M.Sc. Microbiology **2018-2019**

Semester - II

18MBP205C **BIOPROCESS ENGINEERING** **4H – 4C**

Instruction Hours / week:L: 4 T: 0 P: 0 **Marks:** Internal: **40** External: **60** Total: **100**

End Semester Exam: 3 Hours

COURSE OBJECTIVES

This course 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 bio processes may be natural isolates; laboratory selected mutants or genetically engineered organisms.

COURSE OUTCOME

This course will enable the students to design the various microbial fermentation products and their production, purification for various applications.

UNIT–I

Design of a basic fermenter, bioreactor configuration, design features, computer control of fermentation process, measurement and control of process. Types of Bioreactors and its functions.

UNIT – II

Transport phenomena in fermentation: Gas- liquid exchange and mass transfer, oxygen transfer, critical oxygen concentration, heat transfer, aeration/agitation, its importance. Sterilization of Bioreactors, nutrients, air supply, products and effluents, process variables and control, scale-up of bioreactors.

UNIT – III

Growth of cultures in the fermenter. Importance of media in fermentation, media formulation and modification . Kinetics of growth in batch culture, continuous culture with respect to substrate utilization, specific growth rate, steady state in a chemostat, fed-batch fermentation, yield of biomass, product, calculation for productivity.

UNIT – IV

Down streaming process of microbial products (Peptides, Biopolymers, surfactants, Enzymes) - separation, extraction and purification, drying and crystallization.

UNIT – V

Isolation, selection and improvement of microbial cultures. Strain improvement for the selected organism: Use of recombinant DNA technology, protoplast fusion techniques for strain improvement. Improvement of characters other than products and its application in the industry. Preservation of cultures after strain improvement programme.

SUGGESTED READINGS

TEXT BOOKS

1. Demain, A.L., and Davies, J.E., (1999). *Manual of Industrial Microbiology and Biotechnology*. (2nd ed.). A.S.M. Press, Washington, D.C.

2. Hugo, W.B., and Russell, A.D., (1998). *Pharmaceutical Microbiology*. (6th ed.). Publisher Blackwell Science Ltd.
3. Mansi, E.M.T., and Bryce, C.F.A., (2002). *Fermentation Microbiology and Biotechnology*. Taylor and Francis, New York.
4. Patel, A.H. (2003). *Industrial Microbiology*. Macmillan India Ltd. New Delhi

REFERENCES

1. Reed, G. (2002). *Presscott and Dunn's Industrial Microbiology*. (5th ed.). CBS Publishers, New Delhi.
2. Shuler, M.L., and Kargi, F., (2005). *Bioprocess Engineering Basic Concepts*. Pearson Education, New Delhi.
3. Stanbury, P.T., and Whitaker, A., (2005). *Principles of Fermentation Technology*, Pergamon Press, NY.
4. Waites, M. J. (2007). *Industrial Microbiology*. Blackwell Publishing Company. UK.

WEBLINKS

1. http://www.biologydiscussion.com/industrial-microbiology-2/fermentor-bioreactor-history-design-and-its-construction/55756

UNIT I

Duration	Topic to be covered	Reference
1	Design of a basic fermenter, Bioreactor configuration	T1:43-69
1	Design features	T1:97-104
1	Computer control of fermentation process	T2:188-215
1	Measurement and control of process	T2:188-215
1	Types of Bioreactors and its functions.	R1:760-764
1	Reactors for specialized applications: Tube reactors	R1:765-773
1	Packed bed reactors	R2:223-225
1	Fluidized bed reactors, Cyclone reactors, Trickle flow reactors	R2:375-395
1	Revision	

TOTAL HOURS – 9 h

T1 Demain, A.L., and Davies, J.E., (1999). *Manual of Industrial Microbiology and Biotechnology*. (2nd ed.). A.S.M. Press, Washington, D.C.

T2 Hugo, W.B., and Russell, A.D., (1998). *Pharmaceutical Microbiology*. (6th ed.). Publisher Blackwell Science Ltd.

R2 Reed, G. (2002). *Presscott and Dunn's Industrial Microbiology*. (5th ed.). CBS Publishers, New Delhi.

R2 Shuler, M.L., and Kargi, F., (2005). *Bioprocess Engineering Basic Concepts*. Pearson Education, New Delhi.

UNIT II

Duration	Topic to be covered	Reference
1	Transport phenomena in fermentation: Gas- liquid exchange	R1: 85-88
1	Mass transfer in fermentation	R2: 231-244
1	Oxygen transfer and critical oxygen concentration and Heat transfer in fermentation	R2: 231-244
1	Aeration/agitation, its importance.	R3:333-351
1	Sterilization of Bioreactors, nutrients	R3:379-430
1	Air supply, products and effluents	R3:823-830
1	Process variables and control	R3:823-830
1	Scale-up of bioreactors	R4:318-322
1	Revision	

TOTAL HOURS – 9 h

R1 Reed, G. (2002). *Presscott and Dunn's Industrial Microbiology*. (5th ed.). CBS Publishers, New Delhi.

R2 Shuler, M.L., and Kargi, F., (2005). *Bioprocess Engineering Basic Concepts*. Pearson Education, New Delhi.

R3 Stanbury, P.T., and Whitaker, A., (2005). *Principles of Fermentation Technology*, Pergamon Press, NY.

R4 Waites, M. J. (2007). *Industrial Microbiology*. Blackwell Publishing Company. UK.

UNIT III

Duration	Topic to be covered	Reference
1	Growth of cultures in the fermenter.	R1: 315-317
1	Importance of media, Media formulation and modification in fermentation.	R1: 315-317
1	Kinetics of growth in batch and continuous culture with respect to substrate utilization.	R1: 315-317
1	Steady state in a chemostat, fed-batch fermentation.	R2:24-411
1	Yield of biomass, product, and calculation for productivity.	R2:24-411
1	Storage of cultures for repeated fermentations	R2:24-411
1	Scaling up of process form shake flask to industrial fermentation.	R2:24-411
1	Revision	
1	Unit test	

TOTAL HOURS – 9 h

R1 Reed, G. (2002). *Presscott and Dunn's Industrial Microbiology*. (5th ed.). CBS Publishers, New Delhi.

