Semester – VI

15MBU601 INDUSTRIAL MICROBIOLOGY 5H – 5C

Instruction Hours / week: L: 5 T: 0 P: 0 **Marks:** Internal: **40** External: **60** Total: **100**

End Semester Exam: 3 Hours

SCOPE

To acquire the knowledge in the large scale production of industrial product, providing the trends to cater the needs of industry

OBJECTIVES

Industrial microbiology encompasses the use of microorganisms in the manufacture of food or industrial products. The use of microorganisms for the production of food, either human or animal, the microorganisms used in industrial processes may be natural isolates; laboratory selected mutants or genetically engineered organisms.

UNIT – I

History and development of Industrial microbiology - Industrially important strains – screening methods, preservation techniques – strain development – mutation, recombinant DNA technology and plasmid fusion.

UNIT – II

Media for industrial fermentation process - media formulation, sterilization of media – Fermentor designing - body construction, mass transfer, oxygen transfer, effect of viscosity- Scale up process.

UNIT – III

Fermentation types – submerged fermentation, solid state fermentation, batch fermentation, continuous fermentation – Types of fermentors – Tower, fed batch and airlift, Components of CSTR – Down stream process - cell disruption, centrifugation, precipitation, intracellular and extra cellular liquid extraction and product formulation.

UNIT – IV

Role of microorganism in the pharmaceutical sciences - Microbial spoilage, preservation and Sterilization of pharmaceutical products.

UNIT – V

Role of precursors and steering agents - in production of antibiotics, vitamins and enzymes - standardization and Quality control of Pharmaceutical products – Antiseptics, disinfectants, Injectables, IV fluids and pyrogen testing.

TEXT BOOKS

1. Casida, L.E. Jr., 2003. Industrial Microbiology. New Age International Publishers, New Delhi.
2. Demain, A. L. and J. E. Davies, 1999. Manual of Industrial Microbiology and Biotechnology. 2nd Edition, A.S.M. Press, Washington, D.C.
3. Hugo, W.B. and A.D. Russell, 1998. Pharmaceutical Microbiology. 6th Edition, Publisher Blackwell Science Ltd.
4. Mansi, E.M.T. and C.F.A. Bryce, 2002. Fermentation Microbiology and Biotechnology. Taylor and Francis, New York.
5. Patel, A.H., 2003. Industrial Microbiology. Macmillan India Ltd. New Delhi.

2018

III B. Sc Microbiology –Industrial Microbiology – 15MBU601
(2015-2018 Batch, VI semester)

LECTURE PLAN - UNIT -I			
S. no	Lecture duration(Hr)	**Topics covered**	**Supporting materials**
1	1	History	T1 1-3
2	1	Development	T1 4-7
3	2	Industrially important strains	T1 7-15
4	2	Screening methods	T2 15-20
5	1	Preservation methods	T2 21-25
6	2	Strain development	T2 26-30
7	1	Mutation	T1 114-117
8	2	Recombinant DNA technology	T1 114-117
9	2	Plasmid fusion	T1 114-117
10	1	Revision of unit I	
11	1	Unit I test	
		Total hours	16 hours
		LECTURE PLAN - UNIT -II	
S. no	Lecture duration(Hr)	**Topics covered**	**Supporting materials**
1	1	Media	T2 43-54
2	2	Fermentation process	T2 43-54
3	1	Media formulation	T2 43-54
4	1	Sterilization of media	T2 54-64
5	2	Fermentor designing	T2 64-70
6	1	Body construction	T2 70-86
7	1	Mass transfer	T1 114-134
8	1	Oxygen transfer	T1 114-134
9	1	Effect of viscosity	T1 114-134
10	2	Scale up process	T1 114-134
11	1	Revision of unit II	
12	1	Unit II test	
		Total hours	15 hours
		LECTURE PLAN - UNIT -III	
S. no	Lecture duration(Hr)	**Topics covered**	**Supporting materials**
1	1	Fermentation types	R1 11-12
2	2	Submerged fermentation	R1 11-20
3	2	Solid state fermentation	R1 21-23

Prepared by: Dr. S. Dinesh Kumar, Assistant Professor, Dept of Microbiology, KAHE

4	2	Batch fermentation	R1 24-25
5	1	Continuous fermentation	R1 25-27
6	1	Types of fermentors	R1 59-61
7	2	Upstream processing	R1 62-65
8	2	Downstream processing	R1 65-69
9	1	Intracellular liquid extraction	R1 87-95
10	1	Extracellular liquid extraction	R1 87-95
11	1	Product formulation	R1 87-95
12	1	Revision of unit III	
13	1	Unit III test	
		Total hours	18 hours
		LECTURE PLAN - UNIT - IV	
S. no	Lecture duration(Hr)	**Topics covered**	**Supporting materials**
1	2	Role of microbes	R1 171-173
2	1	Pharmaceutical sciences	R1 171-173
3	2	Microbial spoilage of raw materials	R1 171-173
4	2	Microbial spoilage of products	R1 171-173
5	1	Preservation	T2 96-111
6	1	Sterilization	T2 96-111
7	2	Quality check of products	T2 96-111
8	1	Revision of unit IV	
9	1	Unit IV test	
		Total hours	13 hours
		LECTURE PLAN - UNIT -V	
S. no	Lecture duration(Hr)	**Topics covered**	**Supporting materials**
1	1	Role of precursors	R1 257-262
2	1	Role of steering agents	R1 257-262
3	1	Production of antibiotics	R1 267-280
4	1	Production of vitamins	T1 377-387
5	1	Production of enzymes	T1 390-399
6	1	Standardization	R1 287-293
7	1	Quality control	R1 287-293
8	1	Antiseptics	T2 145-157
9	1	Disinfectants	T2 145-157
10	1	Injectables and IV fluids	T2 145-157
11	1	Pyrogen testing	W1
12	1	Revision of unit V	
13	1	Unit V test	
		Total hours	13 hours

Prepared by: Dr. S. Dinesh Kumar, Assistant Professor, Dept of Microbiology, KAHE

2018

Textbooks :	T1: Industrial Microbiology – LE Casida, New Age International Pvt Ltd. T2: Industrial Microbiology – Ah Patel, Macmillan Publishers.
Reference books:	R1- Bioprocess technology – PT Kalaichelvan, MJP Publishers
Website:	W1- www. Microbiology online.com
Journals:	

Prepared by: Dr. S. Dinesh Kumar, Assistant Professor, Dept of Microbiology, KAHE

2018

Industrial fermentation is the intentional use of fermentation by microorganisms such as bacteria and fungi as well as eukaryotic cells like CHO cells and insect cells, to make products useful to humans. Fermented products have applications as food as well as in general industry. Some commodity chemicals, such as acetic acid, citric acid, and ethanol are made by fermentation.[1] The rate of fermentation depends on the concentration of microorganisms, cells, cellular components, and enzymes as well as temperature, pH[2] and for aerobic fermentation[3] oxygen. Product recovery frequently involves the concentration of the dilute solution. Nearly all commercially produced enzymes, such as lipase, invertase and rennet, are made by fermentation with genetically modified microbes. In some cases, production of biomass itself is the objective, as in the case of baker's yeast and lactic acid bacteria starter cultures for cheesemaking. In general, fermentations can be divided into four types:[4]

- Production of biomass (viable cellular material)
- Production of extracellular metabolites (chemical compounds)
- Production of intracellular components (enzymes and other proteins)
- Transformation of substrate (in which the transformed substrate is itself the product)

These types are not necessarily disjoint from each other, but provide a framework for understanding the differences in approach. The organisms used may be bacteria, yeasts, molds, algae, animal cells, or plant cells. Special considerations are required for the specific organisms used in the fermentation, such as the dissolved oxygen level, nutrient levels, and temperature.

General process overview

In most industrial fermentations, the organisms or eukaroyotic cells are submerged in a liquid medium; in others, such as the fermentation of cocoa beans, coffee cherries, and miso, fermentation takes place on the moist surface of the medium.[5][6]There are also industrial considerations related to the fermentation process. For instance, to avoid biological process contamination, the fermentation medium, air, and equipment are sterilized. Foam control can be achieved by either mechanical foam destruction or chemical anti-foaming agents. Several other factors must be measured and controlled such as pressure, temperature, agitator shaft power, and viscosity. An important element for industrial fermentations is scale up. This is the conversion of a laboratory procedure to an industrial process. It is well established in the field of industrial microbiology that what works

well at the laboratory scale may work poorly or not at all when first attempted at large scale. It is generally not possible to take fermentation conditions that have worked in the laboratory and blindly apply them to industrial-scale equipment. Although many parameters have been tested for use as scale up criteria, there is no general formula because of the variation in fermentation processes. The most important methods are the maintenance of constant power consumption per unit of broth and the maintenance of constant volumetric transfer rate.[1]

Phases of growth

When a particular organism is introduced into a selected growth medium, the medium is inoculated with the particular organism. Growth of the inoculum does not occur immediately, but takes a little while. This is the period of adaptation, called the lag phase.[7] Following the lag phase, the rate of growth of the organism steadily increases, for a certain period—this period is the log or exponential phase.[7] After a certain time of exponential phase, the rate of growth slows down, due to the continuously falling concentrations of nutrients and/or a continuously increasing (accumulating) concentrations of toxic substances. This phase, where the increase of the rate of growth is checked, is the deceleration phase. After the deceleration phase, growth ceases and the culture enters a stationary phase or a steady state. The biomass remains constant, except when certain accumulated chemicals in the culture lyse the cells (chemolysis). Unless other micro-organisms contaminate the culture, the chemical constitution remains unchanged. If all of the nutrients in the medium are consumed, or if the concentration of toxins is too great, the cells may become scenescent and begin to die off. The total amount of biomass may not decrease, but the number of viable organisms will decrease.

Fermentation medium

The microbes or eukarytic cells used for fermentation grow in (or on) specially designed growth medium which supplies the nutrients required by the organisms or cells. A variety of media exist, but invariably contain a carbon source, a nitrogen source, water, salts, and micronutrients. In the production of wine, the medium is grape must. In the production of bio-ethanol, the medium may consist mostly of whatever inexpensive carbon source is available.

Carbon sources are typically sugars or other carbohydrates, although in the case of substrate transformations (such as the production of vinegar) the carbon source may be an alcohol or something else altogether. For large scale fermentations, such as those used for the production of ethanol, inexpensive sources of carbohydrates, such as molasses, corn steep liquor,[8] sugar cane juice, or sugar beet juice are used to minimize costs. More

sensitive fermentations may instead use purified glucose, sucrose, glycerol or other sugars, which reduces variation and helps ensure the purity of the final product. Organisms meant to produce enzymes such as beta galactosidase, invertase or other amylases may be fed starch to select for organisms that express the enzymes in large quantity.

Fixed nitrogen sources are required for most organisms to synthesize proteins, nucleic acids and other cellular components. Depending on the enzyme capabilities of the organism, nitrogen may be provided as bulk protein, such as soy meal; as pre-digested polypeptides, such as peptone or tryptone; or as ammonia or nitrate salts. Cost is also an important factor in the choice of a nitrogen source. Phosphorus is needed for production of phospholipids in cellular membranes and for the production of nucleic acids. The amount of phosphate which must be added depends upon the composition of the broth and the needs of the organism, as well as the objective of the fermentation. For instance, some cultures will not produce secondary metabolites in the presence of phosphate.[9]

Growth factors and trace nutrients are included in the fermentation broth for organisms incapable of producing all of the vitamins they require. Yeast extract is a common source of micronutrients and vitamins for fermentation media. Inorganic nutrients, including trace elements such as iron, zinc, copper, manganese, molybdenum and cobalt are typically present in unrefined carbon and nitrogen sources, but may have to be added when purified carbon and nitrogen sources are used. Fermentations which produce large amounts of gas (or which require the addition of gas) will tend to form a layer of foam, since fermentation broth typically contains a variety of foam-reinforcing proteins, peptides or starches. To prevent this foam from occurring or accumulating, antifoaming agents may be added. Mineral buffering salts, such as carbonates and phosphates, may be used to stabilize pH near optimum. When metal ions are present in high concentrations, use of achelating agent may be necessary.

Production of biomass

Microbial cells or biomass is sometimes the intended product of fermentation. Examples include single cell protein, bakers yeast, lactobacillus, E. coli, and others. In the case of single-cell protein, algae is grown in large open ponds which allow photosynthesis to occur.[10] If the biomass is to be used for inoculation of other fermentations, care must be taken to preventmutations from occurring.

Production of extracellular metabolites

Metabolites can be divided into two groups: those produced during the growth phase of the organism, called **primary metabolites** and those produced during the stationary phase, called **secondary metabolites**. Some examples of primary metabolites are ethanol, citric acid, glutamic acid, lysine, vitamins and polysaccharides. Some examples of secondary metabolites are penicillin, cyclosporin A, gibberellin, and lovastatin.[9]

Primary metabolites

Primary metabolites are compounds made during the ordinary metabolism of the organism during the growth phase. A common example is ethanol or lactic acid, produced during glycolysis. Citric acid is produced by some strains of *Aspergillus niger* as part of the citric acid cycle to acidify their environment and prevent competitors from taking over. Glutamate is produced by some *Micrococcus* species,[11] and some *Corynebacterium* species produce lysine, threonine, tryptophan and other amino acids. All of these compounds are produced during the normal "business" of the cell and released into the environment. There is therefore no need to rupture the cells for product recovery.

Secondary metabolites

Secondary metabolites are compounds made in the stationary phase; penicillin, for instance, prevents the growth of bacteria which could compete with *Penicillium* molds for resources. Some bacteria, such as *Lactobacillus* species, are able to produce bacteriocins which prevent the growth of bacterial competitors as well. These compounds are of obvious value to humans wishing to prevent the growth of bacteria, either as antibiotics or as antiseptics (such as gramicidin S). Fungicides, such as griseofulvin are also produced as secondary metabolites.[9] Typically secondary metabolites are not produced in the presence of glucose or other carbon sources which would encourage growth,[9] and like primary metabolites are released into the surrounding medium without rupture of the cell membrane.

In the early days of the biotechnology industry, most biopharmaceutical products were made in *E. coli*; by 2004 more biopharmaceuticals were manufactured in eukaryotic cells, like CHO cells, than in microbes, but used similar bioreactorsystems.[6] Insect cell culture systems came into use in the 2000s as well.[12]

Production of intracellular components

Prepared by: Dr. S. Dinesh Kumar, Assistant Professor, Dept of Microbiology, KAHE

Of primary interest among the intracellular components are microbial enzymes: catalase, amylase, protease, pectinase,glucose isomerase, cellulase, hemicellulase, lipase, lactase, streptokinase and many others.[13] Recombinant proteins, such as insulin, hepatitis B vaccine, interferon, granulocyte colony-stimulating factor, streptokinase and others are also made this way.[6] The largest difference between this process and the others is that the cells must be ruptured (lysed) at the end of fermentation, and the environment must be manipulated to maximize the amount of the product. Furthermore, the product (typically a protein) must be separated from all of the other cellular proteins in the lysate to be purified.

Transformation of substrate

Substrate transformation involves the transformation of a specific compound into another, such as in the case ofphenylacetylcarbinol, and steroid biotransformation, or the transformation of a raw material into a finished product, in the case of food fermentations and sewage treatment.

Food fermentation

Ancient fermented food processes, such as making bread, wine, cheese, curds, idli, dosa, etc., can be dated to more thanseven thousand years ago.[14] They were developed long before man had any knowledge of the existence of themicroorganisms involved. Some foods such as Marmite are the byproduct of the fermentation process, in this case in the production of beer.

Ethanol fuel

Fermentation is the main source of ethanol in the production of ethanol fuel. Common crops such as sugar cane, potato, cassava and corn are fermented by yeast to produce ethanol which is further processed to become fuel.

Sewage treatment

In the process of sewage treatment, sewage is digested by enzymes secreted by bacteria. Solid organic matters are broken down into harmless, soluble substances and carbon dioxide. Liquids that result are disinfected to remove pathogens before being discharged into rivers or the sea or can be used as liquid fertilizers. Digested solids, known also as sludge, is dried and used as fertilizer. Gaseous byproducts such as methane can be utilized as biogas to fuel electrical generators. One advantage of bacterial digestion is that it reduces the bulk and odor of sewage, thus reducing space needed for dumping. The main disadvantage of bacterial digestion in sewage disposal is that it is a very slow process.

Prepared by: Dr. S. Dinesh Kumar, Assistant Professor, Dept of Microbiology, KAHE

Agricultural feed

A wide variety of agroindustrial waste products can be fermented to use as food for animals, especially ruminants. Fungi have been employed to break down cellulosic wastes to increase protein content and improve *in vitro* digestibility.

TYPES OF FERMENTATION PROCESSES | INDUSTRIAL MICROBIOLOGY

There are three types of fermentation processes and they are

1. Batch fermentation
2. Continuous fermentation
3. Dual or multiple fermentation

1. **Batch fermentation**

Batch fermentation is carried out in batches. Here, fermentation media is filled up to 80% space by fermentor, and the remaining space is used as head space. Head space plays an important role as some area of a fermentor is required for collection of air, gases, and foam which is produced during the fermentation process. Further, after inoculation of media the fermentor is steam sterilized and after sterilization, the nutrient media is cooled and inoculated with desired volume of inoculums under aseptic condition.

Fermentation process is carried out under optimum growth condition. It is stopped after specific period of time and the fermented media or broth are removed from the fermentor and the desired product is obtained.

The product obtained is passed through recovery and purification process. Later, the fermentor is cleaned and reused for the next batch. In this process, as the fermentation proceeds, the quantity of nutrients from the media gets depleted, and microbes and products increase. In batch fermentation, growth of micro-organism is slower down due to decrease of nutrients.

