation media. Yeast extract is a good source of vitamins and macronutrients. Trace elements such as copper, zinc, iron, cobalt, molybdenum, manganese are all usually found in the unrefined nitrogen sources but may need to be added when using pure sources.

4. **Miscellaneous:**

- The process of fermentation sometimes produces a large amount of gas that forms a layer of foam and hinders the process. To get rid of this problem, antifoaming agents are also added to the fermentation medium. To stabilise pH of the media, mineral buffering salts such as phosphates and carbonates are also added. The addition of chelating agents may also be required when high concentrations of metals are present in the media.

Materials :

The materials required for fermentation depend largely on the specific type of fermentation you are conducting (e.g., alcoholic fermentation for making beer or wine, lactic acid fermentation for making yogurt or sauerkraut). However, here's a generalized list of materials commonly used in fermentation processes:

1. **Microorganisms:**

- Yeast, bacteria, or other microorganisms responsible for the fermentation process. Examples include Saccharomyces cerevisiae for alcoholic fermentation and Lactobacillus species for lactic acid fermentation.

2. **Substrate:**

- The source of fermentable sugars or carbohydrates that the microorganisms will metabolize. This could be:
- **For Alcoholic Fermentation:** Grains (e.g., barley for beer, grapes for wine), fruits, or other sources of sugars (e.g., molasses, honey).

- **For Lactic Acid Fermentation:** Milk (for yogurt), cabbage (for sauerkraut), or other vegetables and fruits.

4. Water:

- Pure water suitable for the fermentation process. Water quality is important as it can affect the outcome of fermentation.

5. Nutrients:

- Depending on the specific fermentation process and the microorganisms involved, additional nutrients may be required. These can include nitrogen sources (e.g., yeast extract, ammonium salts) and vitamins necessary for microbial growth and metabolism.

6. pH Regulators:

- Some fermentations require specific pH conditions for optimal microbial activity. pH regulators like acids or bases may be used to adjust and maintain the pH within the desired range.

7. Additives:

- Depending on the desired product, additives such as hops (for beer), herbs, spices, or flavorings may be added to enhance the flavor and aroma of the final product.

8. Equipment:

- Various equipment is used depending on the scale and type of fermentation. Common equipment includes:
- Fermentation vessels (e.g., fermenters, carboys, barrels)
- Airlocks or blow-off tubes to allow gases to escape while preventing contamination
- Stirring rods or paddles for mixing ingredients
- Thermometers and hydrometers for monitoring temperature and specific gravity (density of the liquid relative to water, used to measure fermentation progress)

9. Sanitizers:

- Cleaning and sanitizing agents are crucial to maintain a sterile environment and prevent contamination by unwanted microorganisms. Common sanitizers include iodophor, bleach, or other food-safe sanitizing solutions.

10. Containers for Storage:

- After fermentation is complete, containers for storage such as bottles, kegs, or jars are needed to store the finished product.

11. Sterilization Equipment:

- For larger-scale fermentations or when dealing with sensitive microorganisms, sterilization equipment like autoclaves or pressure cookers may be necessary to sterilize equipment and ensure a clean environment.

Innoculum Development

- Inoculum development is a critical process in fermentation, where a small amount of actively growing microorganisms (inoculum) is prepared to initiate and propagate the fermentation process efficiently. The goal is to ensure that the inoculum contains a high concentration of viable and healthy microorganisms capable of rapidly fermenting the substrate. Here are the key steps and considerations involved in inoculum development:

Steps in Inoculum Development:

1. **Selection of Microorganisms:**Choose the **appropriate** strain(s) of microorganisms based on the desired fermentation process and characteristics of the final product. This could be yeast strains for alcoholic fermentation (e.g., Saccharomyces cerevisiae for beer or wine) or specific bacterial strains for lactic acid fermentation (e.g., Lactobacillus species for yogurt or sauerkraut).
2. **Propagation:**Start with a small amount of pure culture (starter culture) obtained from a reputable source or previously successful batches. Propagate the culture under controlled conditions to increase its biomass. This can involve sequential transfers into increasingly larger volumes of growth media to scale up the culture.
3. **Inoculum Preparation:**Once the culture has reached an appropriate biomass level (often determined by optical density or cell count), prepare the inoculum for fermentation. This typically involves centrifugation or filtration to concentrate the cells and remove spent media or other impurities.
4. **Viability and Activity Assessment**:Assess the viability and activity of the inoculum to ensure it is suitable for fermentation. This can be done through viability staining, plate counting, or other methods to determine cell count and metabolic activity.