R2 Shuler, M.L., and Kargi, F., (2005). *Bioprocess Engineering Basic Concepts*. Pearson Education, New Delhi.

UNIT IV

Duration	Topic to be covered	Reference
1	Introduction of Down steam Process	T1: 60- 64
1	Biomass separation by centrifugation.	T1: 64-86
1	Biomass separation by filtration, flocculation and other recent developments.	R2: 111-123
1	Extraction: Solvent, two phase, liquid extraction, whole broth, aqueous multiphase extraction.	R2: 111-123
1	Purification of products by different methods.	R3:445-450
1	Drying and crystallization.	R3:438-563
1	Revision	
1	Unit test	

TOTAL HOURS – 9 h

T1 Demain, A.L., and Davies, J.E., (1999). *Manual of Industrial Microbiology and Biotechnology*. (2nd ed.). A.S.M. Press, Washington, D.C.

R2 Shuler, M.L., and Kargi, F., (2005). *Bioprocess Engineering Basic Concepts*. Pearson Education, New Delhi.

R3 Stanbury, P.T., and Whitaker, A., (2005). *Principles of Fermentation Technology*, Pergamon Press, NY.

UNIT V

Duration	Topic to be covered	Reference
1	Isolation and selection of microbial cultures	R1: 9-50
1	Strain improvement for the selected organism	R2: 71-73
1	Use of recombinant DNA technology	R2: 75-77
1	Protoplast fusion techniques for strain improvement.	R3:178-187
1	Improvement of characters other than products	R3:178-187
1	Application in the industrial important strains	R3:178-187
1	Preservation of cultures after strain improvement programme	R3:178-187
1	Revision	
1	Unit test	

TOTAL HOURS – 9 h

R1 Reed, G. (2002). *Presscott and Dunn's Industrial Microbiology*. (5th ed.). CBS Publishers, New Delhi.

R2 Shuler, M.L., and Kargi, F., (2005). *Bioprocess Engineering Basic Concepts*. Pearson Education, New Delhi.

R3 Stanbury, P.T., and Whitaker, A., (2005). *Principles of Fermentation Technology*, Pergamon Press, NY.

Unit I

DESIGN OF A FERMENTOR

A research team led by Chaim Weizmann in Great Britain during the First World War (1914-1918) developed a process for the production of acetone by a deep liquid fermentation using Clostridium acetobutylicum which led to the eventual use of the first truly large-scale aseptic fermentation vessels (Hastings, 1978). Contamination, particularly with bacteriophages, was often a serious problem, especially during the early part of a large-scale production stage. Initially, no suitable vessels were available and attempts with alcohol fermenters fitted with lids were not satisfactory as steam sterilization could not be achieved at atmospheric pressure. Large mild-steel cylindrical vessels with hemispherical tops and bottoms were constructed that could be sterilized with steam under pressure. Since the problems of aseptic additions of media or inocula had been recognized, steps were taken to design and construct piping, joints and valves in which sterile conditions could be achieved and maintained when required. Although the smaller seed vessels were stirred mechanically, the large production vessels were not, and the large volumes of gas produced during the fermentation continually agitated the vessel contents. Thus, considerable expertise was built up in the construction and operation of this aseptic anaerobic process for production of acetone-butanol. The first true large-scale aerobic fermenters were used in Central Europe in the 1930s for the production of compressed yeast (de Becze and Liebmann, 1944).

The fermenters consisted of large cylindrical tanks with air introduced at the base via networks of perforated pipes. In later modifications, mechanical impellers were used to increase the rate of mixing and to break up and disperse the air bubbles. This procedure led to the compressed-air requirements being reduced by a factor of 5. Baffles on the walls of the vessels prevented a vortex forming in the liquid. Even at this time it was recognized that the cost of energy necessary to compress air could be 10 to 20% of the total production cost. As early as 1932, Strauch and Schmidt patented a system in which the aeration tubes were provided with water and steam for cleaning and sterilizing. Prior to 1940, the other important fermentation products besides bakers' yeast were ethanol, glycerol, acetic acid, citric acid, other organic acids, enzymes and sorbose (Johnson, 1971). These processes used highly selective environments such as acidic or anaerobic conditions or the use of an unusual substrate, resulting in contamination being a relatively minor problem compared with the acetone fermentation or the subsequent aerobic antibiotic fermentations. The decision to use submerged culture techniques for penicillin production, where aseptic conditions, good aeration and agitation were essential, was a very importantfactor in forcing the development of carefully designed and purpose-built fermentation vessels. In 1943, when the British government decided that surface

