COURSE TITLE: FOOD AND INDUSTRIAL MICROBIOLOGY
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Fermentation processes: Historical development, components of fermenter and types (i.e. submerged, surface and solid-state fermentation)

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Fermentation is a metabolic process that produces chemical changes in organic substrates through the action of enzymes.

In biochemistry, it is defined as the extraction of energy from carbohydrates in the absence of oxygen.

Fermentation in food processing is the process of converting carbohydrates to alcohol or organic acids using microorganisms yeasts or bacteria under anaerobic conditions.

Fermentation usually implies desirable action of microorganisms. In microorganisms, fermentation is the primary means of producing adenosine triphosphate (ATP) by the degradation of organic nutrients anaerobically. The science of fermentation is known as zymology.

(en.wikipedia.org › wiki › Fermentation)

Historical development

Since ancient times, humans have exploited the fermentation process. The earliest archaeological evidence of fermentation is 13,000-year-old residues of a beer found in a cave near Haifa in Israel.

Another early alcoholic drink made from fruit, rice, and honey, dates from 7000-6600 BC, in the Neolithic Chinese village of Jiahu and wine making dates from 6000 BC in Georgia.

Seven-thousand-year-old jars containing the remains of wine, now on display at the University of Pennsylvania were excavated in the Zagros Mountains in Iran.

There is strong evidence that people were fermenting alcoholic drinks in Babylon 3000 BC, ancient Egypt 3150 BC, pre-Hispanic Mexico 2000 BC and Sudan 1500 BC.

Traditional fermentation technology is more than 3000 year old in India as mentioned in ancient literature.

A sweet substance known as Soma juice prepared by the Vedic Aryans is supposed to be the first product of fermentation in India. The Rigveda (1500 BC) shows that fermentation technology took its first step in connection with the preparation of Soma juice in India.

There is also another drink known as Sura (wine/beer) prepared by fermentation. So, it is believed that acetic fermentation was known to India since the early times.

Curd is another very popular fermentation product described in the Rigveda . It still remains a popular food and the technology of curdling milk is also found in a number of texts associated with Yajurveda.

In the beginning, fermentation was mainly associated with the preparation of spiritual drinks, but later on it was used for other purposes also.

Finally, the term fermentation is derived from the Latin word Fermentum that stands for boiling. The French chemist Louis Pasteur founded zymology (study of fermentation), when in 1856 he connected yeast to fermentation process.

Food fermentation is the conversion of sugars and other carbohydrates into alcohol, organic acids and carbon dioxide. All three products have showed their importance in human uses.

The production of alcohol is made use of when fruit juices are converted to wine, when grains are made into beer and when foods rich in starch, such as potatoes, are fermented and then distilled to make spirits such as gin and vodka.

So, Fermentation is one of the oldest technologies used for food preservation and it is a process of digesting certain substances that leads to chemical conversion of organic substances into simpler compounds.