5. **Inoculation Strategy**:Determine the appropriate inoculation rate based on the fermentation conditions and desired outcomes. Factors such as substrate composition, fermentation vessel size, and fermentation duration influence the optimal inoculation rate.
6. **Adaptation**:In some cases, particularly with wild or non-standard strains, the inoculum may need to be adapted to the specific fermentation conditions (e.g., pH, temperature, substrate availability) to ensure robust fermentation performance.
7. **Inoculum Storage**:If not immediately used, store the inoculum under appropriate conditions (e.g., refrigeration or freezing with cryoprotectants) to maintain viability until it is needed for fermentation.

Considerations For Innoculum Development:

- **Sterility:** Maintain sterile conditions throughout the inoculum development process to prevent contamination and ensure the purity of the culture.
- **Quality Control:** Regularly monitor and test the inoculum for purity, viability, and activity to maintain consistent fermentation performance.
- **Scale-Up Considerations:** Ensure that the inoculum development process can be scaled up as needed to match the size of the fermentation batch or production scale.
- **Strain Characterization:** Understand the specific characteristics and requirements of the chosen microorganism(s) to optimize growth and fermentation efficiency.
- **Environmental Conditions:** Control environmental factors such as temperature, pH, oxygen availability, and nutrient levels to support optimal growth and metabolism of the inoculum.

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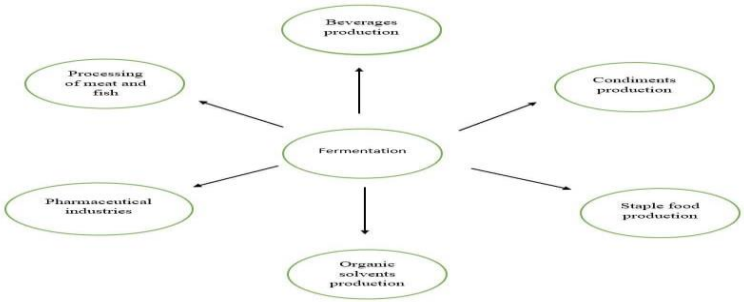
COMPUTER APPLICATIONS OF FERMENTATIONTECHNOLOGY

The document discusses the use of computer applications in fermentation processes. It describes three main functions: data logging to store sensor data, data analysis to clean and analyze stored data, and process control to send signals to pumps and valves based on sensor readings.

Computers are now used for logging process data, analyzing the data, and controlling fermentation processes. Sensors are used to monitor important factors like temperature,pH, dissolved oxygen, and mineral/nutrient levels to provide data inputs for computer control and modeling of fermentation.



FNet is a simple-to-use software for monitoring fermentation and cell culture processes with the MINIFOR laboratory fermentor and bioreactor.

Fermentation is a natural process through which microorganisms like yeast and bacteria convert carbs — such as starch and sugar — into alcohol or acids. The alcohol or acids act as a natural preservative and give fermented foods a distinct zest and tartness.

Fermentation is an anaerobic biochemical process. In fermentation, the first process is the same as cellular respiration, which is the formation of pyruvic acid by glycolysis where net 2 ATP molecules are synthesised. In the next step, pyruvate is reduced to lactic acid, ethanol or other products