Advantages of batch fermentation

It requires less space, there are fewer chances of contamination and this process is easy to handle. The disadvantage is that it is a time consuming process and it requires more time for cleaning, sterilization, cooling. The yield of the product is also low.

2. **Continuous fermentation process.**

In continous fermentation process fermentation runs continuously without emptying of fermentation tank. It involves continous addition of fresh media and withdrawal of fermentation product is constant.Each and every cell in fermentation media should be in log phase and not in stationary phase.The rate of addition of media should satisfy nutrient requirement of fermentation organism if rate of addition of media is slow there are chances that cells enter in stationary phase.There are three types of continuous fermentation processes.

i] Single stage continuous fermentation

ii] Single stage recycle continuous fermentation

iii] Multi-stage continuous fermentation

A] Single stage continuous fermentation

In Single stage Continuous Fermentation, the fermentor is used continuously. In this process, media is added at a specific rate as well as the product formed is removed at the same rate. The input of media and output of fermented media is balanced.

B] Single stage recycle continuous fermentation.

In Single stage Recycle Continuous Fermentation, a single fermentor is used continuously. The rate of inoculation of fermentation media and the rate of withdrawal of fermented broth is same. In this process, a portion of fermented broth plus withdrawal culture is recycled to the fermentor.

C] Multi-stage continuous fermentation.

Prepared by: Dr. S. Dinesh Kumar, Assistant Professor, Dept of Microbiology, KAHE

In Multi-stage Continuous Fermentation process, two or more fermentors are operated continuously in sequence. In this fermentation process first fermentor is used for growth of cells because in some fermentation process the product is formed after cell multiplication. Here effluent of first fermentor acts as in-fluent for next fermentor.

The continuous fermentation techniques are operated by two operating systems that are chemostsat and turbidostat

Advantages of Continuous Fermentation process

1. Product is formed continuously.
2. Good product yield is obtained.
3. The inoculation of culture is carried only once.
4. Continuous fermentation process save time and labour work.

Disadvantages of Continuous Fermentation process

1. This process is a bit complicated and difficult to operate.
2. There are more chances of contamination.
3. As the fermentation process is operated continuously, the operator should have more knowledge about fermentation, microbial behaviour and growth.

3. **Dual or Multiple fermentation process**

In this fermentation process, two or more types of micro-organisms are used. In some fermentation process some micro organism are unable to form desired product so here multiple micro-organisms are used.

In dual fermentation, first one type micro-organism is inoculated and this micro-organism makes the fermentation media suitable for growth of second type micro-organism. It reduces the oxidation reduction potential for anaerobic fermentation of second micro-organism, which in turn produces the desired product. The second micro-organism also removes toxic products produced by the first micro-organism.

Inoculation of micro-organism in fermentation media can be done in different ways.

1. First micro-organism is inoculated in media and allowed to grow and then second micro-organism is inoculated in media for product formation.
2. Sometimes both micro-organisms are inoculated simultaneously in fermentation media and fermentation is carried out.

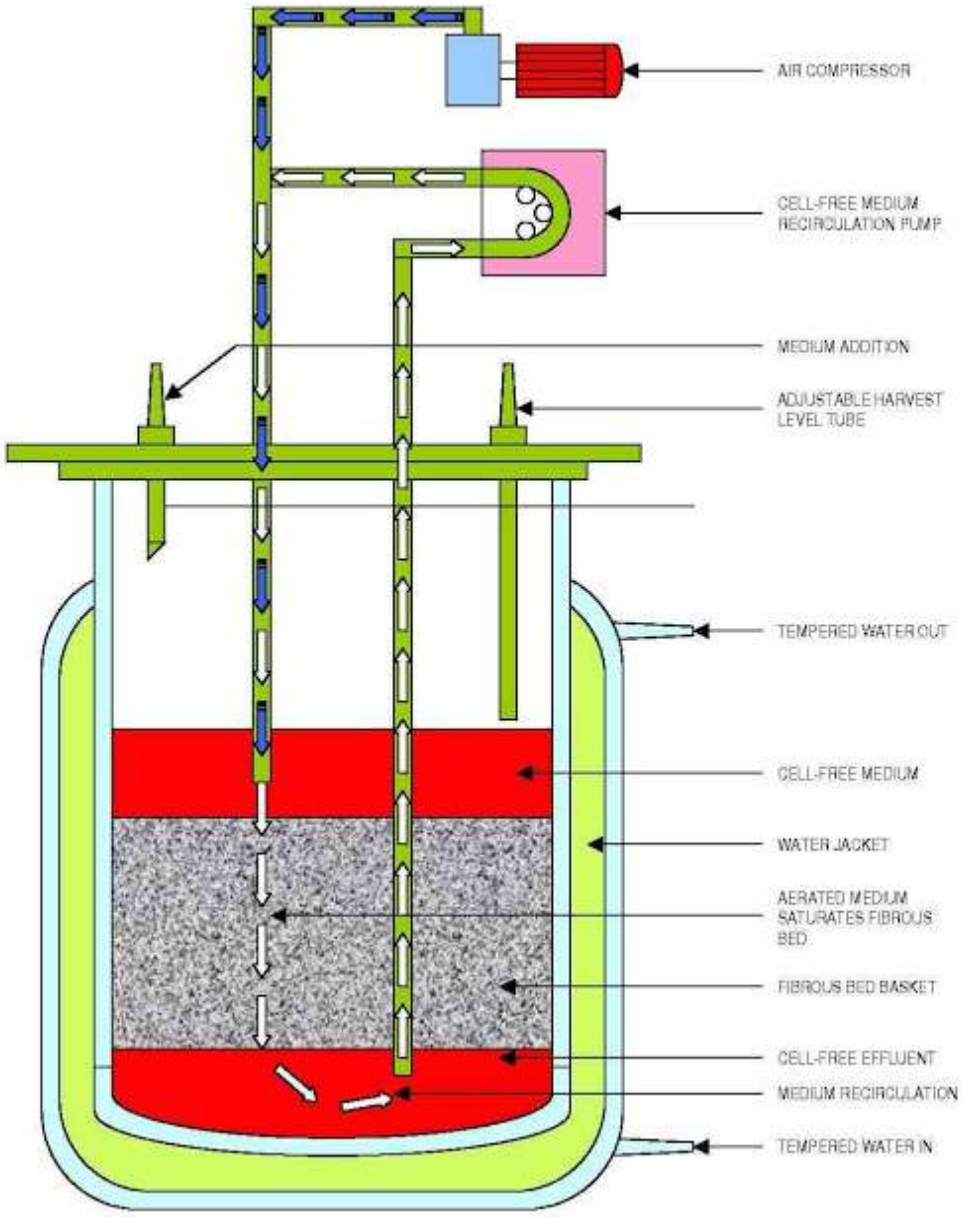
Different Types of Fermentors / Bioreactors

Different Types of Fermentors / Bioreactors

The heart of the fermentation or bioprocess technology is the Fermentor or Bioreactor. A bioreactor is basically a device in which the organisms are cultivated to form the desired products. it is a containment system designed to give right environment for optimal growth and metabolic activity of the organism.

A fermentor usually refers to the containment system for the cultivation of prokaryotic cells, while a bioreactor grows the eukaryotic cells (mammalian, insect cells, etc).

2018



Types of Bioreactor

1. Continuous Stirred Tank Bioreactor
2. Airlift Bioreactor
3. Fluidized Bed Bioreactor
4. Packed Bed Bioreactor
5. Photobioreactor
6. Membrane Bioreactor

Continous Stirred Tank Bioreactor

Prepared by: Dr. S. Dinesh Kumar, Assistant Professor, Dept of Microbiology, KAHE

2018

In Continous Stirred Tank Bioreactor, the contents of the vessel no longer vary with time, this applies to the hold up of micro-organisms and the concentration of the components of the medium in the fermentor.Steady state conditionscan be achieved by either **Chemostatic** or **Turbidostatic** principles.The former involves the adjustment of the flow rate of the fermentor to an appropriate and constant value and allowing the micro-organisms, substrates and biochemical product concentration to attain their natural levels. The turbidostat requires an experimental determination of the turbidity (ie, indirect measurement of microbial concentration). This thus used to control the flow rate. Both these methods have been employed in practice, though the former is obviously the simpler from every view point.

The most successful continuous systems to date have been those employing yeasts and bacteria, in which the desired products are the cells or primary metabolites, compounds that form the chemical 'inventory' of a microbe, (e.g. enzymes and amino acids), or some product clearly associated with growth or energy producing mechanisms (e.g. the production of alcohol).

The most widely used continous process based on CSTF (Continous Stirred Tank Fermentor) is the activated sludge process used in waste water treatment industry.

In continuous processing the autocatalytic (a reaction in which one of the products of the reaction increases the overall rate of a reaction) nature of microbiological reactions takes on a further significance. This is because the presence of one of the products, additional micro-organisms, enhances the overall rate of reaction. In the absence of micro-organisms no reaction can take place. Therefore, it is essential to retain at least a portion within the fermentor. It follows that if the flow rate is raised to a high value, then all the micro-organisms will be swept from the fermentor, and the conversion will cease. This phenomenon is commonly known as 'Wash-out'. if micro-organisms are fed to the fermentor simultaneously with the substrate feed, the problems associated with wash-out are abated, and the reaction proceeds normally.

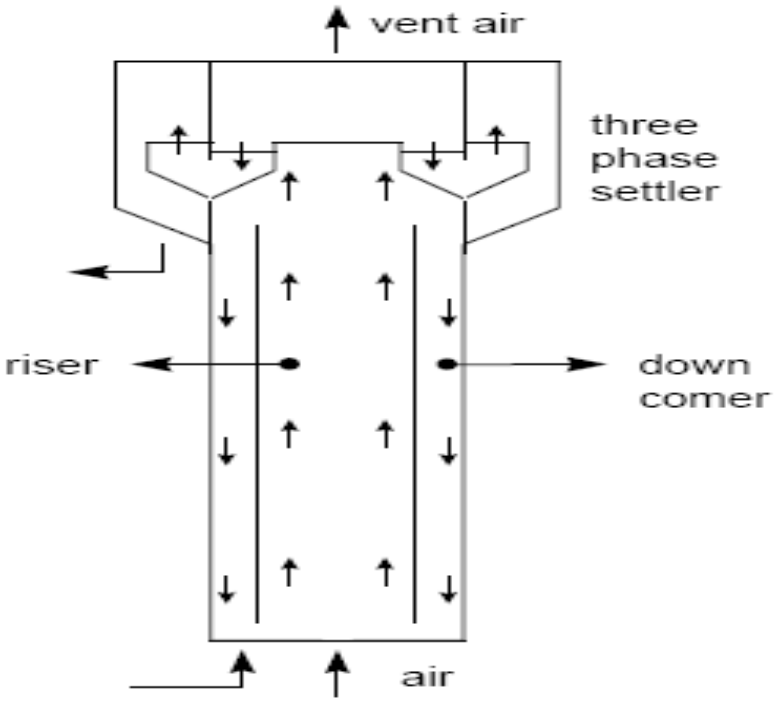
Advantages of Stirred Tank Bioreactor

1. Continuous operation
2. Good temperature control
3. Easily adapts to two phase runs
4. Good control
5. Simplicity of construction
6. Low operating (labor) cost
7. Easy to clean

Airlift Bioreactor

Prepared by: Dr. S. Dinesh Kumar, Assistant Professor, Dept of Microbiology, KAHE

2018



This kind of fermenter works on the principle of an air lift pump. It is of two kinds:

1. Internal loop type
2. External loop type.

The reactor's volume is determined by its capacity, kinetic data, and specific growth rate of the organism used. The rate of airflow of the reactor depends on the volumetric mass transfer coefficient in the reactor system. It is a uniform cylindrical cross type and has an internal loop or external loop riser configuration, diverging converging. The external loop riser configuration is adjustable and the change in the configurationimproves the O2 transfer rate vis-a-vis mass transfer coefficient for a particular rate of airflow. This helps provide required particular dissolved O2 concentration for specific microbial system. This reactor reduces the operating cost for pumping air through the bioreactor.

Prepared by: Dr. S. Dinesh Kumar, Assistant Professor, Dept of Microbiology, KAHE

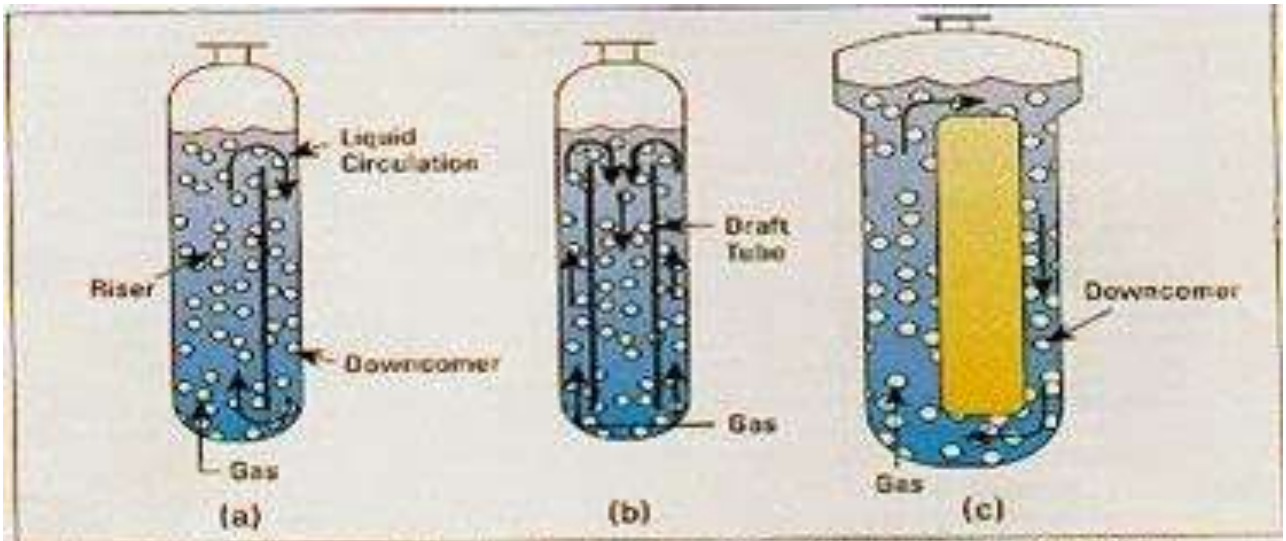


Figure 1. Airlift reactors: (a) split-cylinder internal-loop; (b) draft-tube internal-loop; and (c) external-loop.

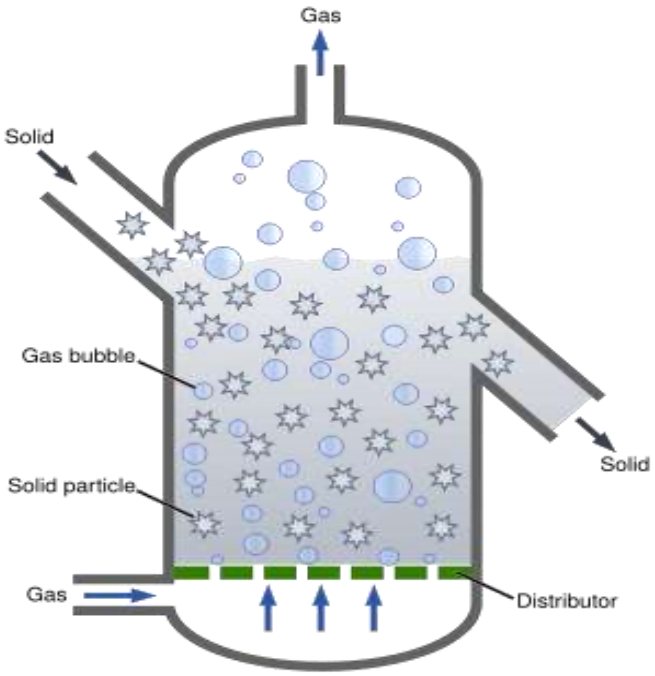
Advantages:

- Simple design with no moving parts or agitator for less maintenance, less risk of defects.
- Easier sterilization (no agitator shaft parts)
- Low Energy requirement vs stirred tank : Obviously doesn't need the energy for the moving parts (agitator shaft).
- Greater heat-removal vs stirred tank: At the Airlift bioreactor it doesn't need the heat plate to control the temperature, because the Draught-Tube which is inside the bioreactor can be designed to serve as internal heat exchanger. It is difference to the Stirred tank bioreactor that needs the heat coat or plate surrounding the tank to make warm bioreactor. It is clear enough that the Airlift bioreactor has greater heat-removal compare to Stirred tank.
- Very low cost

Fluidized Bed Bioreactor

This is a characteristic of beds of regular particles suspended in an up flowing liquid stream.
If an additional gas phase is involved, there is a tendency for the particles in the bed to become less evenly distributed.

Prepared by: Dr. S. Dinesh Kumar, Assistant Professor, Dept of Microbiology, KAHE



There are two important features of the beds of mixed particle sizes:
(i) The increase in porosity from the bottom to the top of the bed, and
(ii) The decreased particle movement when compared with beds containing particles of constant size.
Since porosity or voidage is a measure of the local free space within a bed, it also represents a measure of the microbial hold-up when expressed as wet volume per unit bed volume. Thus, a variation in microbial hold-up is to beexpected within a 'fluidised bed' fermentor on fluidisation, the smaller particles rise relative to the largerparticles, and produce a situation where the smaller particles are at the top and the larger particles are at the bottom of the bed.