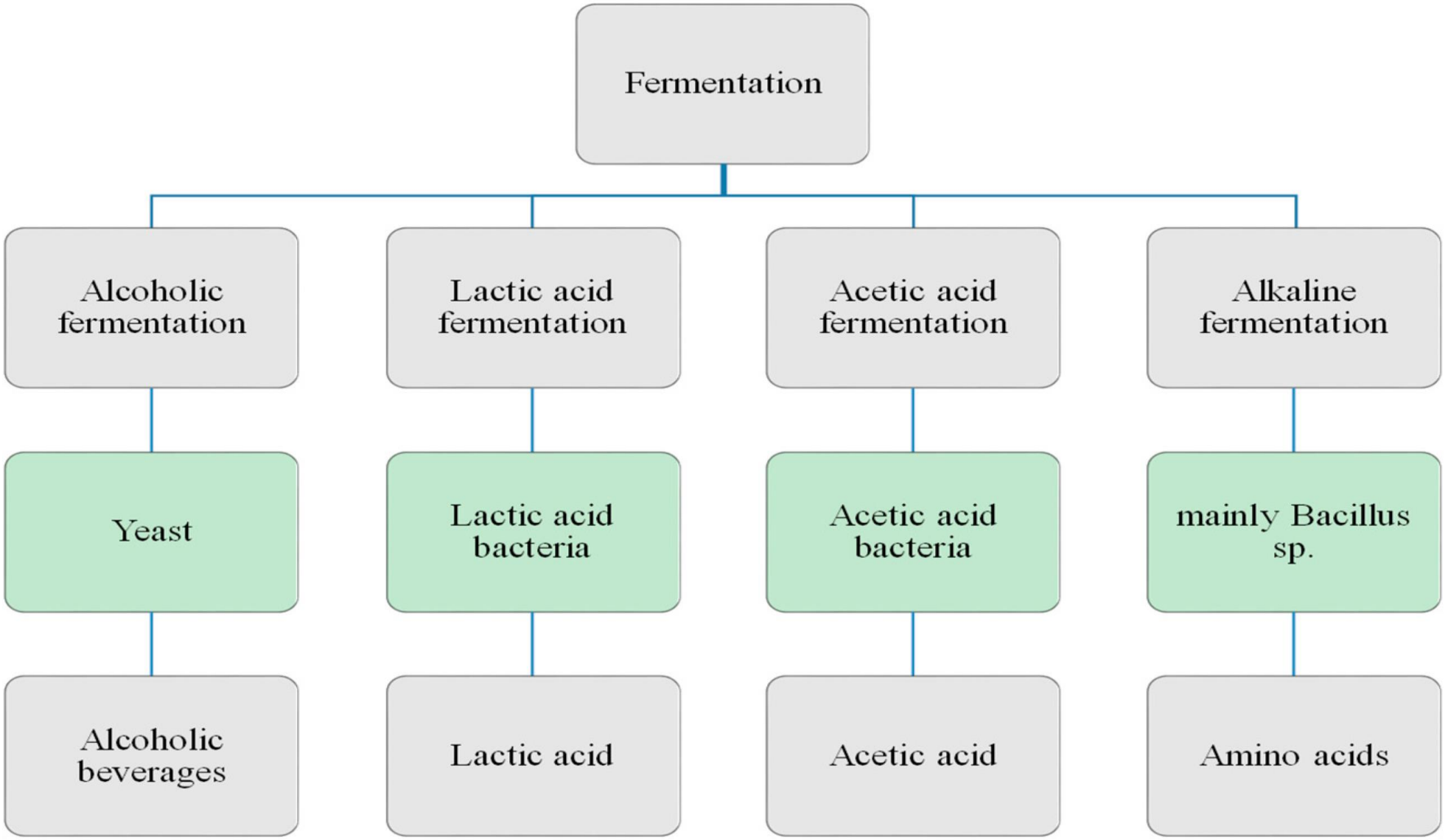
Over the centuries, it has evolved, been refined and diversified. Today a variety of fermented foods is produced both in industrialized and developing countries. A wide range of raw materials is used as substrates in fermentation and resulted end products derived from fermentation are major constituents of the human diet all over the world. Many benefits are attributed to fermentation. It preserves and enriches food, improves digestibility, and enhances the taste and flavour of foods.

Fermentation systems may be liquid, also known as submerged or solid state, also known as surface.