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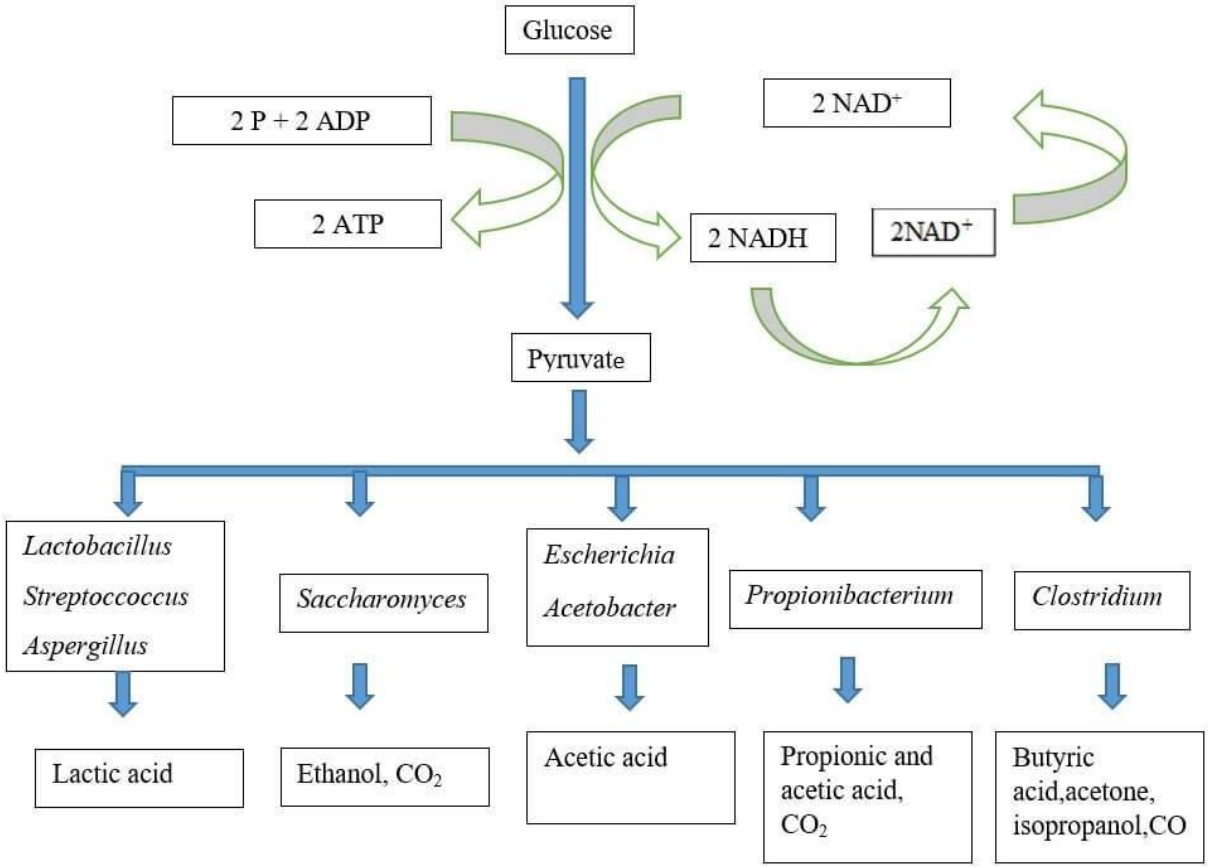

Fermentation technology has wide application for the production of products such as organic solvents (acetone, alcohols), fermented beverages (wine, beer, whisky), and other products like enzymes, amino acids, vitamins, pharmaceuticals etc.

Fermentation is based on the principle of Anaerobic respiration for deriving energy from the breakdown of carbohydrates such as glucose. In this process, glucose is first broken to pyruvate by glycolysis. The pyruvate is then converted to alcohol or lactic acid along with the regeneration of NAD.
Based on the mode of cultivating the microorganisms, fermentation can be classified as batch, continuous, or fed-batch.

Louis Pasteur was a French chemist and microbiologist celebrated for his research in vaccinations, pasteurization, and fermentation. His explorations led to extraordinary discoveries in the awareness of the causes and prevention of disease, fermentation, andgerm theory. Reading time: 4 min.

The major products of fermentation technology produced economically on a large scale industrial basis are wine, beer, cider, vinegar, ethanol, cheese, hormones, antibiotics, complete proteins, enzymes and other useful products.

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Upstream processing

The cultured microorganism. This involves the procedure for selecting a suitable microorganism. Strain improvement to enhance the yield & productivity. Maintenance ofstrain sterility. Preparation of suitable inoculum.

The fermentation medium.