As the smaller particles have the lowest settling velocity, the bed arranges itself, so that the smaller particles may be in the region of the highest porosity and the lowest linear velocity. The tower fermentor (developed for the continuous production of beer) is based upon these general principles (Ault et al, 1969). In this process yeast flocs are maintained in suspension by the upward movement of the nutrient medium. Moreover, any entrained particles are returned by means of a sedimentation device at the top of the tower.
Essentially, the fermentor consists of a vertical cylinder with an aspect ratio (length to diameter) of approximately 10:1 At the top of the tower a separator is provided to induce the gas bubbles produced by th,e reaction, to coalesce and escape from the liquid phase.

Within the separator there is a quiescent lone, free of the rising gas, so that the yeast may settle and return to the main body of the tower, and clear beer can be removed. A flocculent yeast (i.e. a yeast capable of achieving relatively large floc sizes) is essential for an alcoholic fermentation in a PBP at acceptable flow rates, otherwise a large proportion of the yeast would be washed out. As a result of this, an insufficient yeast concentration is

maintained. A mean yeast concentration of 25 % by weight (expressed as centrifuged wet weight) is typical with values as high as 30-35% by weight at the bottom of the tower, and as low as 5-10% by weight at the top.

A significant feature of the tower is the progressive and continuous fall in the specific gravity of the nutrient medium between the bottom and the top of the tower. There is an initial rapid fall at the bottom of the tower. It is followed by a slower fall over the middle and the top of the tower. This gradual fall in the specific gravity is due to the fermentation of the sugars.

Advantages of Fluidized Bed Reactor:

- Uniform Particle Mixing: Due to the intrinsic fluid-like behavior of the solid material, fluidized beds do not experience poor mixing as in packed beds. This complete mixing allows for a uniform product that can often be hard to achieve in other reactor designs. The elimination of radial and axial concentration gradients also allows for better fluid-solid contact, which is essential for reaction efficiency and quality.
- Uniform Temperature Gradients: Many chemical reactions require the addition or removal of heat. Local hot or cold spots within the reaction bed, often a problem in packed beds, are avoided in a fluidized situation such as an FBR. In other reactor types, these local temperature differences, especially hotspots, can result in product degradation. Thus FBRs are well suited to exothermic reactions. Researchers have also learned that the bed-to-surface heat transfer coefficients for FBRs are high.
- Ability to Operate Reactor in Continuous State: The fluidized bed nature of these reactors allows for the ability to continuously withdraw product and introduce new reactants into the reaction vessel. Operating at a continuos process state allows manufacturers to produce their various products more efficiently due to the removal of startup conditions in batch process.

Photobioreactor

Advantages of Photobioreactor

- Cultivation of algae is in controlled circumstances, hence potential for much higher productivity
- Large surface-to-volume ratio. PBRs offer maximum efficiency in using light and therefore greatly improve productivity. Typically the culture density of algae produced is 10 to 20 times greater than bag culture in which algaeculture is done in bags – and can be even greater.
- Better control of gas transfer.
- Reduction in evaporation of growth medium.
- More uniform temperature.
- Better protection from outside contamination.
- Space saving – Can be mounted vertically, horizontally or at an angle, indoors or outdoors.
- Reduced Fouling – Recently available tube self cleaning mechanisms can dramatically reduce fouling.

Membrane Bioreactor

Membrane bioreactors successfully applied to various microbial bioconversions such as alcoholic fermentation, solvents, organic acid production, waste water treatment, etc.

In membrane bioreactor the soluble enzyme and substrate are introduced on one side of ultrafilter membrane by means of a pump. product is forced out through the membrane. membrane holds back the enzyme. good mixing in the reactor can be achieved by using a stirrer.

The most widely used membrane materials includes polysulfonte, polyamide and cellulose acetate.

Advantages of Membrane Bioreactor

1. The loss of enzyme is reduced.
2. Enzyme lost by denaturation can be make up by periodic addition of enzyme.
3. Substrate and enzyme can be easily replaced.

Downstream processing refers to the recovery and purification of biosynthetic products, particularly pharmaceuticals, from natural sources such as animal or plant tissue or fermentation broth, including the recycling of salvageable components and the proper treatment and disposal of waste. It is an essential step in the manufacture of pharmaceuticals such as antibiotics, hormones (e.g. insulin and humans growth hormone), antibodies (e.g. infliximab and abciximab) and vaccines; antibodies and enzymes used in diagnostics; industrial enzymes; and natural fragrance and flavor compounds. Downstream processing is usually considered a specialized field in biochemical engineering, itself a specialization withinchemical engineering, though many of the key technologies were developed by chemists and biologists for laboratory-scale separation of biological products.

Downstream processing and analytical bioseparation both refer to the separation or purification of biological products, but at different scales of operation and for different purposes. Downstream processing implies manufacture of a purified product fit for a specific use, generally in marketable quantities, while analytical

bioseparation refers to purification for the sole purpose of measuring a component or components of a mixture, and may deal with sample sizes as small as a single cell.

Stages

A widely recognized heuristic for categorizing downstream processing operations divides them into four groups which are applied in order to bring a product from its natural state as a component of a tissue, cell or fermentation broth through progressive improvements in purity and concentration.

Removal of insolubles is the first step and involves the capture of the product as a solute in a particulate-free liquid, for example the separation of cells, cell debris or other particulate matter from fermentation broth containing an antibiotic. Typical operations to achieve this are filtration, centrifugation, sedimentation, precipitation, flocculation, electro-precipitation, and gravity settling. Additional operations such as grinding, homogenization, or leaching, required to recover products from solid sources such as plant and animal tissues, are usually included in this group.

Product isolation is the removal of those components whose properties vary considerably from that of the desired product. For most products, water is the chief impurity and isolation steps are designed to remove most of it, reducing the volume of material to be handled and concentrating the product. Solvent extraction, adsorption, ultrafiltration, and precipitation are some of the unit operations involved.

Product purification is done to separate those contaminants that resemble the product very closely in physical and chemical properties. Consequently steps in this stage are expensive to carry out and require sensitive and sophisticated equipment. This stage contributes a significant fraction of the entire downstream processing expenditure. Examples of operations include affinity, size exclusion, reversed phase chromatography, ion-exchange chromatography, crystallization and fractional precipitation.

Product polishing describes the final processing steps which end with packaging of the product in a form that is stable, easily transportable and convenient. Crystallization, desiccation, lyophilization and spray drying are typical unit operations. Depending on the product and its intended use, polishing may also include operations to sterilize the product and remove or deactivate trace contaminants which might compromise product safety. Such operations might include the removal of virusesor depyrogenation.

Prepared by: Dr. S. Dinesh Kumar, Assistant Professor, Dept of Microbiology, KAHE

A few product recovery methods may be considered to combine two or more stages. For example, expanded bed adsorption(Vennapusa *et al.* 2008) accomplishes removal of insolubles and product isolation in a single step. Affinity chromatographyoften isolates and purifies in a single step.

Applications of Microorganisms in the Pharmaceutical Sciences

There has long been a tendency, especially in medical and pharmaceutical circles, to regard microbes as harmful entities to be destroyed. However, as will be described in this chapter, the exploitation of microorganisms and their products has assumed an increasingly prominent role in the diagnosis, treatment and prevention of human diseases. Nonmedical uses are also of significance, e.g. the use of bacterial spores (Bacillus thuringiensis) and viruses (baculoviruses) to control insect pests, the fungus *Sclerotinia sclerotiorum* to kill some common weeds, and improved varieties of Trichoderma harzianum to protect crops against fungal infections.

Early treatment of human disease

The earliest uses of microorganisms to treat human disease can be traced to the belief that formation of pus in some way drained off noxious humours responsible for systemic conditions. Although the spontaneous appearance of pus in their patients' wounds satisfied most physicians, deliberate contamination of wounds was also practised. Bizarre concoctions of bacteria such as 'ointment of pigs' dung' and 'herb sclerata' were favoured during the Middle Ages. Both early central European and South American civilizations cultivated various fungi for application to wounds. In the nineteenth century, sophisticated concepts of microbial antagonism were developed following Pasteur's experiments demonstrating inhibition of anthrax bacteria by 'common bacteria' simultaneously introduced into the same culture medium. Patients suffering with diseases such as diphtheria, tuberculosis and syphilis were treated by deliberate infection with what were then thought to be harmless bacteria such as staphylococci, Escherichia coli and lacto-bacilli. Following their discovery in the early part of this century, bacterial viruses (bacteriophages) were considered as potential antibacterial agents, an idea that soon fell into disuse. This idea has recently been revived but has been criticized because of the possibility of transferring antibiotic resistance genes from phage to host bacteria.

1.2 Present-day exploitation

Some of the most important and widespread uses of microorganisms in the pharmaceutical sciences are the production of antibiotics and vaccines and the use of microorganisms in the recombinant DNA industry. However, there are a variety of other medicinal agents derived from microorganisms including vitamins, amino

acids, dextrans, iron-chelating agents and enzymes. Microorganisms as whole or subcellular fractions, in suspension or immobilized in an inert matrix are employed in a variety of assays.

Microorganisms have also been used in the pharmaceutical industry to achieve specific modifications of complex drug molecules such as steroids, in situations where synthetic routes are difficult and expensive to carry out.

- Applied and Environmental Microbiology
- Bacteriology
- Microbial Taxonomy
- Microbiology and Ecology
- Microbiology and Biochemistry
- Microbiology and Physiology
- Microbiology and Immunology
- Mycology
- Antibiotics in anti-aging
- Novel antibacterial drug discovery
- Modern Antibiotics: Emerging trends, Barriers and Opportunities
- Prevention of microbial infection
- Safe use of anti-infective agents
- Antibiotics versus good bacteria in gut
- Policies to stimulate drug development and discovery
- Role of computational biology
- Resistance and re-emerging theories
- Biosafety, biosecurity & biodefense
- Broadening participation in science
- Business & management of science
- Enhancing laboratory & research experiences
- Public outreach & informal science education
- Early treatment of human disease
- Present-day exploitation
- Pharmaceuticals produced by microorganisms

Prepared by: Dr. S. Dinesh Kumar, Assistant Professor, Dept of Microbiology, KAHE

- Dextrans
- Vitamins, amino acids and organic acids
- Iron-chelating agents
- Enzymes
- Streptokinase and streptodornase
- l-Asparaginase
- Neuraminidase
- b-Lactamases
- Applications of microorganisms in the partial synthesis of pharmaceuticals
- Production of antibiotics
- Steroid biotransformations
- Chiral inversion
- Antibiotic bioassays
- Microbiological assays
- Radioenzymatic (transferase) assays
- Vitamin and amino acid bioassays
- Phenylketonuria testing
- Carcinogen and mutagen testing
- Mutations at the gene level
- The Ames test
- Use of microbial enzymes in sterility testing
- Immobilized enzyme technology
- Use of microorganisms as models of mammalian drug metabolism
- Insecticides
- Evaluation of possible synergistic antimicrobial combinations
- Kinetic kill curves
- Emerging innovations
- Sources and control of contamination
- In manufacture
- Hospital manufacture

Prepared by: Dr. S. Dinesh Kumar, Assistant Professor, Dept of Microbiology, KAHE

- The extent of microbial contamination
- Factors determining the outcome of a medicament-borne infection
- The route of administration
- Resistance of the patient
- Effect of preservative concentration, temperature and size of inoculum
- Factors affecting the ‘availability’ of preservatives
- Effect of product pH
- Efficiency in multiphase systems
- Effect of container or packaging
- Quality assurance and the control of microbial risk in medicines
- Quality assurance in formulation design and development
- Good pharmaceutical manufacturing practice (GPMP)
- Quality control procedures
- Post-market surveillance
- Antimicrobial stewardship

Prepared by: Dr. S. Dinesh Kumar, Assistant Professor, Dept of Microbiology, KAHE

INDUSTRIAL MICROBIOLOGY
15MBU601
III BSc MICRIOBIOLOGY

UNIT-I

Industrial microbiology deals with areas of microbiology involving econom
In history of industrial microbiology, the period of ignorance is
The period of discovery in history of industrial microbiology is from
The period of industrial development in history of industrial microbiology is
Bread was 1st baked around ----------B.C
Wine is produced from
The compound Microscope produced by Zaccharies Jensen had no provision for
Anton von Leewanhoek was able to obtain magnification upto ----------diam
In middle of last century fermentation was consider to be a ------process.
Fermentation was first described as chemical process by
In 1873,----------described that yeastsare involved in fermentation process fc
In fermentation, yeast converts ----------to----------and -----
who isolated the microbes associated fementation.
The optimum temperature condition for fermentation process was in range from----
Pasteur identified the organisms involved in the transformation of sugar to ----------
Lactic acid organism is a ----------
who made an important discovery that fermentation takes place in absence of oxyge
In 1861 pasteur did experiments on ---------and----------fermentation
#VALUE!
The process of sterilization of wine introduced by Pasteur is called
which method is most satisfory method for long time preservation of microbes
The major antibiotics such as streptomycin and neomycin etc were isolated from----
During 1910-1920,----------and---------were produced by Industrial fermentation
During 1920-1930,---------- was produced by industrial fermentation
During 1930-1940, first vitamin to be produced by industrial fermentation is------
The acetone-butanol fermentation is also called as ----------process
The important quality of production strain is
The screening techniques involves ----------and----------
Primary screening technology involves the isolation of new microbial species exhib
Crowded plate technique is an example of ----------screening
The primary screening technique which is employed for a detect and isolating antib
Enrichment culture technology was designed by soil microbiologist----------
which technique is largely employed to identify the growth factor producing strain e
Neutral red, bromothymol blue dyes are added to partly buffered nutrient agar medi
Example of enrichment substrate used is
which screening helps in segregation microbe that have real potential in fermentatic
The suitable protective medium used at the ----------is 10% inositol in diss
The fermentation product produced by the identified industrial strain should be -----

The selected industrial strain is----------by secondary screening.
The process of lyophilization was first applied to microfungi on layers scale by -----
The important criteria in handling the industrially productive strain is to prevent
Mutation is done by----------and----------methods
Phosphorous pentoxide silica gel/freezing trap are examples of----------
In multivalent regulatory mechanism of a branched biosynthetic pathway, ----------
The two categories of mutants are----------and----------
The wild strain of *Coryrebacterium glutamicus* secretes both -------and----------
The mutant strain of *Coryrebacterium glutamicus* produces upto ----------g of
The oil used in oil overlay method is
The lysine biosynthesis, the end products lysine and threonine inhibit the enzyme.
An analogue of threonine is ----------
The analogue is ----------to sensitive mutant cells in the population.
An example of analogue resistant mutant is ----------capable of excreating threor
An example of revertant mutant is----------for the enzyme threonine deaminase.
.----------mutants are important in fermentation industry as produce high yields
Microbes are grown in especially designed vessels called----------, containing speci
Secondary metabolites are produced during _____.
Wine is produced from ----------
The great break through came with observation of----------under simple micros
Anton van Leewanhoek observed ----------in his own scarpings, water.
----------made an important discovery that fermentation takes place in absence c

OPT I	OPT 2	OPT 3	OPT 4
from costly substrates	from cheaper and dis	from unavailable substrat	from foreign countrie
pre-1800	1800-1900	post-1900	post-2000
pre-1800	1800-1900	post-1900	post-2000
pre-1800	1800-1900	post-1900	post-2000
1000	2000	3000	4000
malt	molasses	grapes	sugarcane
resolution power	focusing	light facility	specimen holding
150-300	160-270	140-250	150-200
biological	physical	chemical	electrical
Pasteur	Robert Koch	Liebig	Anton van Leewanho
Pasteur	Schwann	Robert Koch	Berzelius
carbohydrate, alcohol, carbonic	fatty acids, alcohol, c	sugar, alcohol, carbonic a	starch, alcohol, carbo
Schwann	Bertholet	Pasteur	Koch
20-40	30-50	40-60	20-30
pyruvic acid	lactic acid	citric acid	stearic acid
fungi	bacteria	virus	protozoa
Schwann	Pasteur	Koch	Bertholet
butyric acid and acetic acid	acetone and butanol	lactic acid and acetic acid	acetic acid and citric a
Dumas and Napoleon 111	Dumas and Flemming	Napoleon111 and Schwa	Duman and Leewanh
pasteurization	ultrafiltration	low temperature,high hol	high temperature,low
mineral oil overlay	lyophilisation	cryopreservation	periodic transfer
Bacillus sp	*Staphylococcus sp*	*Streptococcus sp*	*Streptomyces sp*
ethanol and glycerol	lactic acid and amyla	acetone and n-butanol	acetone and lactic aci
lactic acid	acetic acid	citric acid	glutanic acid
riboflavin	vitamin B12	vitamin C	vitamin D
Watsmann	Walksman	Websmann	Weizmann
should be a high yielding strain	unstable biochemical	produce underisable subs	not easily cultivate
primary and secondary	secondary and tertiary	primary and quartenary	secondary and quarte
desined color	desired shape	desired quality	desired property
primary	secondary	tertiary	quartenary
crowded plate technique	auxanography	enrichment culture techni	use of indicator dye
Pasteur	Koch	Ehrlich	Beijerinck
crowded plate technique	auxanography	enrichment culture techni	use of indicator dye
vitamins	growth factors	organic acids	amines
nutrient broth	cellulose powder	peptone	minimal media
primary	secondary	tertiary	quartenary
Commonwealth Mycological In	American type cultur	African type culture colle	Indian Mycological Ir
old	novel	gold	critical

optimized	priotized	compared	deselected
Raper and Alexander	Thomas and Alexand	Koch and Alexander	Koch and Thomas
stability	contamination	oxidation	reduction
physical and chemical	chemical and politica	physical and botanical	chemical and zoologi
crypreservation	desiccant	preservants	mineral oil overlay
single	double	triple	single, double, triple
autotropic mutants and mutants	phototropic mutants a	auxenic mutants and mut	auxotropic mutants ar
lysine and threonine	lysine and methionine	threonine and methionine	threonine and pectin