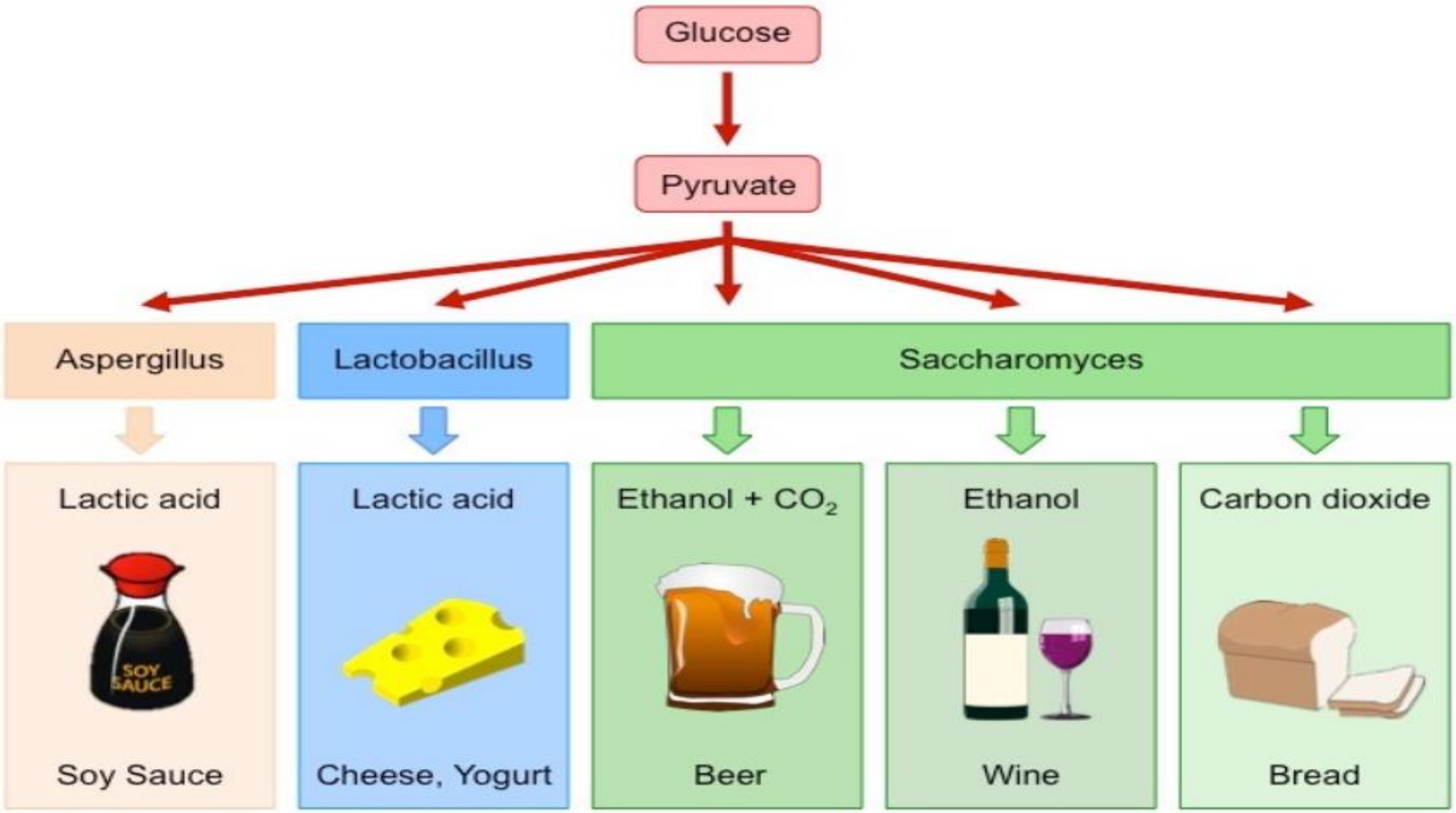
Most fermentors used in industry are of the submerged type, because the submerged fermentor saves space and is more susceptible to engineering control and design.

Much work still needs to be done to identify the best fermentation technique for each bioactive compound.

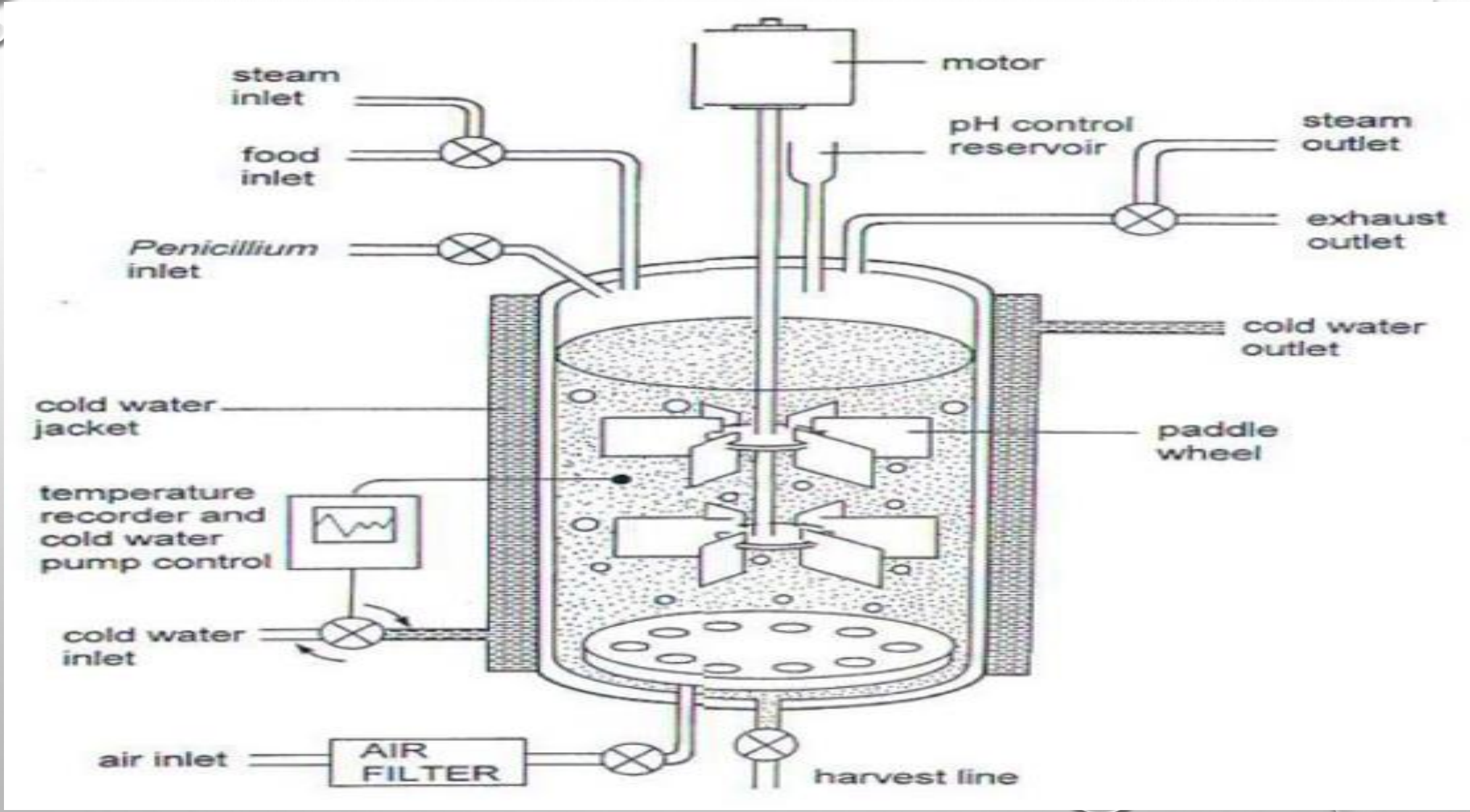
Schematic representation of the common types of fermentation, the microorganisms involved, and the end products