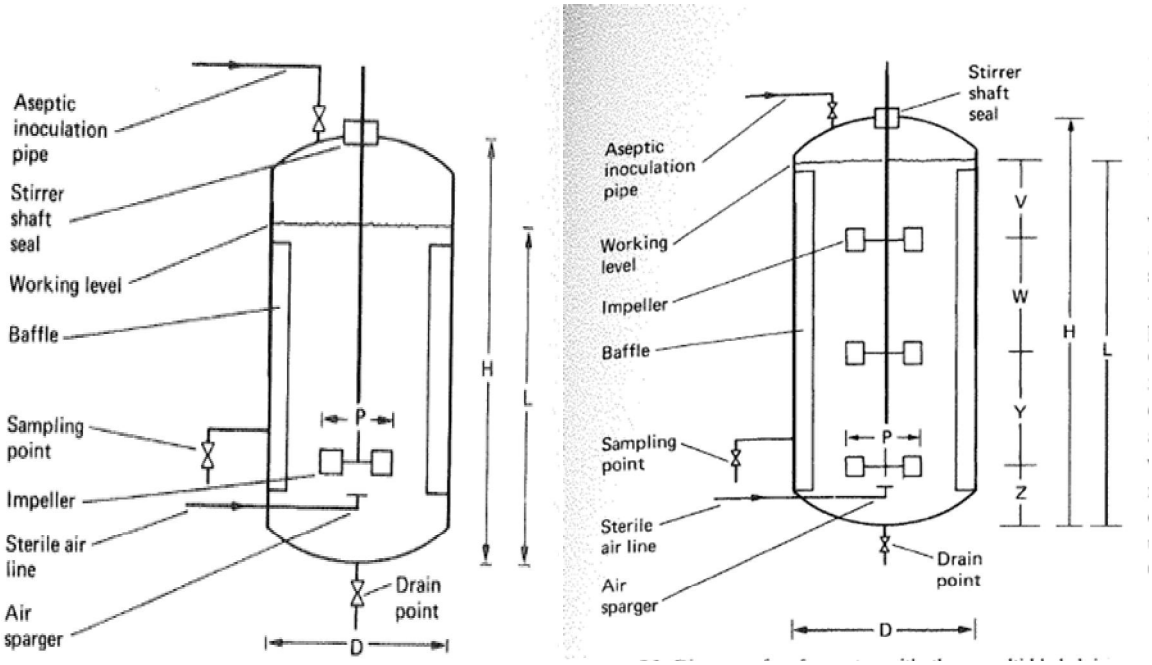
culture production was inadequate, none of the fermentation plants were immediately suitable for deep fermentation, although the Distillers Company solvent plant at Bromborough only needed aeration equipment to make it suitable for penicillin production (Hastings, 1971). Construction work on the first large-scale plant to produce penicillin by deep fermentation was started on 15th September 1943, at Terre Haute in the United States of America, building steel fermenters with working volumes of 54,000 dm3 (Callahan, 1944). The plant was operational on 30th January 1944. Unfortunately, no other construction details were quoted for the fermenters.

BASIC FUNCTIONS OF A FERMENTER FOR MICROBIAL OR ANIMAL CELL CULTURE

The main function of a fermenter is to provide acontrolled environment for the growth of microorganismsor animal cells, to obtain a desired product.In designing and constructing a fermenter a number ofpoints must be considered:

1. The vessel should be capable of being operatedaseptically for a number of days and should bereliable in long-term operation and meet therequirements of containment regulations.
2. Adequate aeration and agitation should be providedto meet the metabolic requirements ofthe micro-organism. However, the mixing shouldnot cause damage to the organism.
3. Power consumption should be as low as possible.
4. A system of temperature control should be provided.
5. A system of pH control should be provided.
6. Sampling facilities should be provided.
7. Evaporation losses from the fermenter shouldnot be excessive.
8. The vessel should be designed to require theminimal use of labour in operation, harvesting,cleaning and maintenance.
9. Ideally the vessel should be suitable for a rangeof processes, but this may be restricted becauseof containment regulations.
10. The vessel should be constructed to ensuresmooth internal surfaces, using welds instead of flange joints whenever possible.
11. The vessel should be of similar geometry toboth smaller and larger vessels in the pilot plantor plant to facilitate scale-up.
12. The cheapest materials which enable satisfactoryresults to be achieved should be used.
13. There should be adequate service provisions for individual plants.

The first two points are probably the most critical. Itis obvious from the above points that the design of afermenter will involve co-operation between experts inmicrobiology, biochemistry, chemical engineering, mechanical engineering and costing. Although many different types of fermenter have been described in the literature, very few have proved to be satisfactory forindustrial aerobic fermentations. The most commonlyused ones are based on a stirred upright cylinder withsparger aeration. This type of vessel can be produced ina range of sizes from one dm3 to thousands of dm3.



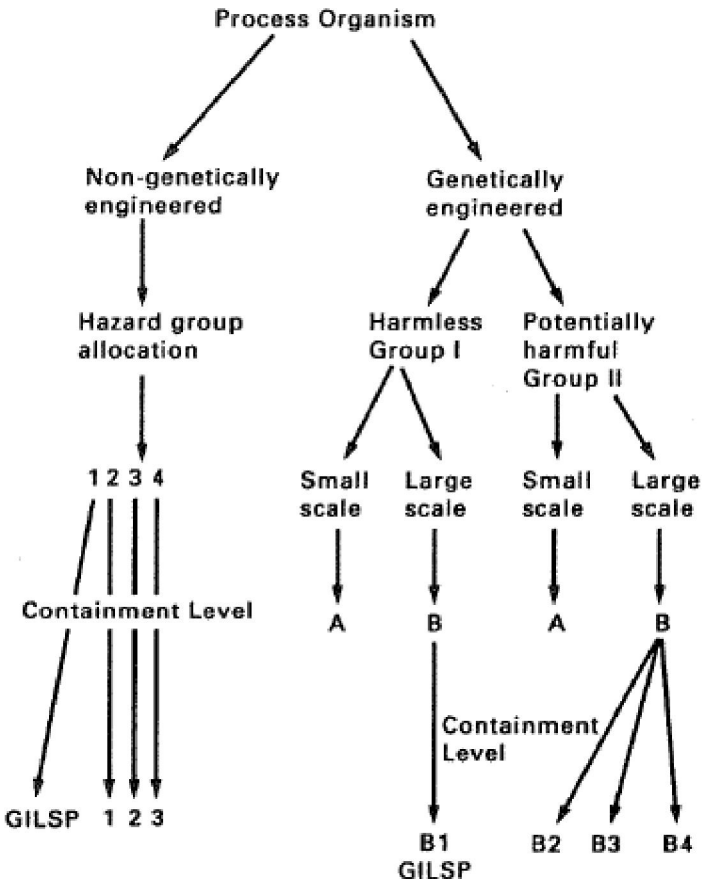
Schematics of a fermentor design (Single and multi bladed impellers)