50	60	70	80
British Pharmacopoeia Medicin	American Pharmacop	Australian Pharmacopoei	African Pharmacopoe
aspartate kinase	homoserine phosphat	serine kinase	tryptophan synthase
α-amino,β-hydroxyvaleric acid	β-amino,β-hydroxyva	ч-amino,β-hydroxyvaleri	)£-amino,β-hydroxyv
ecofriendly	toxic	nutrient	non-toxic
Brevibacterium flavum	*Brevibacterium lactu*	*Brevibacterium aseptum*	*Brevibacterium gluta*
Hydrophiles	*Hydromonas*	*Hydrogenomonas*	*Hydromonothrobis*
auxotrophic	mutants resistant to a	constitutive	mutants sensitive to a
Fermentors	Batch cookers	swap medium	conical flasks
Lag phase	Log phase	Stationary phase	death phase
malt	molasses	grapes	sugarcane
plant	animals	microbes	God
bacteria	fungi	protozoa	viruses
Schwann	Pasteur	Koch	Bertholet

	CORRECT OPTION
s	from cheaper and disposable substrates
	pre-1800
	1800-1900
	post-1900
	4000
	grapes
	focusing
	160-270
	chemical
ek	Liebig
	Schwann
nic acid	sugar, alcohol, carbonic acid
	Pasteur
	30-50
	lactic acid
	bacteria
	Pasteur
acid	butyric acid and acetic acid
oek	Dumas and Napoleon 111
holding ti	pasteurization
	lyophilisation
	Streptomyces sp
d	acetone and n-butanol
	citric acid
	riboflavin
	Weizmann
	should be a high yielding strain
nary	primary and secondary
	desired property
	primary
	crowded plate technique
	Beijerinck
	auxanography
	organic acids
	cellulose powder
	secondary
nstitute	Commonwealth Mycological Institute
	novel

optimized
Raper and Alexander
contamination
cal physical and chemical
desiccant
single, double, triple
ıd mutants autotropic mutants and mutants resistant to analogues
lysine and threonine

ːia Medicir British Pharmacopoeia Medicinal Paraffin oil.
aspartate kinase
aleric acid α-amino,β-hydroxyvaleric acid
toxic
ns *Brevibacterium flavum*
Hydrogenomonas
nalogues. constitutive
Fermentors
Log phase
grapes
microbes
protozoa
Pasteur

2018

Media Formulation

- Thorough analysis is essential to establish a suitable medium for an individual fermentation process
- All most all microbes need water, energy sources, sources of carbon and nitrogen, certain mineral elements and perhaps vitamins plus oxygen if microbes are aerobic
- It is easy to devise a medium containing pure compounds on a small scale but this medium may be unsuitable for use in a large scale fermentation processes
- Following are the criteria imperative to consider while designing a medium for large scale production
- The medium should support the maximum production of yield of product per gram of substrate used
- It should promote maximum accumulation of the product
- The maximum rate of product formation should be achieved
- There should be minimum production of unwanted products
- Constituents of the medium should be available throughout the year at cheaper rate and nearby area
- There should not be any undesirable changes in the consistency of the medium during preparation of media and after sterilization
- There should not be any difficulty in the operations like aeration, agitation during the production process and downstream operations like detection, isolation, extraction, purification and waste treatment

CARBON SOURCES

The rate at which the formation of the product take place is influenced by the rate at which the carbon source is metabolized Many time, sugars which metabolize rapidly results in fast growth of the organism and less productivity of secondary metabolites

Preparation of the Inoculums

Microbial inoculums have to be prepared from the preservation culture so that it can be used for the fermentation process. The aim of inoculum preparation is to select microorganisms with high productivity and to minimize low productive, mutant strains. The process involves several steps:

First generation culture is prepared from the preservation culture on agar slants which is then sub-cultured to prepare “working culture”. At this stage the microorganisms start growing. In small scale fermentation processes working culture is used as inoculum, but for large scale fermentation inoculum preparation involves additional steps.

Prepared by: Dr. S. Dinesh Kumar, Assistant Professor, Dept of Microbiology, KAHE

Second, sterile saline water or liquid nutrient medium containing glass beads is added to the agar slants and shaken so that microbial suspension is prepared. This suspension is transferred to a flatbed bottle which contains sterile agar medium. The microorganisms are allowed to grow by incubating the bottle. NMEICT-MHRD (Govt. of India) Project on - Creation of e-Contents on Fermentation Technology Project control No: RE-02091011297, Christ College, Rajkot, Gujarat, India

Third, the microbial cells from the flat bed bottles are transferred to a shaker flask containing sterile liquid nutrient medium and is placed on a rotary shaker bed in an incubator. Microorganisms grow at a rapid rate due to aeration.

Fourth, microbial cells from the shaker flask can be used as seed culture which are then added to small fermenters and allowed to grow for 1-2 days. This simulates conditions that exist in the larger fermenters to be used for production of metabolites. Finally, the microorganisms are transferred to the main fermentation vessel containing essential media and nutrients.

Culture Medium

Media requirements depend on the type of microorganism being used in the fermentation process, but the basic requirements remain the same source of energy, water, carbon source, nitrogen source, vitamins, and minerals. Designing the media for small scale laboratory purpose is relatively easy, but media for industrial purpose are difficult to prepare.

The culture medium should:

 Allow high yield of the desired product and at fast rate,

 Allow low yield of undesired products,

 Be sterilized easily,

 Yield consistent products i.e., minimum batch variation,

 Be cheap and readily available,

 Be compatible with the fermentation process,

 Not pose environmental problems before, during, or after the fermentation process.

The culture medium will affect the design of the fermenter. For example, hydrocarbons in the media require high oxygen content so an air-lift fermenter should be used. Natural media ingredients are cheap but they have high batch variation. On the other hand pure ingredients (also called defined media or formulated media) have very little batch variation but are expensive. The media should support the metabolic process of the microorganisms and allow bio-synthesis of the desired products.

Carbon & Energy source + Nitrogen source + Nutrients Product(s) + Carbon Dioxide + Water + Heat + Biomass

Media are designed based on the above equation using minimum components required to produce maximum product yield.

Important components of the medium are carbon sources, nitrogen sources, minerals, growth factors, chelating agents, buffers, antifoaming agents, air, steam, and fermentations vessels.

Carbon Sources: Product formation is directly dependent on the rate at which the carbon source is metabolized; also the main product of fermentation determines the type of carbon source to be used. Carbon sources include carbohydrates, oils and fats, and hydrocarbons.

Carbohydrates: These are the most commonly used carbon sources in the fermentation process. Starch is easily available carbohydrate obtained from maize, cereals, and potatoes. It NMEICT-MHRD (Govt. of India) Project on - Creation of e-Contents on Fermentation Technology Project control No: RE-02091011297, Christ College, Rajkot, Gujarat, India

is widely used in alcohol fermentation. Grains like maize are used directly in the form of ground powder as carbohydrate. Malt and beer made from barley grains contain high concentrations of different carbohydrates like starch, sucrose, cellulose and other sugars. Sucrose is obtained from sugar cane and molasses. Molasses is one of the cheapest sources of carbohydrate. It contains high sugar concentration and other components like nitrogenous substances and vitamins and is used in alcohol, SCP (Single-cell Protein), amino acid, and organic acid fermentations.

Extraction and purification of the products is expensive. Sulfite waste liquor is the by-product of the paper industry; it contains carbohydrates and is used in yeast cultivation. Whey is the byproduct of dairy industry. It is used in alcohol, SCP, gum, vitamins, and lactic acid fermentation.

Oils and Fats: Vegetable oils are used as a carbon source. Oils provide more energy per weight compared to sugars. They also have anti-foaming properties but are generally used as additives rather than as the sole carbon source. Examples are olive oil, cotton seed oil, soya bean oil, linseed oil, and lard (animal fat).

Hydrocarbons: C12-C18 alkanes can be used as carbon sources. They are cheap, and have more carbon and energy content per weight than sugars. They can be used in organic acids, amino acids, antibiotics, enzymes, and proteins fermentation.

Nitrogen Sources: Ammonia, ammonium salts, and urea are the most commonly used nitrogen sources in the fermentation process. Ammonia also serves the purpose of pH control. Other substances used as nitrogen sources are corn-steep liquor, soya meal, peanut meal, cotton seed meal, amino acids, and proteins.

Minerals: Calcium, chlorine, magnesium, phosphorous, potassium and sulfur are the essential minerals for all media. Other minerals like copper, cobalt, iron, manganese, molybdenum, and zinc are needed in trace amounts and are generally present as impurities in other components. The specific concentration on these elements depends on the micro-organism being used.

Growth Factors: Vitamins, amino acids, and fatty acids are used as growth factors in the fermentation process to complement the cell components of the microorganisms.

Prepared by: Dr. S. Dinesh Kumar, Assistant Professor, Dept of Microbiology, KAHE

Chelating Agents: Chelating agents prevent formation of insoluble metal precipitates. They form complexes with the metal ions present in the medium and can be utilized by the microorganisms. Chelating agents are not required in large scale fermentation processes since some of the other ingredients like yeast extract will perform the function of forming complexes with the metal ions.

One example of a chelating agent is EDTA (ethylene diamine tetra acetic acid). EDTA is a versatile, being able to form six bonds with a metal ion. It is frequently used in soaps and detergents because it forms a complex with calcium and magnesium ions. These ions are in hard water and interfere with the cleaning action of soaps and detergents. Other chelating agents are citric acid and pyrophosphates NMEICT-MHRD (Govt. of India) Project on - Creation of e-Contents on Fermentation Technology Project control No: RE-02091011297, Christ College, Rajkot, Gujarat, India

Buffers: Buffers are used to maintain the pH of the medium as microbial growth is affected by the pH changes. Optimum pH for most microorganisms is 7.0. Commonly used buffers are calcium carbonate, ammonia, and sodium hydroxide.

Antifoaming Agents: Microbial process produces a large amount of foam in the fermentation vessel. This is due to microbial proteins or other components of the media. Foaming causes removal of cells from the media and their autolysis, thus, releasing more microbial foam-producing proteins, and aggravating the problem. Foam will reduce the working volume in the fermentation vessel, decrease rate of heat transfer, and deposit cells on the top of the fermenter. The air filter exits become wet allowing growth of contaminating microorganisms. Antifoaming agents are also called surfactants, i.e. they reduce the surface tension in the foam and destabilize the foam producing proteins.

Commonly used antifoaming agents are stearyl alcohol, cotton seed oil, linseed oil, olive oil, castor oil, soy bean oil, cod liver oil, silicones, and sulphonates.

Air: Air is required for aeration and is supplied to the fermenter by means of pumps or compressors. It is sterilized by passing through filters before being introduced. The amount of air required and the extent of purity depends on the fermentation process being carried out.

Steam: Steam is used to sterilize fermenters and other equipment and to control temperature. Continuous dry steam supply is required for the fermentation process and care should be taken to prevent condensation. NMEICT-MHRD (Govt. of India) Project on - Creation of e-Contents on Fermentation Technology Project control No: RE-02091011297, Christ College, Rajkot, Gujarat, India

Prepared by: Dr. S. Dinesh Kumar, Assistant Professor, Dept of Microbiology, KAHE

2018

1. Scale up and process innovation

Even if often no distinction is made between invention and innovation, the difference is definitely not a subtle one.

As a matter of fact, it is not always possible to turn a good idea into an innovation and put it into practice: an invention can sometimes lay unused for ages without paying back in terms of industrial realization and of profitable business.

This review will not go into discussion on issue related to the market, nor will deal with the attitude of companies and management to promote innovation. We shall focus instead on the technical knowledge and on the tools that are necessary to change an invention into a true innovation

The chemical business being mature, the attention should be paid more to the innovation of processes than to the invention of new products.

From this point of view, the study and the development of new reactors, that are able to convert raw material into products with high conversion and selectivity, play an important role in the innovation of the chemical business.

1.1. Main problems in the scaling up of reactors

Scaling up of reactors is a major task for chemical engineers and is the fundamental step in the realization and optimization of industrial plants.

The scale up activity represents the synthesis of the know how accumulated in the various phases of process development from the design of laboratory experiments and the derivation of kinetic correlations, to fluid dynamic experiments, mathematical modeling, design and operation of pilot and industrial plants.

The term ''scale up'' has been usually explained as ''how to design a pilot or industrial reactor able to replicate through a standard methodology the results obtained in the laboratory''.

This is a limiting definition, since experience has shown that it does not really exist a standard way through process innovation: actual production processes are the result of successful decisions, and sometimes of many mistakes.

Crucial factors in the ''scale up'' are not only the technical understanding but also the ability of assuming the risk of the business.

As a matter of fact, in the past, decisions have not always been sufficiently supported by adequate experimental evidences and, even today, industrial plant operation is mainly based on experience. From the above remarks, it can be drawn a broader definition of ''scale-up'', as a mixture of know how, innovative ideas, standard methodologies and basic criteria with a glimmer of entrepreneurship.

With reference to chemical reactors, as the core of a process, there is no general rule and no straightforward procedure to achieve these objectives.

The reasons are many:

- kinetic data are peculiar to the reactive system. Often kinetics are masked by transport phenomena and fluid dynamics to the point that sometimes they have no relevance for the process.
- industrial scale technologies are seldom related to laboratory equipment even if industry is full of enlarged laboratory equipment.
- completely different apparatuses are possible for the same reaction and reactions can be carried out in different phases: solution, suspension, fixed beds, trickle beds, fluidized beds, distillation and extraction columns.
- other issues, often ignored in the development work, such as impurities, aging of catalysts, corrosion, fouling, safety and environmental aspects can represent a major risk for the success.

Prepared by: Dr. S. Dinesh Kumar, Assistant Professor, Dept of Microbiology, KAHE

INDUSTRIAL MICROBIOLOGY
15MBU601
III BSc MICRIOBIOLOGY

UNIT-II

ATCC is ---------- founded in 1925
The specific gravity of oil used in presence of cultures is
.----------is the simplest and common method of maintaining microbial cultures.
The Drying-up of medium encourages good ---------of *Streptomyces sp*
.----------and----------are usually maintained in liquid medium
The mineral oil overlay method was firstused by Bwell and Weston in ----------
Temperature of liquid nitrogen is
In Pencillin fementation the precursor added is ----------
Buffering agents used in media formulation is
Foaming during fermentation process creates
The antifoaming agent used in pencillin fermentation is
The citric acid fermentation *Aspergillus niger* culture is grown at ----------pH va
.----------media is mainly used in fermentation process.
Which is the common raw material source used in fermentation process
----------is rich in biotin, panthothenic acid , thiamine, phorphorus and sulpl
In Beet molasses ----------is limiting compared to cane molasses
----------require biotin for growth in production.
In India there is very large utilization of cane blackstrap molasses in ----------i
The ----------contain 17% sugar, 1%acid and 0.3%ash
In grapes the nitrogen content should be ----------as it may result in underisable
----------is 6.6-7.1% in cheddar whey
Cheese whey is an important raw material in ---------production
.----------and----------are the main sources of starch
Wheat, maize and rice are example of ----------
Cellulose are carbohydrates made of repeating units of --------
In ----------industry the digestion process of wood with calcium bisulfate under h
Sulfite waste liquor contains ----------sugars
Wood molasses syrup contain ------of fermentable sugars
The untreated cellulosic wastes have been used in production of
During manufacture of starch, gluten from corn---------is formed by steeping of corn
The clean, yellow, fine powder prepared from embryo of cotton seed is called as ---
Pharmamedia is used as production media for ---------production.
Initially fermentation industries used corn steep liquor for -------production.
Soya bean meal is used as production medium for ----------
Vegetable oils are used as
which is the most preferred method compared to other agents for mass sterilization.
Sugar containing medium can’t be sterilized by prolonged heating because sugars u

----------are simplest method employed for sterilizing production medium.
Lyophilization is the most satisfactory method of long term preservation of microor
---------- is perhaps the most popular form of suspended metabolism.
Hwang in ---------- year recommends precooling to 7 degree Celsius.
Aim of ATCC is to
Oil should be autoclaved at _____
Lyophilization
Dessicant used in lyophilizer are
_____ is used in storage at very low temp
The soil culture tubes are kept in refrigerator at above _____ temperature
De Becze and liebmann in -------- year used the first large scale fermentor for the pr
Fungus Mycelium should be suspended in _____ medium to maintain its structure.
The volume of inoculum used to cultivate bacteria are
Carbon source used for production of tctracycline is _____
Phosphorous pentoxide silica gel/freezing trap are examples of----------
---------- stressed the importance of the elimination of air and moisture from lyophilized cultures prior to sealing of ampoules.
The main success of fermentation industry depends mainly on ----------
Secondary screening reveals whether the culture is homofermentative and ----------
In fermentation process, the---------of product in various organic solvents has to be
The important criteria in handling the industrially productive strain is to prevent ---
The analogue is ----------to sensitive mutant cells in the population.
----------method is used specially for preserving sporing microbes such as *Bacillus,*
The special medium used in soil culture method is soil, sand and calcium carbonate