Production of Fermented Foods by Bacteria and Yeast (*Saccharomyces*)



Design of an Ideal fermenter



Various components of an ideal fermenter are:

S.No.	Part	Purpose
1	Top plate	cover (made of steel)
2	Clamp	top plate compressed onto vessel using clamp
3	Seal	separates top plate from vessel (glass) to prevent air leakage
4	Vessel	glass, jacketed, steel with ports for various outputs, inputs, probes etc
5	Drive motor	used to drive mixing shaft
6	Drive shaft	mixes the medium evenly with its impeller
7	Marine impeller	for plant tissue culture
8	Baffles	prevent sedimentation on sides and proper mixing
9	Sparger	air supplier / after filtration via membranes – ensures efficient dispersal – by attached to impeller
10	Exit gas cooler	like condenser remove as much moisture as possible from exhaust
11	Inoculation needle	port to add inoculum
12	Feed pumps	regulates the flow rates of additives (medium, nutrients) variable speed
13	Peristaltic pumps	fixed speed pumps – used for continuous sampling
14	Syringe pump	using a syringe – mostly used in batch
15	Exit gas analysis	CO_2 analyzer, O_2 analyzer, mass spectrometer
16	Sample pipe	through which samples are drawn
17	3 way inlet	to insert different probes

Monitoring and controlling parts of fermenter are:

S.No	Part	Use
1	Pt100	temperature sensor (platinum resistance electrode)
2	Foam probe	kept above the medium level to sense foam formation
3	pH electrode	senses pH
4	O_2 sensor	Monitors dissolved oxygen level
5	Heater pad	directly heats the medium
6	Cold finger	after direct heating – used to cool the vessel contents (closed coil/pipe to pass cool water)
7	Rotameter	variable air flow meter – indicates rate of air flow into vessel – attached to air sparger
8	Pressure valve	attached to rotameter for safer operation
9	Air pump	supply of air
10	Peristaltic pump	to pump in medium, acids, bases, antifoam

Beneficial properties

Oxygen control	Temperature control	Optimum pH value control
Correct species and strains	Qualitative & quantitative active substances	Closed pure culture
Fully-controlled continuous fermentation	Low risk of contamination	Optimal nutrients

Fermentation is the chemical transformation of organic substances into simplex compounds by the action of enzymes, complex organic catalysts which are produced by microorganism such as yeast, molds or bacteria.

In another words, fermentation is the technique of biological conversion of complex substrates into simple compounds by various microorganism.