Fermentation begins with the inoculation of the growth medium using the desired microorganism. During the lag phase or incubation phase, the microorganisms adapt to their new environment. Cell growth at this point is still slow. Then begins the exponentialgrowth phase in which the growth rate continuously rises.
Fermentation technology is a field which involves the use of microorganisms and enzymesfor production of compounds that have applications in the energy, material, pharmaceutical, chemical and food industries.

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PATETING:

The word patent originates from the Latin patere, which means "to lay open"(i.e., to make available for public inspection). It is a shortened version of the term letters patent, which was an open document or instrument issued by a monarch or government granting exclusive rights to a person, predating the modern patent system. Similar grants included land patents, which were landgrants by early state governments in the US, and printing patents, a precursorof modern copyright.

In modern usage, the term patent usually refers to the right granted to anyonewho invents something new, useful and non-obvious. A patent is often referred to as a form of intellectual property right, an expression which is alsoused to refer to trademarks and copyrights, and which has proponents and detractors see also Intellectual property § The term "intellectual property"

Some other types of intellectual property rights are also called patents in some jurisdictions: industrial design rights are called design patents in theUS, plant breeders' rights are sometimes called plant patents, and utility models and Gebrauchs muster are sometimes called petty
patents or innovation patents. The additional qualification utility patent is sometimes used (primarily in the US) to distinguish the primary meaning from these other types of patents.

Particular types of patents for inventions include biological patents, business method patents, chemical patents and software patents.

Patentability also depends on public policy and ethical standards. Additionally, patentable materials must be novel, useful, and a non-obvious inventive step. A patent is requested by filing a written application at the relevant patent office.

Patents require

a) a series of logical statements clearly delineating the boundaries of the novel aspects of the invention.
b) sufficient disclosure of the invention so that it can be reproduced by others. Patents are granted only for inventions that meet three conditions:novelty, non-obviousness and usefulness

Patent types: The three types of patents are

1) **Utility** patents may be granted to anyone who invents or discovers any new and useful process, machine, article of manufacture, or composition ofmatter, or any new and useful improvement thereof;

2) **Design** patents may be granted to anyone who invents a new, original, andornamental design for an article of manufacture; and

3) **Plant** patents may be granted to anyone who invents or discovers andasexually reproduces any distinct and new variety of plant

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Genetically modified organism (GMO)

It is an animal, plant, or microbe whose DNA has been altered using geneticengineering techniques. For thousands of years, humans have used breeding methods to modify organisms.

Function

Genetic engineering can be done with plants, animals, or bacteria and other very small organisms. With genetic engineering, scientists take the gene for a desired trait in one plant or animal, and they insert that gene into the DNA of another plant or animal. Genes can also be moved from an animal to a plant or vice versa.

The process to create GMOs is different than selective breeding. This involves selecting plants or animals with desired traits and breeding them. Over time, this results in offspring with those desired traits. One of the problems with selective breeding is that it can also result in traits that are not desired.

Genetic engineering allows scientists to select one specific gene to implant. This avoids introducing other genes with undesirable traits. Genetic engineering also helps speed up the process of creating new foods with desired traits.

Genome editing is a newer method that involves adding, removing, or changing the DNA of a plant or animal in a targeted way.

The possible benefits of genetic engineering include:

- More nutritious food
- Tastier food
- Disease- and drought-resistant plants that require fewer environmental resources (such aswater and fertilizer)
- Less use of pesticides
- Increased supply of food with reduced cost and longer shelf life
- Faster growing plants and animals

- Food with more desirable traits, such as potatoes that produce less of a cancer-causing substance when fried

Some people have expressed concerns about GE foods, such as:

- Creation of foods that can cause an allergic or toxic reaction
- Unexpected or harmful genetic changes
- Inadvertent transfer of genes from one GM plant or animal to another plant or animal not intended for genetic modification
- Foods that are less nutritious

These concerns have thus far been unfounded. None of the GMOs used today have caused any of these problems. The US Food and Drug Administration (FDA) assesses all GMOs to make sure they are safe before allowing them to be sold. In addition to the FDA, the US Environmental Protection Agency (EPA) and the US Department of Agriculture (USDA) regulate bioengineered plants and animals. They assess the safety of GMOs to humans, animals, plants, and the environment.