ASEPTIC OPERATION AND CONTAINMENT

Aseptic operation involves protection against contamination and it is a well-established and under stood concept in the fermentation industries, whereas containment involves prevention of escape of viable cells from a fermenter or downstream equipment and is much more recent in origin. Containment guideline swere initiated during the 1970sTo establish the appropriate degree of containment which will be necessary to grow a micro-organism, it,and in fact the entire process, must be carefully assessed for potential hazards that could occur should there be accidental release. Different assessment procedures are used depending on whether or not the organism contains foreign DNA (genetically engineered). Once the hazards are assessed, an organism can be classified into a hazard group for which there is an appropriate level of containment. The procedure which has been adopted within the European Community is outlined. Non-genetically engineered

organisms may be placed into a hazard group (1 to 4) using criteria to assess. risk such as those given by Collins (1992):

1. The known pathogenicity of the micro-organism.
2. The virulence or level of pathogenicity of themicro-organism are the diseases it causes mild or serious?
3. The number of organisms required to initiate aninfection.
4. The routes of infection.
5. The known incidence of infection in the communityand the existence locally of vectors andpotential reserves.
6. The amounts or volumes of organisms used inthe fermentation process.
7. The techniques or processes used.
8. Ease of prophylaxis and treatment.



Categorization of a process micro-organism and designation of its appropriate level of containment at research or industrial sites within the European Federation of Biotechnology

(GILSP = Good Industrial Large Scale Practice).

Once the organism has been allocated to a hazardgroup, the appropriate containment requirements can be applied. Hazard group 1 organismsused on a large scale only require Good IndustrialLarge Scale Practice (GILSP). Processes in this category need to be operated aseptically but no containmentsteps are necessary, including prevention of escapeof organisms. If the organism is placed in Hazardgroup 4 the stringent requirements of level 3 will haveto be met before the process can be operated. Detailsof hazard categories for a range of organisms can be obtained from Frommer et al. (1989).Genetically engineered organisms are classified aseither harmless (Group I) or potentially harmful (GroupII). The process is then classified as either small scale(A: less than 10 dm3) or large scale (B: more than 10dm3) according to guidelines which can be found in theHealth and Safety Executive document (1993). Therefore large scale processes fall into two categories, IE orlIB. IE processes require containment level Bl and aresubject to GILSP, whereas lIB processes are furtherassessed to determine the most suitable containmentlevel, ranging from B2 to B4.Levels B2 to B4 correspond to levels 1 to3 for non genetically engineered organisms.

In future it is possible, under new legislation, that nodistinction will be made between non-genetically engineeredand genetically engineered organisms. The keyfactor will be whether the organism is harmless orpotentially harmful, regardless of its genetic constitution.Containment would then be decided using thescheme which is currently being used for genetically engineered organisms.Other hazard-assessment systems for classifying organismshave been introduced in many other countries.Production and research workers must abide by appropriatelocal official hazard lists. Problems can occurwhen different official bodies place the same organismin different hazard categories. In 1989, the EuropeanFederation for Biotechnology were aware of this problemwith non-recombinant micro-organisms and produceda consensus list (Frommer et ai., 1989).Most micro-organisms used in industrial processesare in the lowest hazard group which only requireGILSP, although some organisms used in bacterial andviral vaccine production and other processes are categorizedin higher groups. There is an obvious incentivefor industry to use an organism which poses a low riskas this minimizes regulatory restrictions and reducesthe need for expensive equipment and associated containmentfacilities.

BODY CONSTRUCTION

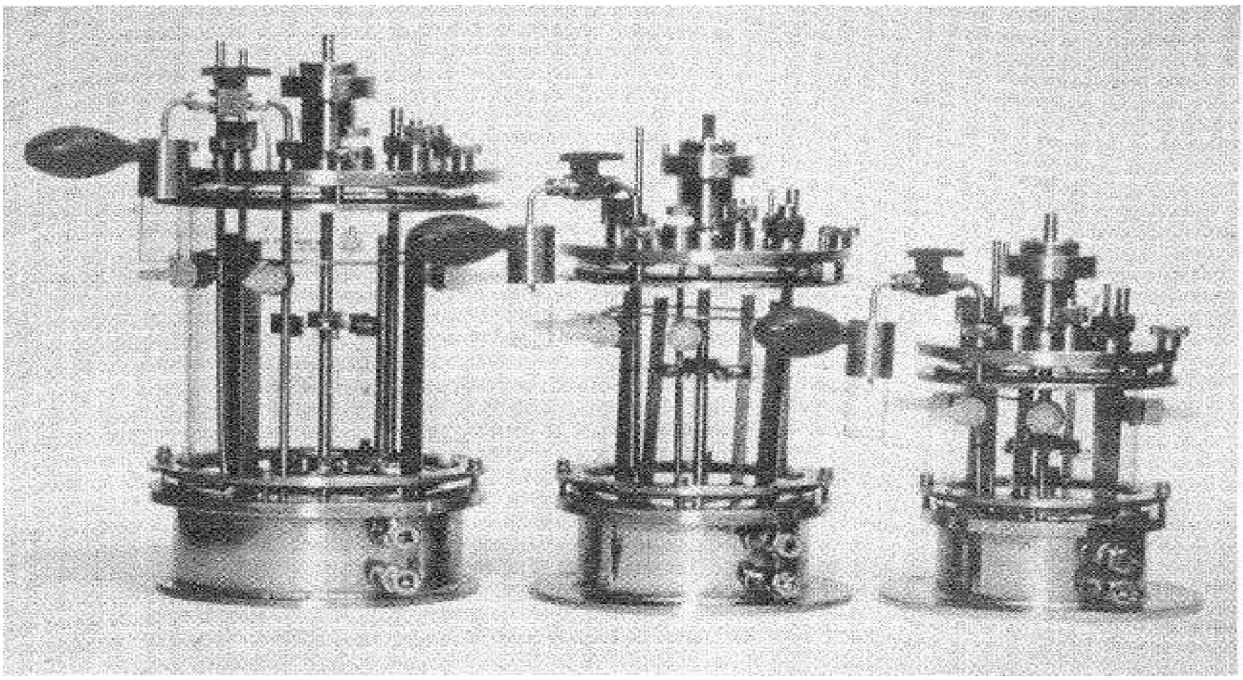
In fermentations with strict aseptic requirements itis important to select materials that can with standrepeated steam sterilization cycles. On a small scale (1to 30 dm3) it is possible to use glass and/or stainlesssteel. Glass is useful because it gives smooth surfaces,is non-toxic, corrosion proof and it is usually easy to examine the interior of the vessel. Two basic types offer menter are used:

1. A glass vessel with a round or flat bottom and a top flanged carrying plate. The largeglass containers originally used were borosilicatebattery jars (Brown and Peterson, 1950). All vessels of this type have to be sterilized by autoclaving.

Cowan and Thomas (1988) state that thelargest practical diameter for glass fermenters is60 cm.

2. A glass cylinder with stainless-steel top and bottom plates. These fermenters may besterilized in situ, but 30 cm diameter is the uppersize limit to safely withstand working pressures(Solomons, 1969). Vessels with two stainless steelplates cost approximately 50% more than those with just a top plate.

At pilot and large scale, when allfermenters are sterilized in situ, any materials used will have to be assessed on their ability to withstand pressuresterilization and corrosion and on their potential toxicity and cost. Walker and Holdsworth (1958), Solomons(1969) and Cowan and Thomas (1988) have discussed the suitability of various materials used in the construction of fermenters. Pilot-scale and industrial scale vessels are normally constructed of stainless steelor at least have a stainless-steel cladding to limit corrosion.



Glass Fermentors of different capacities

The American Iron and Steel Institute (AISI)states that steels containing less than 4% chromium are classified as steel alloys and those containing morethan 4% are classified as stainless steels. Mild steel coated with glass or phenolic epoxy materials has occasionally been used.

Walker and Hold sworth (1958) stated that the extentof vessel corrosion varied considerably and did notappear to be entirely predictable. Athough stain lesssteel is often quoted as the only satisfactory material, ithas been reported that mild-steel vessels were very satisfactory after 12-years use for penicillin fermentations (Walker and Holdsworth, 1958) and mild steel clad with stainless steel has been used for at least 25years for acetone-butanol production (Spivey, 1978).Pitting to a depth of 7 mm was found in a mild-steel fermenter after 7-years use for streptomycin production (Walker and Holdsworth, 1958). The corrosion resistance of stainless steel is thoughtto depend on the existence of a thin hydrous oxide filmon the surface of the metal. The composition of thisfilm varies with different steel alloys and different manufacturing process treatments such as rolling, pickling or heat treatment. The film is stabilized by chromium and is considered to be continuous, non-Zorous,insoluble and self healing. If damaged, the film willrepair itself when exposed to air or an oxidizing agent (CubberIy et a!., 1980). The minimum amount of chromium needed to resistcorrosion will depend on the corroding agent in aparticular environment, such as acids, alkalis, gases,soil, salt or fresh water. Increasing the chromium contentenhances resistance to corrosion, but only gradesof steel containing at least 10 to 13% chromium developan effective film. The inclusion of nickel in highpercent chromium steels enhances their resistance andimproves their engineering properties. The presence ofmolybdenum improves the resistance of stainless steelsto solutions of halogen salts and pitting by chlorideions in brine or sea water. Corrosion resistance canalso be improved by tungsten, silicone and other elements(CubberIy et al., 1980; Duurkoop, 1992).AISI grade 316 steels which contain 18% chromium,10% nickel and 2-2.5% molybdenum are now commonly used in fermenter construction.

TEMPERATURE CONTROL

Normally in the design and construction of a fermentor there must be adequate provision for temperature control which will affect the design of the vessel body. Heat will be produced by microbial activity and mechanical agitation and if the heat generated by thesetwo processes is not ideal for the particular manufacturing gprocess then heat may have to be added to, or removed from, the system. On a laboratory scale little heat is normally generated and extra heat has to be provided by placing the fermenter in a thermostatically controlled bath, or by the use of internal heating coilsor a heating jacket through which water is circulated or by a silicone heating jacket. The silicone jacket consists of a double silicone rubber mat with heating wiresbetween the two mats; it is wrapped around the vessel and held in place by Velcro strips (Applikon, 1989).

Once a certain size has been exceeded, the surface area covered by the jacket becomes too small to remove the heat produced by the fermentation. When this situation occurs internal coils must be used and cold water is circulated to achieve the correct temperature Jackson, 1990). Different types of fermentation will influence the maximum size of vessel that can be used with jackets alone.

AERATION AND AGITATION

The primary purpose of aeration is to provide microorganisms in submerged culture with sufficient oxygen for metabolic requirements, while agitation should ensure that a uniform suspension of microbial cells is achieved in a homogeneous nutrient medium. Theof aeration- agitation system used in a particular fermenter depends on the characteristics of the fermentation process under consideration. Although fineaerators without mechanical agitation have the advantage of lower equipment and power costs, may be dispensed with only when aerationsufficient agitation, i.e. in processes where broths low viscosity and low total solids are used (ArnoldSteel, 1958). Thus, mechanical agitation is usually acquired in fungal and actinomycete. Non-agitated fermentations are normally carried outvessels of a height/diameter ratio of 5:1. Invessels aeration is sufficient to produce high turbulence, but a tall column of liquid does require energy input in the production of the compressed (Muller and Kieslich, 1966; Solomons, 1980).The structural components of the fermenter involved in aeration and agitation are

(a) The agitator (impeller).