FERMENTATION, classified on the basis of substrate used

1. Solid state fermentation (SSF)
2. Submerged fermentation (SmF)

Development of this fermentation techniques has leads to industrial level production of bioactive compounds such as antibiotics, pigments, antioxidants, antitumor agent, bio-surfactants, bioactive peptides etc. The metabolism exhibited by microorganism is different in SSF and SmF.

Submerged fermentation	Solid state fermentation
1. Fermentation may be carried out as batch or continuous	1. Fermentation may be carried out as batch
2. Medium is added in large vessel	2. Medium is added in flat vessel or trays
3. Surface area to volume height ratio is very less	3. Surface area to volume height ratio is very high
4. 5-10% of inoculums is added	4. Less inoculum is added
5. Inoculum is usually in liquid form	5. Inoculum is usually sprayed on surface of medium
6. Product used are usually high as compared to input cost	6. Product yield is comparatively less
7. Lesser space is required	7. More space is required
8. Less contamination	8. More contamination
9. If a batch get contaminated there is a loss of entire batch	9. If a tray gets contaminated then there is a loss of only tray but not the batch
10. Entire fermentation media is utilized by microorganism for growth and product fermentation	10. There is wastage of fermentation media
11. Aeration and agitation of system is possible by use of sparger and impeller	11. Aeration is usually carried out by passing sterile air and no agitation

Submerged fermentation	Solid state fermentation
12. Power consumption is high	12. Power consumption is less
13. Controlling parameters like temperature, pH is easy	13. Controlling parameters like temperature, pH is difficult
14. Foaming occurs	14. Foaming doesn't occurs
15. Automation and use of computer is easy	15. Automation and use of computer is difficult
16. Less labor required	16. More labor required
17. eg penicillin, streptomycin production etc.	17. eg Veniger, amylase production etc.

Substrate Used

Submerged fermentation (SmF)	Solid state fermentation (SSF)
➢ Soluble sugar ➢ Molasses ➢ Liquid media ➢ Fruit and vegetable juices ➢ Sewage / waste water	➢ Wheat bran ➢ Rice and wheat straw ➢ Fruit and vegetable waste ➢ Paper pulp ➢ Bagasses ➢ Coconut coir ➢ Synthetic media

Solid state fermentation (SSF)

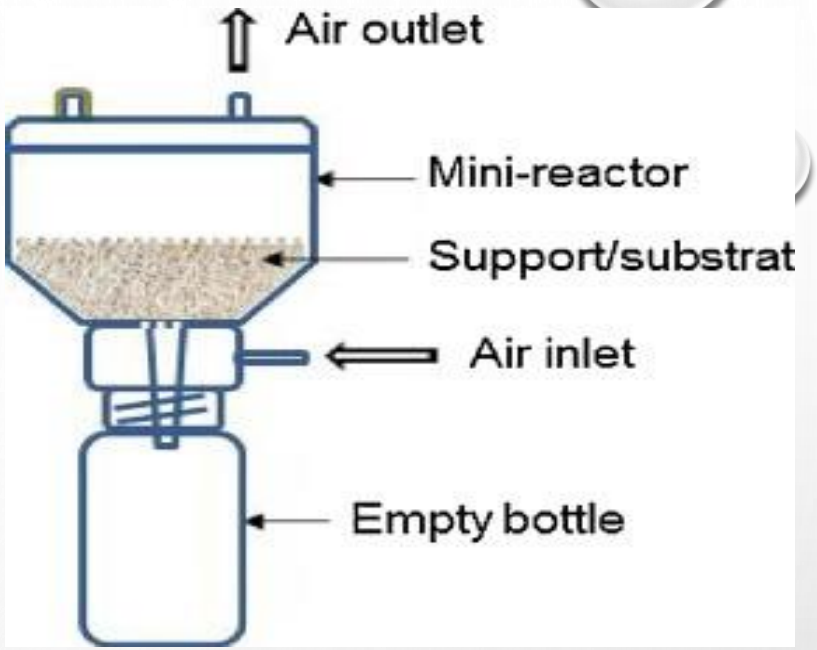
Solid state fermentation (SSF) has been defined as the fermentation process occurring in the absence of free water. Solid state fermentation is a method used for the production of enzymes, which involves the cultivation of microorganisms on a solid substrate, such as grains, rice and wheat bran.