Food Sources

Cotton, corn, and soybeans are the main GNO crops grown in the United States. Most of these are used to make ingredients for other foods, such as:

- Corn syrup used as a sweetener in many foods and drinks
- Corn starch used in soups and sauces
- Soybean, corn, and canola oils used in snack foods, breads, salad dressings, and mayonnaise
- Sugar from sugar beets

Livestock feed

Other GMO crops include one or more varieties of the following:

- Apples
- Alfalfa
- Canola

Side Effects

There are no side effects from consuming GMO foods

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PRODUCTION OF ETHANOL

Ethanol has been produced by the fermentation for thousands of years. This was mostly associated with brewer and distillery industries. In the developing countries, microbial fermentation processes are performed for the production of alcohol. This is mainly because of the cheap raw materials available.

Ethanol as a motor fuel:

- Prior to second world war , ethanol was extensively used as a motor fuel.
- Some motor companies designed some vehicles to run on alcohol (or) petrol (or) mixture of both.
- Brazil was the first country to produce ethanol in large scale by yeast fermentation by utilizing sugarcane and cassara.
- This alcohol used as motor fuel is referred to as "green petrol".

Production of ethanol by fermentation:

In recent years ,many countries have started production of ethanol by fermentation process. The micro organisms and the raw materials used in this production are:

Micro organisms:

Certain yeasts and bacteria are employed for alcohol fermentation. Among the yeasts, saccharomyces cerevisiae, is the most commonly used. While among bacteria, zymomonas mobilis is the most frequently employed for alcohol production.

Raw materials:

There are large number of raw materials that can serve as substrates for alcohol fermentation. They may be broadly categorized in to

1. Saccharides (or) sugary materials.

   Eg : molasses, glucose , sucrose etc….

2. Starchy materials.
   Eg: wheat , rice , maize , potato etc…..
3. Cellulose materials
   Eg : wood , agricultural waste etc….
   Among these molasses is most widely used raw material for alcohol fermentation.

Production process of Ethanol :

Ethanol production can be carried out in to 3 stages :

1. Preparation of fermentation media & inoculum
2. Fermentation proper
3. Recovery

1. Preparation of fermentation media :

These are various raw materials for alcohol production. The most commonly used raw materials are molasses , grains , potatoes & wood wastes.
When molasses is used for fermentation , it is diluted with water . when starchy materials (corn , barley) are used , they have to be first hydrolysed by pre treatment.
This may be done by barley malt, dilute acids (or) fungal amylases. Most frequently barley malt is used. Molasses contain most of the nutrients required for alcohol fermentation. However ammoniumsulphate (or) phosphate are added to the nutrient solution to supply nitrogen and phosphorous. The ph of the medium is adjusted to 4-5 by adding sulfuric acid (or) lactic acid.

Preparation of Inoculum :

After selection of desired organisms , isolate in pure form the inoculum is prepared under aseptic conditions . for this purpose , the organisms are first cultured in flasks under aerobic conditions to increase the size of inoculum.

2. Fermentation proper :

- Production of alcohol is carried out in large fermentors . The ph is maintained around 4.0-4.5. The initial temperature is kept between 21-26 c .
- At the time of fermentation process the temperature raises to around 30 c. It is necessary to usecooling devices & bring down the temperature to less than 27c.
- This is because of the ethanol gets evaporated at temperature above 27c.
- Aeration is required for good growth of the organisms. It takes about 2-3 days for thefermentation to be completed.

3. Recovery of Ethanol :

- The cell mass is separated by centrifugation (or) sedimentation Ethanol from the fermentationcan be recovered by successive distillations.
- By this process it is easy to obtain ethanol around 95% for obtaining more than 95% of ethanol ,special techniques of distillations have to be required like analyser and rectifier.

Stillages in alcohol production:

- Large volume of wastes , which are technically referred to as stillages are formed during the process of alcohol fermentation.

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SCREENING METHODS:

DEFNITION: A first step method to establish the presence of a substancein a population for the purposes of estimating risk. Food intake is combined with likely chemical concentration to create an estimate of chemical exposure.