(b) Stirrer glands and bearings.

(c) Baffles.

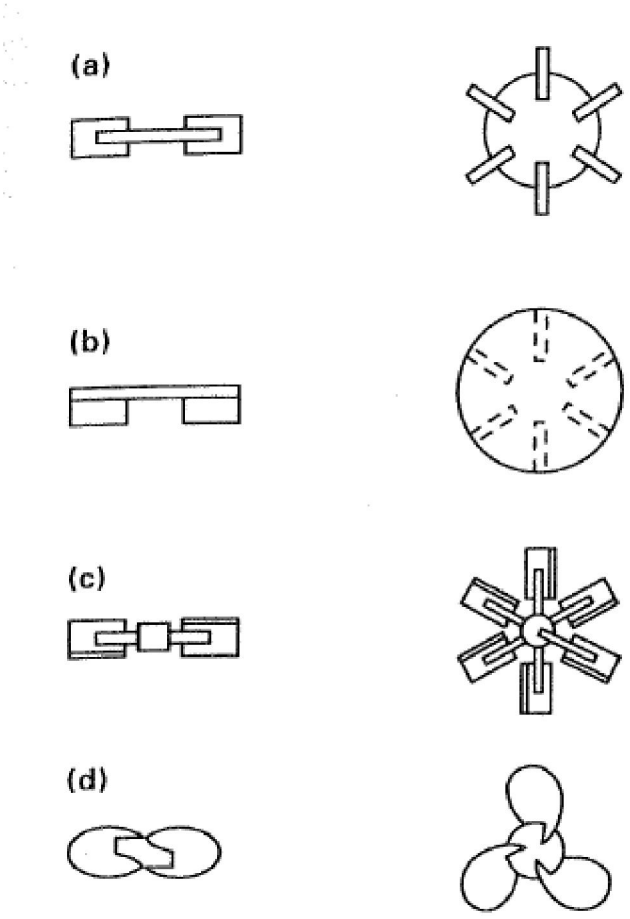
(d) The aeration system (sparger).

THE AGITATOR (IMPELLER)

The agitator is required to achieve a number ofmixing objectives, e.g. bulk fluid and gas- phase mixing,air dispersion, oxygen transfer, heat transfer, suspensionof solid particles and maintaining a uniform environmentthroughout the vessel contents. It should bepossible to design a fermenter to achieve these conditions;this will require knowledge of the most appropriateagitator, air sparger, baffles, the best positions fornutrient feeds, acid or alkali for pH control and antifoam addition.

Agitators may be classified as disc turbines, vaned discs, open turbines of variable pitch and propellers. The disc turbine consistsof a disc with a series of rectangular vanes set in avertical plane around the circumference and the vaneddisc has a series of rectangular vanes attached vertically to the underside.

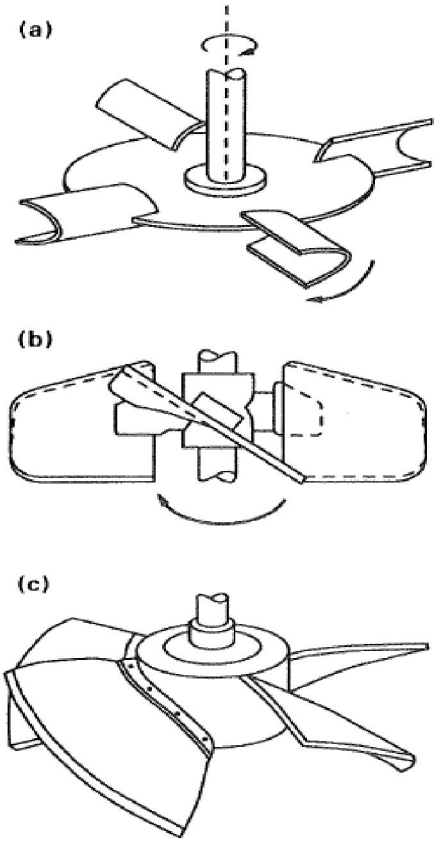
Air from the sparger hits the underside of the disc and is displaced towards the vanes where the air bubbles are broken up into smaller bubbles. The vanes of a variable pitch open turbine andthe blades of a marine propeller are attached directly to a boss on the agitator shaft. In this case the air bubbles do not initially hit any surface before dispersion by the vanes or blades.



Types of agitator - (a) disc turbine; (b) vaned disc; (c)open turbine, variable pitch; (d) marine propeller.

Four other modern agitator developments, the Scaba6SRGT, the Prochem Maxflo T, the Lightning A315and the Ekato Intermig, which arederived from open turbines, will also be discussed forenergy conservation and use in high-viscosity broths.Since the 1940s a Rushton disc turbine of one-thirdthe fermenter diameter has been considered the optimumdesign for use in many fermentation processes. Ithad been established experimentally that the disc turbinewas most suitable in a fermenter since it couldbreak up a fast air stream without itself becomingflooded in air bubbles (Finn, 1954). This flooding conditionis indicated when the bulk flow pattern in thevessel normally associated with the agitator design (radialwith the Rushton turbine) is lost and replaced by acentrally flowing air-broth plume up the middle of thevessel

with a liquid flow as an annulus. The propeller and the openturbine flood when V, (superficial velocity, i.e. volumetricair flow rate/cross-sectional area of fermentedexceeds 21 m h - \ whereas the flat blade turbine cantolerate a V, of up to 120 m h -1 before being flooded,when two sets are used on the same shaft. Besidesbeing flooded at a lower V, than the disc turbine, thepropeller is also less efficient in breaking up a streamof air bubbles and the flow it produces is axial rather than radial (Cooper et at., 1944). The disc turbine wasthought to be essential for forcing the sparged air in to the agitator tip zone where bubble break up would occur.