SSF employs natural raw materials as carbon source such as cassava, barley, wheat bran, sugarcane bagasse, various oil cakes like palm kernel cake, soybean cake, ground nut oil cake, fruit pulps (e.g. apple pomace), saw dust, seeds (e.g. tamarind, jack fruit), coffee husk and coffee pulp, tea waste etc.

Important Features

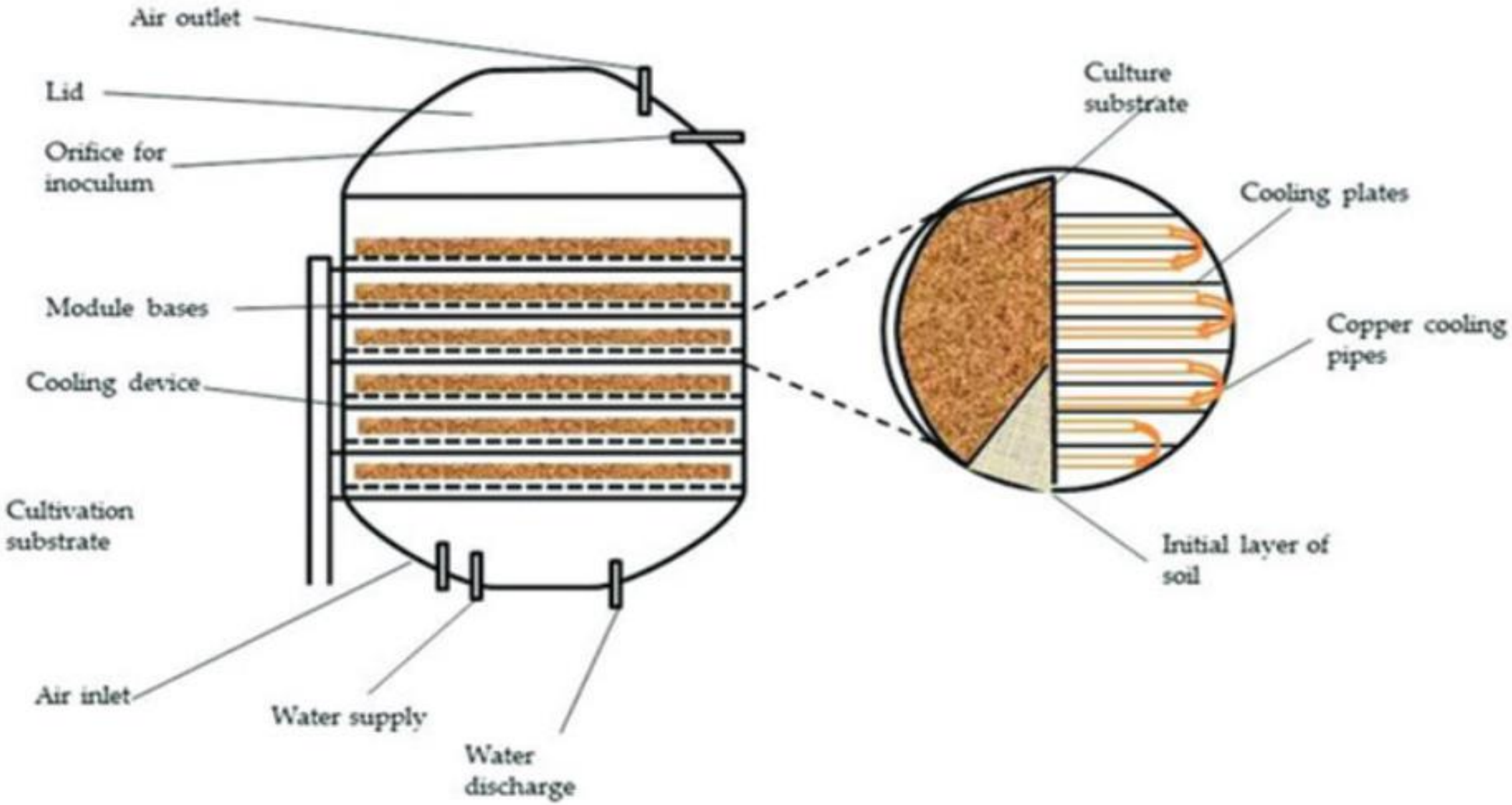
- ✓ Solid state fermentation has been defined as the fermentation process occurring in the absence or near absence of free water utilizing the solid substrate.
- ✓ It is a biomolecule manufacturing process used in the food, pharmaceutical, cosmetic, fuel and textile industries. These biomolecules are mostly metabolites generated by microorganisms grown on a solid support selected for this purpose.
- ✓ This technology for the culture of microorganisms is an alternative to liquid or submerged fermentation, used predominantly for industrial purposes

Why solid state fermentation ?