A producer strain should possess the following characters:

1. It should be able to grow on relatively cheaper substrates.
2. It should grow well in an ambient temperature preferably at 30-40°C.This reduces the cooling costs.
3. It should yield high quantity of the end product.
4. It should possess minimum reaction time with the equipmentused in a fermentation process.
5. It should possess stable biochemical characteristics.
6. It should yield only the desired substance without producingundesirable substances.
7. It should possess optimum growth rate so that it can be easilycultivated on a large scale.

They are:

1. Primary screening,
2. Secondary screening

PRIMARY SCREENING OF MICROORGANISMS:

Primary screening may be defined as detection and isolation of the desiredmicroorganism based on its qualitative ability to produce the desired product like antibiotic or amino acid or an enzyme etc. In this process desired microorganism is generally isolated from a natural environment like soil, which contains several different species. Sometimes the desired microorganism has to be isolated from a large population of different species of microorganisms.

The following are some of the important primary screeningtechniques:

(i) The crowded plate technique

(ii) Indicator dye technique

(iii) Enrichment culture technique

(iv) Auxano graphic technique

3. Technique of supplementing volatile and organic substrates

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SECONDARY SCREENING OF MICROORGANISM:

Primary screening helps in the detection and isolation of microorganisms from the natural substrates that can be used for industrial fermentations for the production of compounds of human utility, but it cannot give the details of production potential or yield of the organism. Such details can be ascertained by further experimentation.

METHODS OF SECONDARY SCREENING:

Secondary screening gives very useful information pertaining to the newly isolated microorganisms that can be employed in fermentation processes of commercial value. These screening tests are conducted by using petri dish containing solid media or by using flasks or small fermenters containing liquid media. Each method has some advantages and disadvantages. Sometimes both the methods are employed simultaneously.

Liquid media method is more sensitive than agar plate method because it provides more useful information about the nutritional, physical and production responses of an organism to actual fermentation production conditions. Erlenmeyer flasks with baffles containing highly nutritive liquid media are used for this method. Flasks are fully aerated with glass

baffles and continuously shaken on a mechanical shaker in order to have optimum product yield.

There are several techniques and procedures that can be employed for secondary screening. However, only a specific example of estimation of antibiotic substance produced by species of Streptomyces, is described in the following paragraph. Similar methods could be used for the detection and isolation of microorganisms capable of producing other industrial products.

(i) Giant Colony Technique:

This technique is used for isolation and detection of those antibiotics, which diffuse through solid medium. Species of Streptomyces, is capable of producing antibiotics during primary screening. The isolated Streptomyces culture is inoculated into the central area of a sterilized petri plates containing nutrient agar medium and are selected. The plates are incubated until sufficient microbial growth takes place.

Cultures of test organism, whose antibiotic sensitivity is to be measured are streaked from the edges of plate's upto but not touching the growth of Streptomyces and are further incubated to allow the growth of the test organisms. Then the distance over which the growth of different test organisms is inhibited by the antibiotic secreted Streptomyces is measured in millimeters.

The relative inhibition of growth of different test organisms by the antibiotic is called inhibition spectrum. Those organisms whose growth is inhibited to a considerable distance are considered more sensitive to the antibiotic than those organisms, which can grow close to the antibiotic. Such species of Streptomyces, which have potentiality of inhibiting microorganisms is preserved for further testing.

(ii) Filtration Method:

This method is employed for testing those antibiotics which are poorly soluble in water or do not diffuse through the solid medium. The Streptomyces is grown in a broth and its mycelium is separated by filtration to get culture filtrate. Various dilutions of antibiotic filtrates are prepared and added to molten agar plating medium and allowed tosolidify.

Later on cultures of various test organisms are streaked on parallel lines on the solidified medium and such plates are incubated. The inhibitory effect of antibiotic against the test organisms is measured by their degreeof growth in different antibiotic dilutions.

(iii) Liquid Medium Method:

This method is generally employed for further screening to determine the exact amount of antibiotic produced by a microorganism like Streptomyces.

Erlenmeyer conical flasks containing highly nutritive medium are inoculated with Streptomyces and incubated at room temperature. They are also aerated by shaking continuously and vigorously during incubation period to allow Streptomyces to produce the antibiotic in an optimum quantity.

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