OPT I	OPT 2	OPT 3	OPT 4
American type culture collectio	African type culture c	Auxenic type culture coll	Australian type cultur
0.821-0.860	)0.865-0.890	0.752-0.812	0.718-0.835
serial subculture	lyophilisation	cryopreservation	dessication
growth	sporulation	storage	collection
Bacteriophages and *Actinomyce*	Bacteriophages and *c*	Viruses and *Acetobacter*	Bacteriophages and *S*
1945	1947	1949	1950
-130˚C	-150˚C	-176˚C	-196˚C
phenyl acetic acid	benzyl alcohol	Benzene	pyridine
mono and dihydropotassium ph	triiodosodium phosph	Tetraiodiosodium phosph	monophosphates
oxidation	reduction	contamination	production
lard oil	lard oil with ocetadec	decanol	mustard oil with deca
low	high	medium	very high
synthetic	semi-synthetic	non-synthetic	differential
food waste	agricultural waste	industrial toxic waste	Biofuel waste
cane molasses	beet molasses	fruit molasses	cheese molasses
biotin	pyridoxine	thiamine	pantothenic acid
Bacteria	yeasts	viruses	phages
textile	animal fodder	alcohol	dyes
rust	just	must	bust
high	medium	low	no
total solids	protein	lactose	fat
lactic acid	aspartic acid	glutamic acid	citric acid
molasses and cereals	cheese whey and tube	cereals, roots and tubers	cereals and cornsteep
molasses	cereals	roots	tubers
α-glucose	β-glucose	α-galactose	β-galactose
cheese, cheese whey	starch, starch liquor	paper pulp, sulfite waste	wood, molasses
1%	2%	3%	4%
60-80%	65-85%	70-90%	75-95%
ethanol	single cell protein	fuel	vitamins
sulfite waste liquor	corn steep liquor	wood molasses syrup	distillers soluble
corn-steep liquor	soya bean meal	Pharmamedia	distiller’s soluble
penicillin	streptomycin	tetracycline	griseofulvin
mushroom	penicillin	vitamin	organic acid
penicillin	streptomycin	tetracycline	griseofulvin
animal feed	antifoams	mushroom production me	pH adjustment
steam	UV light	ethidium bromide	chlorine
reaction with contaminants	charring	caramelization	reaction with phospha

continous fermentors	batch cookers	filtration	radiation
long term	short term	both a and b	none of the above
overlaying cultures with minera	lyophilization	nitrogen storage	none of the above
1966	1969	1974	1980
Prevent contamination	To maintain antibiotic	Preserve cultures	None of the above
15 lb/in2 for 2 hr	30 lb/in2 for 2 hr	45 lb/in2 for 2 hr	60 lb/in2 for 2 hr
Short term preservation of mic	long term preservatio	killing of Microorganism	None of the above
Phosphorus	silica gel	Magnesium	Copper
glycerol	mineral oil	Paraffin wax	All the above
5-80 C	4-60 C	2-40 C	6-80 C
1941	1944	1948	1940
Dimethyl sulphoxide	Rose Bengal medium	Sabouraud's dextrose agar medium	nutrient agar
0.1-2%	0.5-5%	3-5%	06-Apr
Molasses	Corn steep liquor	Barley	None of the above
crypreservation	desiccant	preservants	mineral oil overlay
Meryman	Dewald	leogetring	Louis Pasteur.
production temperature	inoculums developme	production strain	production medium
zygofermentative	heterofermentative	perifermentative	afermentative
solubility	insolubility	emulsion	viscosity
stability	contamination	oxidation	reduction
ecofriendly	toxic	nutrient	non-toxic
agar slant cultures	agar deep cultures	soil cultures	cryopreservation
20%,78% and 2%	18%,78% and 4%	10%,88% and 2%	30%,68% and 2%

	CORRECT OPTION
e collectio	American type culture collection
	)0.865-0.890
	serial subculture
	sporulation
treptomyce	Bacteriophages and *clostridium*
	1947
	-196˚C
	phenyl acetic acid
	mono and dihydropotassium phosphates
	contamination
nol	lard oil with ocetadecanol
	low
	non-synthetic
	agricultural waste
	cane molasses
	biotin
	yeasts
	alcohol
	must
	low
	total solids
	lactic acid
liquor	cereals, roots and tubers
	cereals
	β-glucose
	paper pulp, sulfite waste liquor
	2%
	65-85%
	single cell protein
	corn steep liquor
	Pharmamedia
	tetracycline
	penicillin
	streptomycin
	antifoams
	steam
ıtes	caramelization

batch cookers
long term
lyophilization
1966
Preserve cultures
15 lb/in2 for 2 hr
long term preservation of Microorganisms
silica gel
glycerol
5-80 C

1944

Dimethyl sulphoxide

0.5-5%
Molasses
desiccant
Dewald

production strain
heterofermentative
solubility
contamination
toxic
soil cultures
20%,78% and 2%

2018

Coagulation, flocculation and precipitation

Abstract

Fermentation is one of the most widely used reaction types to produce a wide range of chemical products. The downstream processing of a fermentation broth is a vital step to first isolate, and then purify, the wanted product. This report will focus on several chemical techniques within this; coagulation, flocculation and precipitation. Whilst coagulation and flocculation are involved in solid-liquid separation, precipitation is more often used later in purification, but the control of all three is highly important in achieving a success fermentation processing.

Background

Downstream processing is specifically for bioprocess purification. The main objective of the whole process is to separate, recover and purify products. Figure 1 presents the flowsheet of the basic steps of downstream processing. It contains first the removal of solids, then the primary purification stage, secondary purification and concentration, and final product isolation. The target of the solids removal stage for recovering the extracellular product is to separate and remove large particles with techniques such as centrifugation (P.F. Stanbury, 2017). Ultrafiltration, ion-exchange, liquid-liquid extraction and precipitation are some of techniques used within the primary isolation stage to fractionate or extract into fractions. After that, fractional precipitation or chromatographic techniques are used to purify and concentrate the product from any impurities. Afterwards, products are isolated by modification of flow-stream and drying may be needed as the final procedure (P.F. Stanbury, 2017).

This report focuses on three product separation techniques; coagulation, flocculation and precipitation. Mostly, coagulation and flocculation are applied in the solids removal stage, whilst precipitation is mainly in primary isolation stage.

Coagulation

Coagulation refers to the destabilization of a colloidal system by the action of additives (coagulants.) In the case of proteins, heat or mechanical stress cause a change in the charge of the system or the dispersed particles, enhancing their approximation and generation of micro-flogs. (Mazille & Dorothee, 2011)

The aggregation of particles, or generation of micro-flocs, is a fast process that takes only a few minutes. The process is driven by Van der Waal bonding forces and requires vigorous but short mixing and agitation in order to achieve its top efficiency. This guarantees that most of the particles or proteins are affected by the coagulant. (Mazille & Dorothee, 2011)

Two cases of coagulation can be distinguished; the chemical coagulation and the bio-coagulation (or denaturation) of proteins. The chemical coagulation usually refers to a change in the surface charge of the particles allowing particles to come closer. On the other hand, bio-coagulation refers to a change in the structure of, or the unfolding of, the protein followed by aggregation of proteins caused by coagulants, heat or vigorous mixing.

Chemical coagulation is widely used in waste water treatment. It allows the removal of colloidal particles and soluble substances which have length of 10nm to 10 µm. (Koohestanian, et al., 2008). The most common coagulants for waste water treatment that neutralize the negative charge of colloids are: inorganics such as aluminum sulfate, aluminum chloride, polyamine chloride, ferric sulfate, ferrous sulfate and ferric chloride or organics like polyamines and PolyDADMAC (ChemTreat, 2016). Many different types of reactors are used, with the most common being: (EPA, 2002)

- Mechanical mixers in which rotating paddles, turbines or propellers do the agitation.
- Hydraulic mixers that use the turbulence of flow or baffles and diffusers in a vessel to mix the coagulant.
- Pump mixing using a pump to insert the coagulant in the most turbid location of the vessel or pipeline.

Coagulation of proteins is usually referred as denaturation. This process is widely used in downstream processing for removing proteins and tannins from beer and to create new products such as cheese or desserts like meringues.

Prepared by: Dr. S. Dinesh Kumar, Assistant Professor, Dept of Microbiology, KAHE

2018

Beer is produced from barley which contains proteins and tannins. Tannins give a bitter taste to beer and are usually attached to proteins, therefore they must be removed in order to achieve good taste and decrease the turbidity of the beer. When the beer broth is heated, the proteins unfold exposing their internal hydrophilic part, allowing the generation of new hydrogen bonds between proteins and so the generation of clogs. This process is called “Hot break” and is one of the main downstream processes for beer purification. (Braukaiser, 2009) CHEM E3140- Bioprocess technology II Estefania Isaza 05/12/2016 Owain Dawson Yang Wang

Coagulation is also used in cheese making. The casein milk proteins are unfolded due to the addition of lime, lactic acid or bacteria (remit), before the casein coagulates and traps fat between the new caseins bonds, producing cheese.

Flocculation

In a lot of ways, flocculation is very similar to chemical coagulation. Like coagulation, the chemical basis is that particles, generally cells, are kept apart in a suspension via electrostatic repulsion, due to negative charges on their surface (Talib et al. 2016). Flocculation can be thought of as aggregating the micro-flocs generated into much larger flocs that then settle on the base of the vessel. Hence both are generally involved in the same stage of the downstream processing – the initial solid-liquid separation.

One key area for flocculation is within centrifugations, which are commonly performed within downstream processing to separate the solids from the liquid via density differences. The settling rate of the cells is increased greatly with diameter, hence meaning that the larger the aggregate, the faster and more well defined the separation of it from the liquid (P.F. Stanbury, 2017).

Following centrifugations, one or more filtrations are generally performed. Here comes into play the second useful aspect of flocculation – that the larger flakes mean that there are few particles capable of blocking the filter. The strength of them also results in a filter cake that is easier to remove. On top of this, some systems, such as in microbial biomass production, are not compatible with filter aids. The use of flocculation helps to overcome this and keep the process working well (P.F. Stanbury, 2017).

An additional use of flocculation in downstream processing is for example in the harvesting of microalgae to produce biofuel. Due to the low cost of the final product, centrifugation is not performed to separate, nor are simple sedimentations or screenings because of the very small size of cells. Pre-flocculation to concentrate the cells is therefore vital, and can even allow separation by an extremely low cost gravity sedimentation (Vandamme et al. 2013).

To overcome the electrostatic repulsion between particles, flocculation, or clarification, agents often are required to be added. Generally, like for coagulants, ionic compounds such as aluminium sulfate and ferric chloride are regularly used to induce flocculation (Heitner 2000). Additionally, the cells may not first want to aggregate due to their varying shapes meaning that fitting together issterically difficult. Therefore, high molecular weight polymer bridges, particularly cationic ones, are also used extensively (P.F. Stanbury, 2017).

Within downstream processing, an example of used flocculation agents are chitosan and synthetic cationic polyacrylamide within the recovery of 1,3-propanediol from fermentation broth (Hao et al. 2006; Xiu & Zeng 2008). This is a different use for flocculation, being within the recovery and purification from cell debris, instead of the initial solid-liquid separation. Generally, the same compounds used as the coagulants also work for the flocculation in the solid-liquid separation stage.

The occurrence of flocculation is heavily dependent on several factors. These include the nature of the cells (or other separated component), as well as the medium itself, for example its own ionic character. Changing many different properties also influences flocculation – such as raising temperatures increasing the rate of it or a changing pH altering the ionic character of the cell surfaces and medium.

A different case of the use of flocculation is in the brewing of beer (Gassara et al. 2015). Here, flocculation of the yeast cells occurs naturally anyway within the fermentation broth, thus allowing the cells to be easily separated. The control of this is highly important; if it happens too early then the taste can be reduced, whilst if it doesn’t happen then yeasty

deposits are left in the final beer product. The separated cells can also then be used in further fermentations, reducing costs.

The main additional use of flocculation is waste-water management; removing small particles contaminants, as well as pathogens (Ayekoe et al. 2016). It can also be used within the beneficiation of ores to remove inorganic particles from the waste streams, or also within the Bayer process for the inorganic residue from bauxite digestion. There are also many other cases where it is used. In these processes, other agents can be used, such as calcium hydroxide (lime) and even proteins like albumin (Heitner 2000).

Precipitation

Precipitation is a process that is used for product purification or recovery, especially in the primary purification. Figure 3 shows the basic mechanism of precipitation in a simple way (Diana Romanini, 2013). It can be attached to different stages for the product recovery process (P.F. Stanbury, 2017). Meanwhile, precipitation can also be used as a partial process for enrichment and concentration. Thus, the operating volume of the following process is lower than it was previously. Through the precipitation stage, products can be obtained directly or by further technique after a cell lysate (P.F. Stanbury, 2017). The mechanism of precipitation is that soluble compounds in solution become insoluble due to different chemical reaction parameters, e.g. pH. For instance, ethanol precipitation is an approach for protein precipitation by adding ethanol as an anti-solvent.

To perform the precipitation process, there are several agents in use.
1. Agents that aim to change solution pH conditions to precipitate products (P.F. Stanbury, 2017). The principle of this agents is that pH is changed to reach the compound isoelectric point, at which point no overall charge is present in the solution and solubility decreased.

2. Salts such as sodium sulfate are one agent in protein product recovery and fractionation, which is called salting-out also. (P.F. Stanbury, 2017) Salts added into solution bond stronger with water than protein, leading to protein precipitation (Michael L. Shuler, 2002). There is an equation which explains the relationship between solubility of proteins in solution and ionic strength, as listed below:

$\log S/S_0 = -K's(I)$
where solubility (S, g/l) works as function of solution ionic strength (I). S_0 is the protein solubility when I=0. K's is the salting-out constant value, which depends on temperature and pH of solution (Michael L. Shuler, 2002). A plot of this is shown in Figure 4. The basic mechanism of precipitation (Diana Romanini, 2013) illustrates the relationship of ion concentration and protein solubility. The equation below explains the connection of ionic molar concentration and ionic charge with ionic strength, where Ci is the ionic molar concentration and Zi is ionic charge: (Michael L. Shuler, 2002)
$I = 12Ci*Zi2$
Figure 4 Effect of inorganic salts on solubility of a typical protein (Michael L. Shuler, 2002)
Protein solubility is decreasing logarithmically along with ionic strength.
3. An organic solvent is added into a solution, resulting in precipitation (P.F. Stanbury, 2017). This method can precipitate proteins also. The principle of this method is to precipitate proteins by reducing the dielectric constant of the solution (Michael L. Shuler, 2002). One application of using organic solvents to precipitate protein is that of an active enzyme—pectinase precipitation. Chilled acetone or ethanol is used as the organic addition (S.A. Singh, 1999).

4. Polymers are also a possible precipitation agent (P.F. Stanbury, 2017). Non-ionic polymers such as polyethylene glycol (PEG) are normally used and those polymers work as organic solvents.
5. In addition to cell aggregation, polyelectrolytes can also be applied in compounds precipitation (P.F. Stanbury, 2017).

6. Triazine dyes, which are called protein binding dyes, bind to some categories of proteins and perform the precipitation process (P.F. Stanbury, 2017).

7. Affinity precipitants are the most studied and utilized precipitation agents nowadays, since these agents can bind and precipitate proteins selectively (P.F. Stanbury, 2017).

8. Temperature is not actually an agent, but it can lead to selective precipitation phenomenon in the purification step for various thermostable products and in the deactivation of cell proteases (Michelle Y.T. Nga, 2006).

Conclusion

Coagulation, flocculation and precipitation are three processes involved in the purification and separation of products from a fermentation broth. Coagulation and flocculation are both used within the solid-liquid separation stage of the downstream processing, with coagulation being the faster initial process. The stability of the colloidal suspensions is broken by coagulation, forming micro-flogs, before flocculation helps increase the size of the grouped particles to aid sedimentation.
Precipitation is more generally used within purification and concentration, later in the process. It can also be within the solid-liquid separation however if the products are dissolved in solution.
The use and control of all three techniques is important for efficient downstream processing.

Prepared by: Dr. S. Dinesh Kumar, Assistant Professor, Dept of Microbiology, KAHE

2018

Prepared by: Dr. S. Dinesh Kumar, Assistant Professor, Dept of Microbiology, KAHE

INDUSTRIAL MICROBIOLOGY
15MBU601
III BSc MICRIOBIOLOGY

UNIT-III

Microbes are grown in especially designed vessels called----------, containing speci
Fermentation tank should be provided with ports for ---------- addition.
----------are used in side of fermentors to avoid vortex formation
)----------tanks are used in production of all and lactic acid fermentation
The ----------in stainless steel fermentor gives resistance to halogen salts, lodine a
-----are used to regulate the addition of medium,nutrients, defoamers.
The fermentor vessel ----------should be increased with scale.
-------between top plate and vessel is very important to maintain airtight / aseptic co
---------device is used for giving air into fermentor
----------type of bubbles facilitate high oxygen transfer than ----------bub
The ideal aspect ratio for a fermentor is
The number of baffles used in fermentor of diameter 3dm3 is
)----------spargers are used widely large scale fermentation process
---------removes enough moisture from the gas leaving fermentor and prevent exces
In high quality bioreactor, all the processes in fermentation are controlled by
In-line, on-line and off-line are types of ----------
Example of In-line sensors are
----------sensors don't form integral part of fermentor.
)----------is generated due to mixing by agitator and microbes action on substrat
--------are semiconductors of Iron, Michel oxides exhibiting large change in resistar
Stainless steel ---------- sensors are used for temperature measurements in ferm
Gas flow rate is measured by ----------
The liquid flow rate is measured by using
Which gauge is used for measuring pressure under aseptic condition
-------is used for measuring the speed of agitator
Peristaltic pump is mainly used for addition of ---------and--------
Dissolved oxygen in fermentation process medium measured by using ----------
If initially foam has started forming interior ----------has been used to control fo
Oxygen diffuses from tubing into medium is measured by
pH denotes the presence of ----------in aqueous solution
The voltage difference between two electrodes is used to determine ----------of unl
----------,----------and---------- are three distinct areas of computer functio
----------system controls the addition of liquid from reservoir to fermentator
At ---------- cultures may be aerated by means of the *shake-flask technique* where
In ---------- fermentations broth or culture is aerated by *stirrers or agitators.*
In ---------- bioreactor are so designed that adequate supply of oxygen is obtained
In ---------- bioreactor are so designed that adequate supply of oxygen is obtained

Maximum ----------production may be achieved by satisfying the organism's max
_____ is to provide microorganisms in submerged culture with sufficient oxygen f
_____ ensures uniform suspension on microbial cells.
---------device is used to introduce air in fermenter
Aeration and agitation of a liquid medium may lead to the formation of
In fermentation, it is very important to find the ----------or---------- of product
---------and----------developed methods to analyse the biomass and product conce
The capacity of the batch fermentors _____
Large fermentors range from _____

Clogging problems occur in _____
Sparger size ranges from
The first pilot fermenter was erected in India at Hindustan Antibiotic ltd, Pune in the --------- year.
-------- type of bioreactor is used for vinegar production.
---------- is used for sep and other algal protein production.
---------- is used as a enzyme bioreactor.
---------- are provided to maintain constant temperature inside the bioreactor

The impeller should be ---------- of the vessel diameter.
Range of fermentation tank used in enzyme production.
-------- fermenter is called as elongated non-mechanically stirred fermenter
_____ fermentor is a gas tight baffled rise tube connected to a down comer tube.