- Simple and cost effective
- Less effluent release, reduce pollution
- Aeration is easy
- Resembles the natural habitat of some fungi and bacteria
- SSF utilizes solid substrate, thus nutrient rich waste materials can be easily recycled as substrate
- Substrate are used very slowly and steadily so the same substrate can be used for longer fermentation period
- SSF is best suited for fermentation techniques involving fungi and microorganism that require less moisture content

Solid state (substrate) fermentation



Factors Involved in SSF Process

- **Selection of Micro-organisms**
- **Substrate**
- **Process Optimization**
- **Product Isolation & Purification**

Selection of Micro-organism -- This is one of the key factor for improved yields of the product. Bacteria, Yeast and Filamentous Fungi can be used. Filamentous Fungi has shown better results growing in the solid substrate fermentation.

Substrate -- Substrate also plays important role in determining the growth of micro-organisms, there by increasing the product yield. Substrate is chosen such a way that it should provide physical support as well as nutrients to the growing culture.

Substrate is of two types:

One is Specific substrate, which requires suitable value-addition and / or disposal.

The second is for producing a specific product from a suitable substrate.

Process Optimization – It is the optimization of physico-chemical and Biochemical Parameters which includes size, initial moisture, pH and pre-treatment of the substrate, Relative humidity, temperature of incubation, agitation and aeration and age and size of the inoculum. Nutrient Supplementation such as N, P and trace elements and supplementation of additional carbon source and inducers. Extraction of product and its purification.

Applications: Solid State fermentation is being employed in various fields ranging from pharmacology to bioremediation, covering various aspects of biodiversity conservation.

These are the applications of Solid State fermentation --
Production of Industrial Enzymes as almost all the known microbial enzymes can be produced under SSF systems. Enzymes of industrial importance, like proteases, cellulases, ligninases, xylanases, pectinases, amylases, phenolic acid esterases, microbial rennets, oligosaccharide oxidases etc. using SSF systems.

Production of Bio pesticides -- The infamous Bacillus thurengenesis (Bt)'s protein can be produced in large scale in order to address the issues of pest attacks-yield damage.

Applications of Solid State fermentation

-Food Industry	Traditional Food Fermentations	Koji, Tcznpch, Rae, Attickc, Fermented cheeses
	Mushroom Production & spawn	Agaricus, Pleurotus, Shn-take
	Bioconversion By-products	Sugar pulp Bagasse Composting, Detoxication
	Food Additives	Flavours. Dyestuffs.
Agriculture	Biocontrol , Bioinsecticide	Beauveria Metarhizium, Tricho derma
	PlantGrowth Hormones / Enhancers	Giberellins, Rhizobium, Trichoderma
Industrial Fermentation	Enzymes production	Amylases, Cellulases Proteases, Pectinases, Xylanases
	Antibiotic production	Pencillin, feed & Probiotics
	Organic acid Production	Citric acid, Fumaric acid,etc
	Fungal Metabolites	Alkaloids
	Ethanol Production	Malting and Brewing