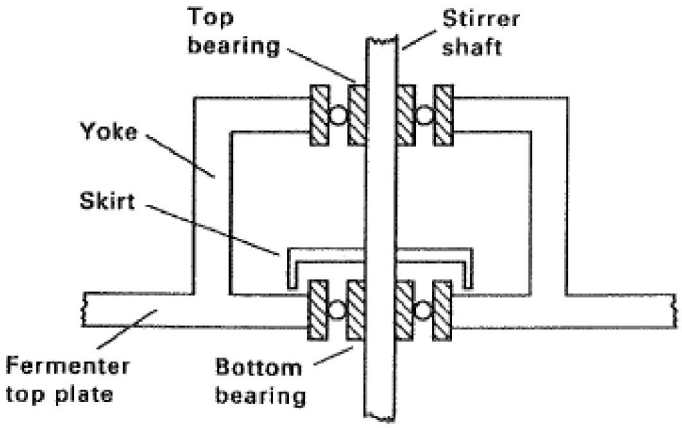
Different types of Agitator (a) Scaba agitator; (b) Lightnin' A315agitator (four blades) and (c) Prochem Maxflo T agitator

In other studies it has been shown that bubble breakup occurs in the trailing vortices associated with allagitator types which give rise to gas-filled cavities and provided the agitator speed is high enough, good gas dispersion will occur in low-viscosity broths (Smith,1985). It has been also shown that similar oxygen-transfer efficiencies are obtained at the same power input per unit volume, regardless of the agitator type.

STIRRER GLANDS AND BEARINGS

The satisfactory sealing of the stirrer shaft assembly top plate has been one of the most difficult problems to overcome in the construction of fermentation equipment which can be operated aseptically for long periods. A number of different designs have been developed to obtain aseptic seals. The stirrer shaft can enter the vessel from the top, side (Richards, 1968) or bottom of the vessel. Top entry is most commonly used, but bottom entry may be advantageous if more space is needed on the top plate for entry ports, and the shorter shaft permits higher stirrer speeds to be used by eliminating the problem of the shaft whipping at high speeds. Originally, bottom entry stirrers were considered undesirable as the bearings would be submerged. Chain etal. (1952) successfully operated vessels of this type, and they have since been used by many other workers. Mechanical seals can be used for bottom entry provided that they are routinely maintained and replacedat recommended intervals (Leaver and Hambleton,1992).One of the earliest stirrer seals described was thatused by Rivett, Johnson and Peterson (1950) in alaboratory fermenter. A porous bronze bearingfor a 13-mm shaft was fitted in the centre of thefermenter top and another in a yoke directly above it.

The bearings were pressed into steel housings, whichscrewed into position in the yoke and the fermentertop. The lower bearing and housing were covered witha skirt-like shield having a 6.5 mm overhang whichrotated with the shaft and prevented air-borne contaminantsfrom settling on the bearing and workingtheir way through it into the fermenter.



Simple stirrer seal

BAFFLES

Four baffles are normally incorporated into agitated vessels of all sizes to prevent a vortex and to improve aeration efficiency. In vessels over 3-dm3 diameter sixor eight baffles may be used (Scragg, 1991). Baffles are metal strips roughly one-tenth of the vessel diameter and attached radially to the wall. The agitation effect is onlyslightly increased with wider baffles, but drops sharply with narrower baffles (Winkler, 1990). Walker andHoldsworth (1958) recommended that baffles should be installed so that a gap existed between them and thevessel wall, so that there was a scouring action aroundand behind the baffles thus minimizing microbial growthon the baffles and the fermenter walls. Extra cooling coils may be attached to baffles to improve the coolingcapacity of a fermenter without unduly affecting the geometry.

THE AERATION SYSTEM (SPARGER)

A sparger may be defined as a device for introducingair into the liquid in a fermenter. Three basic types ofsparger have been used and may be described as theporous sparger, the orifice sparger (a perforated pipe)and the nozzle sparger (an open or partially closedpipe). A combined sparger-agitator may be used inlaboratory fermentersand is discussed brieflyin a later section.

POROUS SPARGER

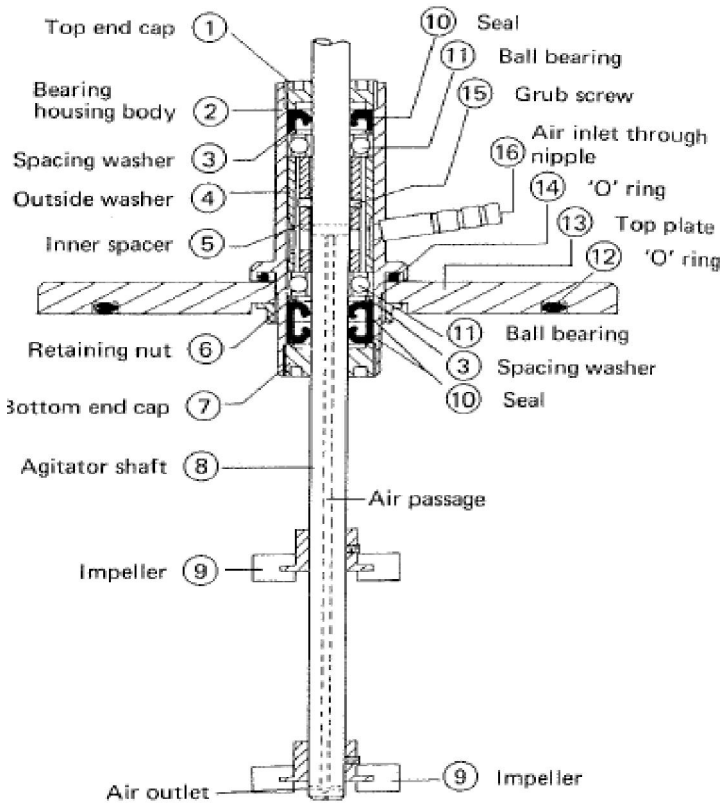
The porous sparger of sintered glass, ceramics ormetal, has been used primarily on a laboratory scale innon-agitated vessels. The bubble size produced fromsuch spargers is always 10 to 100 times larger than thepore size of the aerator block (Finn, 1954). Thethroughput of air is low because of the pressure dropacross the sparger and there is also the problem of thefine holes becoming blocked by growth of the microbialculture.