Multiple air lift fermenter Is designed by
The inoculum level introduced into a production tank is usually _____.
-------- fermenter is called as elongated non-mechanically stirred fermenter
_____ fermentor is a gas tight baffled rise tube connected to a down comer tube.

OPT I	OPT 2	OPT 3	OPT 4
Fermentors	Batch cookers	swap medium	conical flasks
contamination	medium	inoculums	foam
spargers	bearing glands	rotameter	baffles
stainless steel	glass	copper	wooden
chromium	molybdenum	nickel	tungsten
syringe pumps	peristaltic pumps	feed pumps	pressure pumps
diameter	thickness	height	design
baffles	sealing	sparger	clamp
sparger	baffles	shaft	bearings box
larger, smaller	smaller, larger	medium, large	very small, very medi
03:01	04:01	05:01	06:01
2 TO 4	4 TO6	6 TO 8	8 TO 10
porous	orifice	nozzle	combined sparger agi
baffles	heat exchange	cooler	exit gas cooler
agitator	)aeration	process controller	cooler
foamers	agitator glands	shaft	sensors
Ion-specific sensors	mass spectrophotome	antifoam probe	medium addition prob
in-line	on-line	off-line	fermentor
energy	heat	resistance	current
mercury in-glass thermometers	electrical resistance	thermistors	electrical impedance
Pt 100	Pl 100	Pb 100	Ps 100
thermometers	rotameters	pistonmeters	torsion dynamometer
thermometers	thermal mass flowme	pistonmeters	torsion dynamometer
peristaltic	diaphragm	diaguls	bourbon tube
voltmeter	torsion dynamometer	ammeter	taometer
medium and inoculums	acid and base	buffers and antifoamers	salts and growth facto
galvanic electrode	pH electrode	thermometers	thermistors
antifoamers	mechanical foam brea	water	controlling agitator/ae
galvanic electrode	paramagnetic gas ana	platinum electrode	thermistors
hydrogen ion	hydroxyl ion	carboxyl ions	carbonyl ion
temperature	pH	moisture	dissolved oxygen
logging of process data	data analysis	process control	logging of data, data a
analog control	direct control	direct digital control	human control
pilot scale	laboratory-scale	industrial scale	semi-industrial scale
Pilot- and industrial-scale	pilot scale	industrial scale	laboratory-scale
bubble columns	CSTR	PACKED BED	FLUIDISED BED
AIR LIFT	CSTR	PACKED BED	FLUIDISED BED

waste	biomass	toxic	
Aeration	Agitation	Impeller	Baffler
Aeration	Agitation	Sparger	Baffler
Spargers	Impellers	Baffles	Turbines
Acid	Alkali	Foam	Air
gases or distribution	transport or energy	productivity or conversio	concentration or cons
Hump and Honey	Humphery and Coone	Hughes and Humphery	Hyhes and Cooney
10 – 12 litre	12 – 15 litre	20 -40 litre	6 – 8 litre
2000 – 5,000 gallons	5000 – 10,000 gallons	10,000 gallons	none of the above.
Bacteria	Algae	Mycebial	Mycorhiza
1/64 – 1/32 inch	1/32 – 1/18 inch	1/48 – 1/32 inch	1/24 – 1/12 inch
1920	1930	1940	1950
packed tower	photo bioreactor	pulsed column	bubble column
packed tower	photo bioreactor	pulsed column	bubble column
packed tower	photo bioreactor	pulsed column	bubble column
baffles	cooling coils	stirrer gland	sparger
1\1	none of the above	1\4	1\3
1500 30,000	1000 – 30,000 c	gallons	none of the above
Tower	Airlift	Cylindraconical	Deep jet
Tower	Air lift	Cylinder conical	Deep jet
Bakker etal	Okabe etal	Bacon etal	Dawsa
0.5-5%	5 – 25%	20 – 40%	50%
Tower	Airlift	Cylindraconical	Deep jet
Tower	Air lift	Cylinder conical	Deep jet

CORRECT OPTION

Fermentors
inoculums
baffles
wooden
molybdenum
feed pumps
thickness
sealing
sparger
ıum smaller, larger

05:01

6 TO 8
tator nozzle
exit gas cooler
process controller
sensors
ɔe antifoam probe
off-line
heat
thermistors
Pt 100
rotameters
thermal mass flowmeter
diaphragm
torsion dynamometer
ɔrs acid and base
galvanic electrode
ɛration spe mechanical foam breaker
paramagnetic gas analyzer
hydrogen ion
pH
ıanalysis an logging of data, data analysis and process control
direct digital control
laboratory-scale
Pilot- and industrial-scale
bubble columns
AIR LIFT

biomass
Aeration
Agitation
Spargers
Foam
umable productivity or conversion yield
Humphery and Cooney
10 – 12 litre
2000 – 5,000 gallons

Bacteria
1/64 – 1/32 inch
1950

packed tower
photo bioreactor
pulsed column
cooling coils

1\3
1500 30,000
Tower
Air lift

Bakker etal
5 – 25%
Tower
Air lift

2018

Role of Microbes in Food and Industrial Microbiology

Microorganisms, particularly the bacteria and fungi, have served humans since hundreds of years for the purpose of food, drugs, and other high-value chemical products. The use of microbes for fermentation is known since Neolithic age. Microbes not only give a good taste, texture and smell to the foods, but also produce certain inhibitory compounds that help in stopping food spoilage thus increasing the storage and safety of food.

Lactobacilli are important in the production of foods that require lactic acid fermentation, notably dairy products [yogurt and cheese], fermented vegetables [olives, pickles, and sauerkraut], fermented meats [salami], and sourdough bread. The use of Lactobacilli in the food industry has a long history, and the functions of the bacteria in the industrial setting have been well studied [1]. The genera important members of this group are *Lactobacillus, Leuconostoc, Pediococcus* and *Streptococcus*. These organisms are heterotrophic and generally have complex nutritional requirements due to lacking of many biosynthetic capabilities. Consequently, most species have multiple requirements for amino acids and vitamins [2]. Metchnikoff winner of a Nobel Prize for his pioneering descriptions of phagocytosis, was interested in the ageing process. While modern research on this topic concentrates on the maintenance of non-mutated DNA sequences, Metchnikoff focused on the gut micro-biota as a source of intoxication from within [3]. According to Metchnikoff, the

bacterial community residing in the large bowel of humans was a source of substances toxic to the nervous and vascular systems of the host. These toxic substances, absorbed from the bowel and circulating in the bloodstream, contributed to the ageing process. Gut bacteria were thus identified as the causative agents of "autointoxication." The offending bacteria were capable of degrading proteins [putrefaction], releasing ammonia, amines, and indole, which, in appropriate concentrations, were toxic to human tissues. The term 'Probiotic' first coined by Lilleyand Stillwell [4] in an entirely different context to describe substances secreted by one type of microorganism that stimulated the growth of another [probiotic to contrast with antibiotic], was subsequently used to describe "organisms and substances which contribute to intestinal

microbial balance" [5]. Fuller's definition [6] for probiotic "a live

microbial feed supplement which beneficially affects the host animal

by improving its intestinal balance," has been widely used. Similary,

fungi especially *Saccharomyces* sps. have found historic use in baking

and brewing industry. Particularly *S. cerevisiae* plays an important

role in revenue generation from brewing industry so much so that *S.*

cerevisiae was declared official state microbe by the state of Oregon in

2013. Other fungi such as *Aspergillus* sps. and *Penicillium* sps. have also

found use in food industry particularly due to the enzymes obtained

Prepared by: Dr. S. Dinesh Kumar, Assistant Professor, Dept of Microbiology, KAHE

from them [7]. Fungi have an important role in probiotics preparation as well.

The best example of drug production by microbes lies in antibiotics production. The Penicillin was discovered in 1928 by Scottish scientist Alexander Fleming and later several derivatives of it brought a kind of revolution in the world of antibiotics. However in the present time, microbes are used for production of secondary metabolites and combinatorial biosynthesis of drugs, thus paving way for newly discovered antibiotics, such as candin, semisynthetic versions of older antibiotics such as ketolides; older under-utilized antibiotics such as teicoplainins; and new derivatives of older undeveloped narrowspectrum antibiotics such as streptogramins [8]. In addition, many antibiotics are used as antitumor agents, immunosuppressive agents, anti-migraine agents, enzymes inhibitors etc. Another major class of pharmaceutical products obtained from microbes is of recombinant proteins where eukaryotic production system such as yeast is preferred over prokaryotic system due to its ability to carry out post translational modifications in the expressed proteins. The approved protein products produced exclusively in *Saccharomyces cerevisiae* are hormones [insulin, insulin analogues, non-glycosylated human growth hormone somatotropin, glucagon], vaccines [hepatitis B virus surface antigen - in the formulation of 15 out of the 28 yeast derived products-] and virus-like particles [VLPs] of the major capsid protein L1 of human papilloma virus type 6, 11, 16 and 18, urate oxidase from *Aspergillus flavus*, granulocyte-macrophage colony stimulating factor,

albumin, hirudin of *Hirudo medicinalis* and human platelets derived growth factor [9].

Bio-fuels research has gained momentum due to the fact that natural fuel resources are fast depleting and cannot be replenished. Microbes have an active role to play in production of bio-fuels. The most commonly used bio-fuels are bio-ethanol obtained from starch, fast emerging cellulosic ethanol and biodiesel from vegetable oils. Cellulosic ethanol has gained momentum owing to the easily available non-food and non-fodder raw material in the form of agriculture waste and forest residue. The enzyme cocktails required for hydrolysis of plant cell wall polysaccharides into monomers are derived from microbes and are mainly comprised of glycoside hydrolases [10]. The fermentation of these monomers into ethanol requires the use of microbes particularly yeast and *coli* [11]. Many other approaches such as Consolidated Bioprocessing [CBP] [12], Simultaneous Saccharification and Fermentation [SSF] [13], are being attempted in order to bypass the need for expensive enzyme cocktails. Such processes require microbes that can be developed either by engineering of the native cellulolytic mechanism of the microbe to improve product related properties, or by engineering a non-cellulolytic organism that exhibits high product yield and titer by expressing heterologous cellulose system [14]. Besides, microbes such as Clostridia are being developed for improved production of advanced fuels such as butanol and isopropanol which are considered superior fuels than ethanol due to higher energy density and being non-hygroscopic in nature [15].

The huge amount of data available from various genomic, transcriptomic, proteomic and metabolomic studies has paved ways to engineer and program microorganisms to produce desirable fuel and other products of commercial importance. Synthetic biology allows design and construction of new genetic parts such as promoter, transcription factors, ribosomal binding sites, degradation tags and transcriptional terminators that do not exist in nature but can be assembled and used for reprogramming biological systems to our advantage [16]. Microbial production of chemicals presents an alternative to ubiquitous chemical synthesis methods. Biosynthetic production is attractive because it can use a broad assortment of organic feed stocks, proceed under benign physiological conditions, and avoid environmentally deleterious byproducts. Biosynthetic alternatives are being pursued for a wide range of chemicals, from bulk commodity building blocks to specialty chemicals. Fortunately, natural sensors exist for a wide array of industrially relevant chemicals, including aliphatic hydrocarbons, short-chain alcohols, sugars, amino acids, polymer building blocks, and vitamins [17]. Microbes can be exploited in industries for production of various products useful to human and other animals. By way of engineering the microbes, there are more possibilities to exploit microbes in a better way.

Prepared by: Dr. S. Dinesh Kumar, Assistant Professor, Dept of Microbiology, KAHE

INDUSTRIAL MICROBIOLOGY
15MBU601
III BSc MICRIOBIOLOGY

UNIT-IV

Ancillary equipments in fermentors means the ----------
The sterilization temperature of the fermentation equirement is
After sterilization, all parts of fermentor are kept sterile by maintaining at ----------
There should be no permanent direct connection below ----------and----------parts
High quality valves such as----------should be used where joints are needed connec
Mycellium undergoes autolysis with raise in _____
Example for non ionic detergents
Silicon compound are example are of inert ------- agent
---------- is the main compound in corn steep liquor
Impeller are used in the fermentor helps in
_____ or _____ is added to adjust pH if too acidic.
_____ chromatography separates according to the affinity of the protein, for th
-------- chromatography is a powerful and highly selective purification technique.
Microbial cells and other insoluble materials are normally separated from the harve
Salts such as ammonium and ----------are used for the discover of protein
Dextrans can be precipitated out of a broth by the addition of _____.
---------- method is used for large scale enzyme purification
Ultrasonication has frequency of ----------khz
---------- damage the cell membrane and lead to the release of intracellular compc
_____ caused by a sudden change in salt concentration will cause disruption of a
_ is the separation process where the solvent molecules are passed to flow through
_____ of any product is often the last stage of a manufacturing process.
--------- drier is mostly used for drying of biological materials
_____ device are the most economical available for handling large volumes.
------- drying is an important operation in the production of biological and pharmac
_____ is an established and final purification of a diverse range of compounds.
_____ are metal strips roughly one- tenth of the vessel diameter and attached radic
A combined sparger and agitator may be used in ---------fermenter
---------were first used as careeier for antifoam in antibiotic processes
Which is the by-product after starch extraction from maize.
Chemically defined amino acid media devoid of protein are used in production of _
_____does not appear to play a nutritional role in the metabolism of fungi.
_____ is used to carry out microbiological process on batch basis.
Small lab fermentor is in the size range of
Larger fermentor range from _____ gallous.
pH control is achieved by _____ device.
_____ consists of circular discs to which blades are fitted with bolts.
Size of the holes in the sparger ranges from_____

Steady state condition can be achieved by operation on _____ principles.
CSTF is expanded as
The temperature of _____ in the production tanks is satisfactory during fermenta
Industrial alcohol production can be carried out in very large fermentor upto _____
_____ chromatography separates according to the affinity of the protein, for the surface of the solid matrix
-------- chromatography is a powerful and highly selective purification technique.