Problems in Solid state (substrate) fermentation

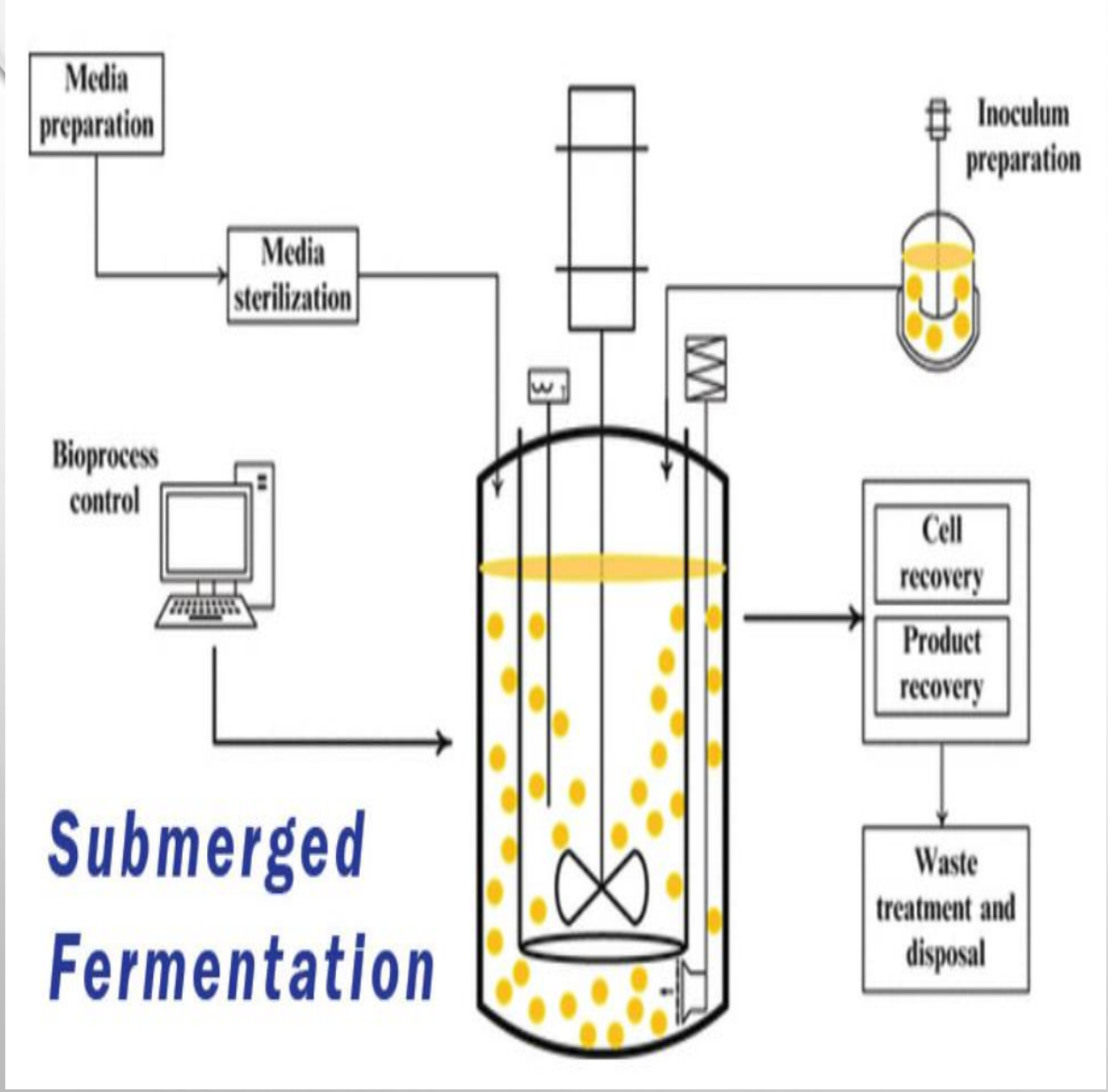
- Heat Transfer: One of the main difficulty is to control the temperature during the fermentation process.
- Heat is generated during the metabolic activities of micro-organisms, since the substrate used has low thermal conductivity heat removal will be slow.
- When the heat generated goes beyond certain level, which will result in product denaturation and will affect growth of microbe, ultimately ending up in reduction in yield and quality of the product.

Submerged fermentation

Submerged fermentation / liquid fermentation

It is a techniques of cultivation of microorganism in liquid broth which breaks down the nutrient to release the desired bio-active compound into solution. In this method, selected microorganism are grown in closed vessels containing a broth rich in nutrients and high concentration of oxygen. In SmF substrate are utilized quite rapidly hence need to be constantly replaced or supplemented with nutrients. Bacteria that requires high moisture content or high water activity are best suited for submerged fermentation.

Applications of Submerged Fermentation (SmF) / Liquid Fermentation (LF) -- SmF utilizes free flowing liquid substrates, such as molasses and broths. The bioactive compounds are secreted into the fermentation broth. The substrates are utilized quite rapidly that's why substrate needs to be constantly replaced / supplemented with nutrients. This fermentation technique is best suited for microorganisms such as bacteria that require high moisture. An additional advantage of this technique is that purification of products is easier. SmF is primarily used in the extraction of secondary metabolites that need to be used in liquid form.



- ✓ More that 75% of the industrial enzymes are produced using SmF, one of the major reasons is that SmF supports the utilization of genetically modified organisms to a greater extent than SSF.
- ✓ In SmF, the accumulation of variety of intermediate metabolites results in lowered enzyme activity and production efficiency
- ✓ Based on research, certain bioactive compounds have found to be produced in higher quantities in SSF, whereas other compounds have been extracted using SmF

THANK YOU