Microbial cells and other insoluble materials are normally separated from the harvested broth by_____ or _____.
----------is added along with stainless steel fermentor during construction im

In antibiotic manufacturing processes, the fermentation time ranges from_____
Micro filtration refers to the separation of suspended material such as bacteria by using a membrane with spore sizes of_____

Chromatography is based on the_____
The purification and recovery of the production after fermentation is called_____
Batch fermentation is also called_____
The capacity of laboratory fermentors is_____
Cell lysis becomes an important operation if the product is _____
Precipitation is done by_____
If the product formed is extracellular then the method which is not used is_____
Which process uses a porous medium for the separation of the solid material from g
A centrifuge is used to separate molecules on the basis of their --------
Which is the preferred method for clarification of wine_____ ?
Chilled_____ and_____ can be used in the precipitation of proteins mainly
Which separation technique is based on differential partitioning between two phase
Two phase aqueous extraction requires ----------

OPT I	OPT 2	OPT 3	OPT 4
seed tank	fermentation medium	extra connection	antifoamers
120°C – 15 min	120°C – 20 min	115°C – 15min	115°C – 20min
positive	negative	no	zero
medium and air	sterile and non-sterile	mixing and air	probes and medium
plastic	β-hydroxybutyrate	silica gel	rubber
pH value	temp	Ionic conc	none
Tween 80	Tween 20	Tween 40	none of the above
Antibacterial	Antifoam	Anti fungal	Antiprotozal
Lactic acid	Amino acid	Tartaric acid	Lactose
Aeration	Antifoaming	Agitation	Absorption
Ammonia	Sodium hydroxide	Both a or b	Sulphuric acid
Adsorption	Affinity	Ion exchange	Column
Adsorption	Affinity	Ion exchange	Column
Filtration	Centrifugation	Filtration OR centrifugat	Sedimentation
NH3SO4	Na2SO4	CaCl2	Ca
Methanol	Ethanol	Butanol	Alcohol
Liquid shear	Solid shear	Ultrasonication	Freeze thawing
200	2	20	2000
Osmotic shock	Alkali treatment	Detergent	Enzyme
Osmotic shock	Alkali	Protease	SDS
Ultra filtration	Reverse osmosis	Liquid membranes	pumping
Filtration	Centrifugation	Drying	Packing
Freeze	Spray	Drum	Tray
Freeze	Spray	Drum	Tray
Freeze	Drum	Spray	Tray
Drying	Crystallization	Filtration	HPLC
Sparger	Baffler	Magnetic devices	Impellers
Laboratary	Tower	Airlift	Batch
Oils	Fats	Carbohydrate	Acids
Corn steep liquor	Barley	Molasses	Soybean oil
Acids	Vitamin	Vaccines	Antibiotics
Chlorine	Fluoride	Copper	Cadmium
Batch fermentor	Continuous fermento	Fed batch fermentor	Semi continuous ferm
1-21+	0.5-11+	1-101+	5-101+
5000-10000	100-1000	1000-5000	10000-20000
Anti titrator	Aerator	Baffler	Impeller.
Impellers	Sparger	Baffler	Aerator
1/64-1/32	1/32- 1/64	1/32- 1/32	1/64 -1/64

Chemostatic	Turbidostatic	Both chemostat and turbi	Photostat
Continuous stirred tank ferment	Continuous solid tank	Cell suspended tank ferm	Continuous solid type
80°F	70°F	90°F	100°F
12500	125	25000	100000
Adsorption	Affinity	Ion exchange	Column
Adsorption	Affinity	Ion exchange	Column
Filtration	Centrifugation	filtration or centrifugation	Sedimentation
chromium	nickel	tungsten	silicone
2-3 weeks	1-2 weeks	4-5 weeks	2-4 weeks
0.02 to 10μm	1-10A°	20-30μm	10-200A°
different rate of movement of the solute in the column	separation of one solute from other constituents by being captured on the adsorbent	different rate of movement of the solvent in the column	solvent differnces
Upstream process	Downstream process	Surface fermentation	None of these
Closed system	Open system	Fed-batch system	None of these
12–15 liters	2000 gallons	500 liters	10000 gallons
Extra cellular	Heat labile	Toxic	Intracellular
PEG(polyethylene glycol)	Triazine dyes	Ammonium and sodium s	PEG, triazine, ammor
Reverse osmosis	Ultra filtration	Chromatography	Freeze thawing
Precipitation	Filtration	Centrifugation	Foam separation
Size	Shape	Density	Size, shape and densi
Precipitation	Chromatography	Centrifugation	Foam separation
Ethanol, Ethane	Acetone, Ketone	Glycol, Glycerol	Ethanol, Acetone
Filtration	Precipitation	Centrifugation	Chromatography
Low water content and low surf	Low water content an	High water content and l	High water content ar

	CORRECT OPTION
	extra connection
	120°C – 20 min
	positive
	sterile and non-sterile
	rubber
	pH value
	Tween 20
	Antifoam
	Lactic acid
	Agitation
	Both a or b
	Adsorption
	Affinity
	Filtration OR centrifugation
	Na2SO4
	Methanol
	Liquid shear
	20
	Osmotic shock
	Osmotic shock
	Reverse osmosis
	Drying
	Spray
	Spray
	Freeze
	Crystallization
	Baffler
	Laboratary
	Oils
	Corn steep liquor
	Vaccines
	Chlorine
ıentor	Batch fermentor
	1-21+
	5000-10000
	Anti titrator
	Impellers
	1/64-1/32

Both chemostat and turbidostat
e fermentor Continuous stirred tank fermentor
80°F
12500
Adsorption

Affinity

filtration or centrifugation

nickel

4-5 weeks

0.02 to 10μm

different rate of movement of
the solute in the column
Downstream process
Closed system
12–15 liters
Intracellular
nium and s PEG, triazine, ammonium and sodium sulphate
Freeze thawing
Filtration
ty Size, shape and density
Centrifugation
Ethanol, Acetone
Chromatography
nd high sur High water content and high surface tension.

2018

EVALUATION OF PYROGENS TESTS IN PHARMACEUTICAL PRODUCTS

A comparison of methodologies for detection of pyrogens in pharmaceutical products was performed. The rabbit pyrogen test was optimized and the dose-response curve was obtained for the 2nd International Standard for bacterial endotoxins, establishing 13.81 EU/mL/kg as the concentration of endotoxin necessary to induce a temperature rise of 0.5ºC. The 0.5ºC cut-off was shown to give results that were more compatible with the pyrogenic doses for humans. The Limulus amoebocyte lysate test (LAL) was standardized with gel-clot and chromogenic endpoints, and used for the comparative evaluation of pharmaceutical products showing good agreement. The use of β-glucan-reactive and non-reactive LAL reagents identified some products with falsepositive results. The interference test was carried out and the specifications validated for some new products as the maximum valid dilution. The results emphasized the importance and limitations of the assays recommended for the evaluation of purity and quality control of parenteral medicinal products, improving the existing methodologies in the context of reduction and replacement in the use of animal models.

Key words: bacterial endotoxin, Limulus amoebocyte lysate, pyrogens, rabbit pyrogen test

INTRODUCTION

Pharmaceutical products intended for parenteral use must be free of pyrogens, which can originate from Gram-negative or Gram-positive bacteria, viruses and fungi. Endotoxins (Lipopolysaccharides, LPS) from Gram-negative bacteria are commonly found in parenteral pharmaceuticals and medical devices and are of particular concern to the pharmaceutical industry. Endotoxins are large molecular weight complexes (~106 Da) associated with, and shed from, the outer membranes of

Prepared by: Dr. S. Dinesh Kumar, Assistant Professor, Dept of Microbiology, KAHE

Gram-negative bacteria (8,14). Endotoxins consist of three distinct chemical regions: a lipid moiety (lipid A) which is linked to a polysaccharide core that is, in turn, linked to O-antigenic side-chains (8,18). Each endotoxin presents a composition and a variable structure that affects its function and biological activity; endotoxin functions include the induction of fever and acute phase proteins, headache and severe hypotensive shock. There is good evidence that the fever response to various exogenous pyrogens (e.g. endotoxin) is mediated by endogenous pyrogens, i.e. pyrogens generated by the host. Endogenous pyrogens have potent pyrogenic and inflammatory activities and include interleukin 1-α (IL-1α), interleukin-1β (IL-1β), tumor necrosis factor α (TNF-α) and interleukin-6 (IL-6) (10,21).

The rabbit pyrogen test based on the intravenous injection of a sterile solution was adopted for many years for the quality control of parenteral preparations (14). Alternatives for the refinement of the test, including the comparison of rabbit responses to two E. coli endotoxin preparations, suggested that the temperature cut-off of 0.6ºC should be decreased to 0.5ºC, as the criterion for a positive result (9,20). However, the rabbit pyrogen test has several drawbacks including low sensitivity, absence of quantitation, unsuitability for some product categories and the involvement of animals (1,6).

The observation that the blood (haemolymph) of the

horseshoe crab became clotted in the presence of the bacterial endotoxins gave rise to the Limulus amoebocyte lysate test (LAL), which is specific and sensitive for endotoxins from Gram-

*Corresponding author. Mailing address. Departamento de Farmácia Industrial, Centro de Ciências da Saúde, Universidade Federal de Santa Maria.

97105-900, Santa Maria, RS, Brasil. Tel/Fax: (+5555) 2208805. E-mail: sdalmora@ccs.ufsm.br

negative bacteria (11,20). However, the test can give falsenegative and false-positive results and, being a test for Gramnegative LPS, it does not detect Gram-positive exotoxins, viruses and fungi (7,13,16). In the LAL test, the LPS-induced reaction can be measured using various approaches, which were adopted together with the rabbit pyrogen test in the collaborative studies that established the 1st and 2nd International Standard for bacterial endotoxins (15,17). However, despite the specification of the LAL test for pharmaceutical products, there remain a number of complex preparations, such as biologicals, without specifications or that cannot be tested by the LAL test (6).

In the context of the development of alternatives for the refinement, replacement and reduction of biological tests, the LAL test and the rabbit pyrogen test are also used for the validation of novel in vitro assays under investigation, based on the use of cell lines, peripheral blood monocytes and whole blood (5,7,13,16).

The aims of the present study were to validate the specifications for the bacterial endotoxins test in parenteral pharmaceutical products, to refine the rabbit pyrogen test, to evaluate the responses of different LAL reagents, and to correlate the results of the two methodologies, thus contributing to the quality control of medicines.

MATERIALS AND METHODS

Reference standard and reagents

The 2nd International Standard for bacterial endotoxins, 10,000 EU/vial (WHO 94/580), was kindly donated by the National Institute for Biological Standards and Control (NIBSC), Herts, UK. Limulus amoebocyte lysate, 0.06 EU/mL was obtained from Endosafe (Charleston; SC, USA), Biowhittaker (Walkersville; MD, USA) and Cape Code (Cape Code; MA, USA). New methylene blue was purchased from Sigma (St. Louis; MO, USA). A number of parenteral pharmaceutical products were used, in some cases different batches of the same product, all within their period of validity. Other reagents and plasticware were purchased as sterile and pyrogen-free and glassware was baked at 250ºC for 1 h prior to use.

Rabbit pyrogen test

This test was carried out according to the literature (3,20), using the New Zealand white rabbit strain.

Limulus amoebocyte lysate test (LAL)

The bacterial endotoxin limits for the parenteral

pharmaceutical products without specifications were calculated as the maximum valid dilution (MVD) (2,20).

Interfering factors test

The inhibition/enhancement test was performed (2,20) on the sample solutions at a dilution less than the MVD, not containing any detectable endotoxins. The geometric mean endpoint concentration of the solutions was determined using the equation M = antilog ($\Sigma e/f$), where: Σe is the sum of the log endpoint concentrations of the dilution series used, and f is the number of replicate test tubes. If the sensitivity of the lysate determined in the presence of the sample solution under test is not less than 0.5 λ and not greater than 2 λ, the sample solution does not contain factors which interfere under the experimental conditions.

Gel-clot assay

The assays were performed in Petri dishes (2,3,20), adjusting the volumes and maintaining constant the relationship between them. Equal volumes of lysate and test solution or standard (usually 10 µL) were added to Petri dishes. The reaction mixture was incubated at 37ºC for 1 h. A number of two-fold serial dilutions were tested and the gel-clot endpoint was determined by adding 1 µL of a 0.2% new methylene blue solution, observing the mixing (negative reaction) or distribution on the surface of the gel (positive reaction). The endotoxin concentration was calculated by multiplying the reciprocal of the greatest dilution

of the test solution that gave a positive endpoint by the sensitivity (to endotoxin) of the lysate preparation; the results were expressed in EU/mL.

Chromogenic assay

The assay was performed in a microplate at 37 ±1ºC (2,20). Fifty µL of the standard or samples were dispensed into the appropriate microplate wells. Then, 50 µL of the Limulus amoebocyte lysate solution were added and the microplate was incubated for 10 min at 37 ±1ºC. One hundred µL of the substrate solution were pipetted and the reaction was stopped after 6 minutes by adding 100 µL of 25% acetic acid. The absorbance was read at 405 nm in a microplate reader and a standard curve plotted. The results were expressed in EU/mL.

RESULTS

Rabbit pyrogen test

The dose-response curve of the 2nd International Standard for bacterial endotoxins was obtained by recording the rise in temperature at 15 minutes intervals for three hours. The regression line was calculated and the concentrations that produced a temperature rise of 0.5ºC and 0.6ºC were calculated as 13.81 EU/mL/kg and 18.57 EU/mL/kg, respectively (Fig. 1).

Limulus amoebocyte lysate test (LAL), gel-clot

The labeled reagent LAL sensitivity of 0.06 EU/mL was confirmed before carrying out the assays. The interfering factors test was performed for the products without specifications

(Table 1), at dilutions not exceeding the MVD spiked with the

2nd International Standard, and the samples returned positive

50

S.L. Dalmora et al.

Table 1. Inhibition/enhancement test of pharmaceutical products, by the Limulus amoebocyte lysate (LAL) assay, with a sensitivity

of 0.06 EU/mL.

Products Endotoxins limit Maximum Sample Minimum Geometric

calculated valid dilution test valid dilution mean EU/mL

Ciprofloxacin 2 mg/mL 0.87 EU/mg 1:29 - - 1:16 0.06

Ketoprofen 100 mg/2 mL 3.50 EU/mg 1:2917 - - 1:32 0.03

Diclofenac 75 mg/3 mL 4.70 EU/mg 1:1958 - - 1:64 0.03

Dipyrone 500 mg/mL 0.70 EU/mg 1:5833 - - 1:10 0.03

Erythropoietin 2000 IU/vial 2.50 EU/2000 IU 1: 42 - - 1:2 0.03

recG-CSF 300 mcg/vial 2 EU/mL 1:33 - - 1:8 0.06

Calcium folinate 50 mg/5 mL 0.60 EU/mg 1:100 - - 1:1 0.04

Fluconazol 2 mg/mL 2.33 EU/mg 1:78 - - 1:2 0.06

Mesna 0.87 EU/mg 1:1458 - - 1:32 0.06

Midazolam 50 mg/10 mL 35 EU/mg 1:2917 - - 1:64 0.06

Pantoprazol 40 mg/10 mL 8.75 EU/mg 1:583 - - 1:256 0.03

Tenoxican 40 mg/mL 8.75 EU/mg 1:2917 - - 1:640 0.03

(-) Negative response.

Table 2. Results of pharmaceutical products by the LAL gel-clot test with different reagents.

Products Endotoxin limit Reagent Limulus amoebocyte lysate (LAL)

EU/mL

Ia IIa IIIb

EU/mL EU/mL EU/mL

Ampicillin 1000 mg/5 mL A 30 153.60 – 307.20 76.80 – 153.60 <0.06

Ampicillin 1000 mg/5 mL B 30 30.72 – 61.44 15.36 – 30.72 <0.06

Insulin 100 U/mL 80 <0.06 <0.06 <0.06

Erythropoietin 2000 IU/vial A 2.50 491.50 – 983 491.50 – 983 245.75 – 491.50

Erythropoietin 4000 IU/vial C 5 3.84 –7.68 7.68 –15.36 <0.06

Gentamicin 80 mg/2 mL 68 <0.06 <0.06 <0.06

Oxacillin 500 mg/5 mL 20 <0.06 <0.06 <0.06

Heparin 5000 IU/mL 150 <0.06 <0.06 <0.06

a β-glucans-reactive; b β-glucans non-reactive.

results, thereby enabling the determination of the minimum valid dilution of the sample free of interference and the endotoxin content in EU/mL.

The comparative results of LAL reagents using the gel-clot endpoint revealed interference with the β-glucan-reactive LAL reagent for ampicillin samples and erythropoietin C, which gave false-positive results (Table 2).

The LAL gel-clot test, more widely used as a qualitative or semi-quantitative test, was compared to the chromogenic, quantitative assay (Table 3) and produced comparable results for all samples with the exception of methylprednisolone, which gave a lower value for the chromogenic method.

The comparative evaluation of pyrogens by the qualitative rabbit pyrogen test and the semi-quantitative LAL test showed

a contamination level that, in most cases, was lower than the sensitivity of the lysate used (Table 4); furthermore, there was good correlation within the two assays.

DISCUSSION

The rabbit pyrogen test was studied in the context of the importance of alternatives that could contribute towards its refinement. A dose of 13.81 EU/mL/kg of the 2nd International Standard for bacterial endotoxins was identified as that which

produced a 0.5ºC rise in temperature (Fig. 1); this value, although variable according to the strain of the animals used, is recommended as a criterion for positive responses (9,17). Despite its shortcomings, the test is recommended by the Pharmacopoeias (2,3,20), and it is important for the validation of new in vitro assays under development (4,7,16).

The Limulus amoebocyte lysate test (LAL) is recommended (2,3,20) for the quality control of medicines, but the specifications need to be validated for new biological medicines, which are produced mainly through recombinant DNA technology (Table 1). The validation studies indicated geometric means between $\geq 0.5\lambda$ and $\leq 2\lambda$, with recovery between 50 and 200% (2,20). This

test is important in order to eliminate false-negative and false-positive results (13,14,16) caused by the interference of the active substance or components of the final product formulation. Thus, the establishment and validation of the specifications for the quality control of the medicinal products tested is recommended.

The evaluation of pharmaceutical products using different LAL reagents (Table 2) showed that some batches of ampicillin and human recombinant erythropoietin, which gave positive results with the β-glucan-reactive reagents, gave negative results when analyzed by the β-glucan nonreactive reagent, thus demonstrating interference.

These observations are important for quality control, assisting in the selection of the LAL reagent and the evaluation of the results obtained for the samples (12,19). It should be recalled that this difference between the reagents could result in the incorrect rejection of batches, mainly when the response levels are near to the maximum valid dilution specified for test compliance.

The comparative tests of pharmaceutical products using the LAL test with chromogenic and gel-clot endpoints gave similar results (Table

3). These experiments are important considering that the gel-clot method, being less expensive and easier to perform, has been used routinely for the quality control of medicines with limits declared as endotoxin units (2,3,20). In the present study, it was also used for comparison with the rabbit pyrogen test, showing good agreement (Table 4) and demonstrating the importance of both methodologies for the development and validation of new pyrogens tests.

Our results show that the rabbit pyrogen test, which is being gradually replaced by the LAL test,

Table 3. Results of pharmaceutical products by the LAL gel-clot and Chromogenic tests.

Products	LAL test Results	
	Gel-clot EU/mL	Chromogenic EU/mL
Cytarabine 100 mg/5 mL	<0.06	0.06 Pass
Diclofenac 75 mg/3 mL	<0.06	<0.06 Pass
Ranitidine 25 mg/mL	1.20 – 2.40	1.37 Pass
Heparin 5000 IU/mL	<0.06	<0.06 Pass
Erythropoietin 4000 IU/vial B	0.48 – 0.96	0.84 Pass
Furosemide 10 mg/mL	<0.06	<0.06 Pass

Methylprednisolone 500 mg/5 mL 245.75 – 491.50 146 Fail

Insulin 100 U/mL <0.06 <0.06 Pass

Vancomycin 500 mg/5 mL 2.40 – 4.80 3.20 Pass

Dexamethasone 4 mg/mL <0.06 <0.06 Pass

Oxacillin 500 mg/5 mL <0.06 <0.06 Pass

Gentamicin 80 mg/mL <0.06 <0.06 Pass

Metoclopramide 10 mg/mL <0.06 <0.06 Pass

recG-CSF 300 mcg/vial <0.06 <0.06 Pass

Vitamin K 10 mg/mL <0.06 <0.06 Pass

Table 4. Results of the parenteral pharmaceutical products by the rabbit pyrogen test and Limulus amoebocyte lysate test.

Products

Endotoxin limit

LAL test

Rabbit

EU/mL test

Amikacin 500 mg/2 mL 0.33 EU/mg <0.06 Pass

Ampicillin 1000 mg/5 mL A 0.15 EU/mg <0.06 Pass

Ampicillin 1000 mg/5 mL B 0.15 EU/mg <0.06 Pass

Ketoprofen 100 mg/2 mL 3.50 EU/mg* <0.06 Pass

Ciprofloxacin 2 mg/mL 0.87 EU/mg* <0.06 Pass

Cytarabine 100 mg/5 mL 0.07 EU/mg <0.06 Pass

Dexamethasone 4 mg/mL 31.30 EU/mg <0.06 Pass

Diclofenac 75 mg/3 mL 4.70 EU/mg* <0.06 Pass

Dipyrone 500 mg/mL 0.70 EU/mg* <0.06 Pass

Enoxaparin 100 mg/mL 0.01 EU/U anti-Xa <0.06 Pass

Erythropoietin 2000 IU/vial A 2.50 EU/2000 IU* 245.75 – 491.50 Fail

Erythropoietin 4000 IU/vial B 5 EU/4000 IU* 0.48 – 0.96 Pass

Erythropoietin 4000 IU/vial C 5 EU/4000 IU* <0.06 Pass

recG-CSF 300 mcg/vial 2 EU/mL* <0.06 Pass

Fluconazol 2 mg/mL 2.33 EU/mg* <0.06 Pass

Calcium folinate 50 mg/5 mL 0.60 EU/mg* <0.06 Pass

Gentamicin 80 mg/2 mL 1.7 EU/mg <0.06 Pass

Mesna 0.87 EU/mg* <0.06 Pass

Methylprednisolone 500 mg/5 mL 0.17 EU/mg 245.75 – 491.50 Fail

Midazolam 50 mg/10 mL 35 EU/mg* <0.06 Pass

Oxacillin 500 mg/5 mL 0.20 EU/mg <0.06 Pass

Pantoprazol 40 mg/10 mL 8.75 EU/mg* <0.06 Pass

Ranitidine 25 mg/mL 7 EU/mg 1.20 – 2.40 Pass

Saline solution 0.9% 0.50 EU/mL 48 – 96 Fail

Glucose 0.5% 0.50 EU/mL 1920 – 3840 Fail

Tenoxican 40 mg/2 mL 8.75 EU/mg* <0.06 Pass

Vancomycin 500 mg/5 mL 0.33 EU/mg 2.40 – 4.80 Pass

*Endotoxin limits calculated – DFI/UFSM.

52

S.L. Dalmora et al.

Figure 1. Linear regression line calculated from three

independent assays of the 2nd International Standard for

Bacterial endotoxins. Concentrations necessary to produce a

temperature rise of 0.5ºC (....) and 0.6ºC (—). Y= 0.21 + 0.021 X

($R2 = 0.92$; $p > 0.05$ for $n = 9$).

continues to be valid and necessary. However, the LAL test validation and the specifications established for the new recombinant biologicals will contribute towards assuring the quality and safety of parenteral pharmaceutical products.

INDUSTRIAL MICROBIOLOGY
15MBU601
III BSc MICRIOBIOLOGY

UNIT-V

The Aspergillus niger spores are developed by growing stock culture onto a solid --
Alpha amylase are produced by _____
Proteases are produced by _____
Acid proteases are produced by the fungi
_____ are usually employed for commercial prodution of ethyl alcohol
The most efficient industrial strain used in production of citric acid is ----------
---------- is often the best carbon substrate for production of citric aci(d)
The optimum pH for citric acid production is ----------
The optimum temperature for citric acid production according to Doelger and Presc
The citric acid fermentation using Aspergillus niger at low pH is highly favourable
The production vessel for citric acid must be made of ---------- as they are le
The citric acid fermentation takes up to ---------- days
----------have various structures, composition and are of low molecular weight co
Natural and semi-synthetic penicillins and cephalosporins are included in ----------
In ---------- group of antibiotics, β-lactum ring is foun(d)
---------- employed in production media yields high amounts of penicillin.
For the commercial production of penicillin high yielding strains have been selecte
The examples of two important naturally occurring pencillin are ---------- and ----
Staphylococcus aureus produces an enzyme called ------- which cleaves the β-lactur
The precursor for penicillin G is ----------
The ---------- medium contains glycerol, cane molasses, corn-steep liquor for produ
For removal of fungal mycelium ----------with rotoary vacuum filter has been em
For extraction of penicillin, ---------- solvent extraction technique is use(d)
The organic solvents such as amylacetate, butyl acetate are used in ---------- solver
The final industrial product is treated with ---------- to eliminate pyrogens.
.---------- is a penicillin without an acyl group, prepared from 6-amino penicillani
---------- of 6-amino penicillanic acid is carried out by using derivative of carboxyl
Citric acid is used as ---------- is food and pharmaceutical industries.
Several species such as Aspergillus clavatus, Penicillium luteum, Penicillium citrin
The foremost important bioparameter in citric acid fermentation is----------
When the ratio of surface area to volume is ---------- there is high yield of citric aci
The recovered fermentation liquor is treated with -------- forming precipitate of calc
Another method for extraction and purification of citric acid is by using tri-n-butyl
The problems faced during recovery of citric acid are --------
The yield of citric acid by submerged fermentation are ----------
In 1970, ---------- yeast was used for submerged culture process of citric acid p
One gram of glucose yields ---------- gms of ethanol in fermentation process.
The bioconversion of sugars to alcohols are mediated by ---------- of years.

The french word “vinaigre” means ----------

The temperature required for fermentation is ----------

There are ---------- stages in alcoholic fermentation.

The primary stage of alcoholic fermentation is carried out in open fermentation vats

The secondary stage of alcoholic fermentation proceeds for several weeks in --------

---------- is generally added in alcoholic production medium.

--------- inhibits the growth and activity of unwanted bacteria and yeasts.

The microorganismis namely Aspergillus niger, Rhizopus niveus, Rhizopus delem

Lipase is produced industrially using ----------.

During completion of enzymatic fermentation, the spent liquid is subjected to -----

The presence of ----------inhibits the formation of α-amylase enzyme.

The enzymes are purified using solvents such as----------

which enzyme is very important in bread making, beer making for flood industry.

The preservation of bacterial α-amylase enzyme in liquid formulation is--------% Na

The optimum pH for amylase industrial fermentation process is----------

Proteases enzymes are a complex mixture of ---------- &----------

which enzyme are unstable & loss of activity can occur during fermentation proces

The enzyme protease is produced from bacteria such as ----------

The optimum temperature & pH for protease enzyme fermentation is----------

Vitamin B12 is formed by linking of a cobinamide linked to a ----------

The nucleotide of cyanocobalamine has ---------- as its base.

Microorganisms namely Streptomyces griseus, Streptomyces olivaceus, Bacillus me

The strain used for vitamin B12 production is -------

The vitamin B12 fermentation takes about ---------- days

After removal of cobalamine from mycelium of Streptomyces olivaceus, ---------- i

The temperature of ---------- is favourable during fermentation of vitamin B12.

The yield of cobalamine is in range of ---------- mg/l at end of fermentation proce

OPT I	OPT 2	OPT 3	OPT 4
production medium	sporulation medium	growth medium	optimization medium
Aspergillus	*Mucor*	*Pencillium*	*fusarium*
B .subtilis	*B.sterothermophiles*	*B.tumefashions*	*none.of the above*
Aspergillus oryzae	*Rhizopus stoleniferou*	*fusarium sp*	*Altemaria sp.*
Sacchcromyces cerevasiae	*Candida albucans*	*Cryptococcus*	*none of the above*
Aspergillus niger	*Aspergillus terreus*	*Aspergillus clavatus*	*Aspergillus oryzae*
sugarcane molasses		wood molasses	corn steep liquor
2.60 to 3.20	3.6 to 4.20	2.20 to 1.60	1.2 to 2.6
25oC to 27oC	26oC to 28oC	28oC to 30oC	27oC to 29oC
sterilization of medium is not ef	citric acid is produce	oxalic acid formation is s	There is high rich of c
stainless steel	iron	aluminium	stainless steel and alu
5 to 8 days	6 to 9 days	7 to 10 days	8 to 11 days
antibiotics	hydrocarbons	proteins	lipids
peptide	antifungal	macrolide	pencillin
peptide	antifungal	macrolide	pencillin
pharmamedia	corn-steep liquor	sulfite-waste liquor	hydrocarbons
Penicillium notatum	*Penicillium flavum*	*Penicillium geiseofulvicu*	*Penicillium chrysoge*
pencillin G and V	penicillin B and V	penicillin M and V	penicillin O and V
amylase	pectinase	protease	penicillinase
phenyl sulphate	phenylacetic acid	phenyl phosphate	phenychloride
production	sporulation	preservation	mother culture
centrifugation	filtration	heating	precipitation
counter current	charcoal	co-current	bi-current
counter current	charcoal	co-current	bi-current
alkali	charcoal	glycerol	acid
natural penicillin	synthetic penicillin	semi-synthetic penicillin	GN penicillin
acylation	chlorination	carboxylation	methylation
acidulant	basifier	precursor	anti-foaming agent
lactic acid	succinic acid	tartaric acid	citric acid
temperature	pH	ratio of surface area to vo	aeration
lower	higher	medium	equal
NaOH	Sulphuric acid	Nacl	Milk of lime
counter current extraction	co-current	bi-current	Tri-current
presence of unconverted sugars	presence of oxalic aci	presence of trace salts	PRESENCE OF unco
80	90	95	92
Candida brumptil	*Candida lipolytica*	*Candida hydrocarbofum*	*Candida albicans*
0.4111	0.5111	0.6111	0.7111
proteins	lipids	saccharides	enzymes

fresh grape juice	fresh wine	sour wine	ethanol
70-80oF	72-82°F	75-80°F	76-82°F
two	three	four	five
2 to 5	3 to 7	4 to 8	5 to 10
closed	open	plastic	iron
sulfur dioxide	sulfur oxide	ethylene gas	nitrous oxide
sulfur dioxide	sulfur oxide	ethylene gas	nitrous oxide
α-amylase	Glucoamyase	Protease	Cellulase
Aspergillus niger	*Rhizopus sp*	*Candida sp*	*Aspergillus niger, Rh*
rapid heating	precipitation	condensation	rapid cooling
glucose	lactose	sucrose	galactose
acetone	alcohols	inorganic-salts(ammoniu	acetone, salts and solv
α-amylases	pectinase	cellulase	proteases
5	10	15	20
5	6	7	8
amylases & amylopectinases	cellulase & cellulobia	proteinases & peptidases	invertase& phosphata
α-amylase	Glucoamyase	Protease	Cellulase
Bacillus subtilis	*Bacillus licheniformis*	*Bacillus subtilis & Bacill*	*Bacillus anthrax*
20-30°C, 7	30-40°C, 7	30-40°C, 8	40-50°C, 7
nucleotide	nucleoside	nucleode	nucleic acid (DNA/RI
5,7-dimethyl benziminazole	5,8-dimethyl benzimi	5-6-dimethyl benziminaz	5,4-dimethyl benzimi
vitamin A	vitamin B12	vitamin C	vitamin D
Streptomyces olivaceus NRRL A	*Streptomyces olivace*	*Streptomyces olivaceus N*	*Streptomyces olivace*
2 to 4	3 to 5	4 to 6	5 to 7
cyanide	chlorine	cobalt	citrate
60oF	70oF	80oF	90oF
0.5-1	0.8-1	1 to 2	1.2-2.5

CORRECT OPTION
sporulation medium
Aspergillus
B .subtilis
Aspergillus oryzae
Sacchcromyces cerevasiae
Aspergillus niger
beet molasses
2.20 to 1.60
26oC to 28oC
contaminat ANS: (c)oxalic acid formation is suppresse(d)
minium stainless steel and aluminium
7 to 10 days
antibiotics
pencillin
pencillin
corn-steep liquor
num *Penicillium chrysogenum*
pencillin G and V
penicillinase
phenylacetic acid
sporulation
filtration
counter current
counter current
charcoal
semi-synthetic penicillin
acylation
acidulant
citric acid
ratio of surface area to volume
lower
Milk of lime
counter current extraction
nverted sugars, oxalic acid, trace salts
90
Candida lipolytica
0.5111
enzymes

sour wine
75-80^{o}F
two
3 to 7
closed
sulfur dioxide
sulfur dioxide
Protease
izopus sp a *Aspergillus niger, Rhizopus sp and Candida*
rapid cooling
glucose
vents acetone, salts and solvents
α-amylases
20
7
se proteinases & peptidases
Protease
Bacillus subtilis & Bacillus lichenifermis
30-40°C, 7
NA) nucleotide
nazole 5-6-dimethyl benziminazole
vitamin B12
us NRRL L *Streptomyces olivaceus NRRL B-1125*
3 to 5
cyanide
80oF
1 to 2

Reg. No. : ----------

[15MBU601]

KARPAGAM ACADEMY OF HIGHER EDUCATION

(Under Section 3 of UGC Act 1956)

COIMBATORE – 641 021

B.Sc. DEGREE EXAMINATION, January 2018

DEPARTMENT OF MICROBIOLOGY

I INTERNAL TEST - SIXTH SEMESTER

INDUSTRIAL MICROBIOLOGY

Time: 2 hours **Maximum: 50 marks**

Date / Session : **Class: III BSc MB**

PART-A

Multiple Choice Questions **20 x 1 = 20 marks**

1. Secondary screening determine the
   a. Inhibitory activity b. Producing ability
   c. optimum condition of the growth d. Checking contamination
2. Nutrient broth containing unusual substrates in ----------
   a. Auxanography b. homofermentation
   c. Crowded plate technique d. Enrichment technique
3. Storage at very low temperature are also known as
   a. Cryogenic storage b. Lyophilization
   b. freezing d. Cold storage
4. Overlaying cultures with mineral oil was used by ----------
   a. Fennel b. Raper & Alexander
   c. Sherf d. Buell & Weston
5. ---------- used to isolate the growth factor producing organisms
   a. Crowded plate technique b. Serial dilution
   c. Auxanography d. Pure culture technique
6. The medium which doesn't contain particular essential nutrient is called as _____.
   a. enriched media b. minimal media
   c. molten agar d. selective media
7. Indicator dye are used in the medium
   a. to detect temp change b. to detect nutrient concentration
   c. to detect ph change d. to detect aw availability.
8. _____ is used in the food industry as nutritional supplement in bread products
   a. Valine b. Lysine
   c. Arginine d. Proline
9. Crowded plate technique is used in the detection _____ producing organisms.
   a. growth factor producers b. aminoacid producers
   c. antibiotic producers d. organic acid producers.
10. Growth factor producing organisms are identified by using
    a. growded plate technique b. enrichment technique
    c. auxanography d. indicator dye method.
11. Chemical stability of the product is identified by _____.
    a. primary screening b. secondary screening
    c. enrichment method d. tertiary method.

12. ---------- culture where medium is fed to the culture resulting in an increases in volume

a. Batch b. Fed batch

c. Continuous d. Semi continuous

13. In -------a portion of the culture is harvested and replaced by an equal volume of medium.

a. Fed batch culture b. Batch culture

c. Semi continuous culture d. Continuous culture

14. Cell without cell wall is called as _____.

a. protoplast b. spleroplast

c. thermoplast d. periplast

15. Among the following which solution is used for the preparation of protoplast?

a. Osmotic b. Hypertonic

c. Isotonic d. Hyphotonic

16. Different microbial species are fused by _____.

a. r-DNA technology b. protoplast fusion

c. mutation d. plasmid fusion

17. Primary metabolites are produced during _____.

a. lag phase b. stationary phase

c. trophophase d. idiophase

18. Secondary metabolites are produced during _____.

a. lag phase b. log phase

c. trophophase d. idiophase

19. The chemical agent which causes mutation is _____.

a. sodium sulfate b. ethyl methyl sulfanate

c. CaCl2 d. ethyl sulfanate

20. CSTF is expanded as

a. Continuous Stirred Tank Fermentor b. Continuous Solid Tank Fermentor

c. Cell Suspended Tank Fermentor d. Continuous Solid Type Fermentor

Part B

Answer all the questions **3x10 = 30 marks**

21. A. Explain in short about history and development of industrial microbiology.

Or

B. Write in detail about the industrially important strains.

22. A. Explain in short about the screening methods.

Or

B. Write in detail about the strain development.

23. A. Give a detailed note media formulation and sterilization

Or

B. What is the structure of fermentor?

****