ORIFICE SPARGER

Various arrangements of perforated pipes have beentried in different types of fermentation vessel with orwithout impellers. In small stirred fermenters the perforatedpipes were arranged below the impeller in theform of crosses or rings (ring sparger), approximatelythree-quarters of the impeller diameter. In most designsthe air holes were drilled on the under surfaces ofthe tubes making up the ring or cross. Walker andHoldsworth (1958) commented that in production vessels,sparger holes should be at least 6 mm (1/4 inch)diameter because of the tendency of smaller holes toblock and to minimize the pressure drop.

In low viscosity fermentations sparged at 1 wm(volume of air- l volume of medium- l minute-I) witha power input of 1 W kg-I, Nienow et ai. (1988) foundthat the power often falls to below 50% of its unaeratedvalue when using a single Rushton disc turbinewhich is one-third the diameter of the vessel and a ringsparger smaller than the diameter of the agitator. If thering sparger were placed close to the disc turbine andits diameter was 1.2 times that of the disc turbine, anumber of benefits could be obtained (Nienow et

ai.,1988). A 50% higher aeration rate could be obtainedbefore flooding occurred, the power drawn was 75% ofthe unaerated value, and a higher KLa could be obtainedat the same agitator speed and aeration rate.These advantages were lost at viscosities of about 100m Pas.Orifice spargers without agitation have been used toa limited extent in yeast manufacture (Thaysen, 1945),effluent treatment (Abson and Todhunter, 1967) andlater in the production of single-cell protein in theair-lift fermenter which are discussed in a later sectionof this chapter (Taylor and Senior, 1978; Smith, 1980)



Agitator-air sparger hybrid

FERMENTER TYPES

The Waldhof-type fermenter

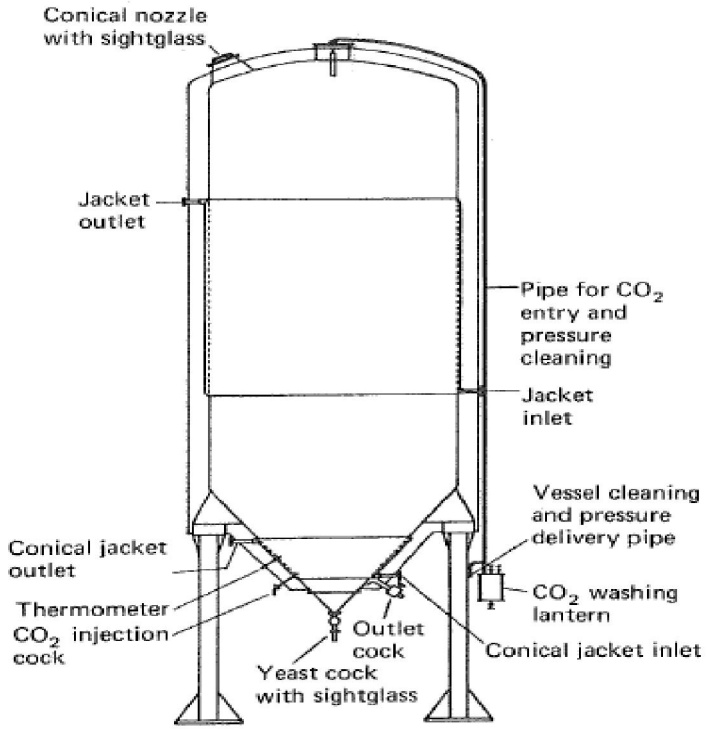
The investigations on yeast growth in SUlphite waste liquor in Germany, Japan and the United States of America led to the development of the Waldh of-type fermenter (Inskeep et al., 1951; Watanabe, 1976).Inskeep et al. (1951) have given a description of a production vessel based on a modification of the originaldesign of Zellstofffabrik Waldhof. The fermenterwas of carbon steel, clad in stainless steel, 7.9 m indiameter and 4.3-m high with a centre draught tube 1.2m in diameter. A draught tube was held by tie rodsattached to the fermenter walls. The operating volumewas 225,000 dm3 of emulsion (broth and air) or 100,000dm3 of broth without air. Non- sterile air was introducedinto the fermenter through a rotating pin-

wheeltype of aerator, composed of open-ended tubes rotating at 300 rpm. The broth passed down thedraught tube from the outer compartment and reducedthe foaming.

The tower fermenter

It is difficult to formulate a single definition whichencompasses all the types of tower fermenter. Theirmain common feature appears to be their height:diameterratio or aspect ratio. Such a definition has beengiven by Greenshields et al. (1971) who described atower fermenter as an elongated non-mechanicallystirred fermenter with an aspect ratio of at least 6:1 forthe tubular section or 10:1 overall, through which thereis a unidirectional flow of gases. Several different typesof tower fermenter exist and these will be examined inbroad groups based on their design.The simplest types of fermenter are those that consistof a tube which is air sparged at the base (bubblecolumns). This type of fermenter was first described forcitric acid production on a laboratory scale (Snell andSchweiger, 1949). This batch fermenter was in the formof a glass column having a height:diameter ratio of 16:1with a volume of 3 dm3. Humid sterile air was suppliedthrough a sinter at the base. Steel et al. (1955) reportedan increase in scale to 36 dm3 for a fermenter of thistype. Pfizer Ltd has always used non-agitated towervessels for a range of mycelial fermentation processesincluding citric acid and tetracyclines (Solomons, 1980;Carrington et al., 1992). Recently Pfizer Ltd sold theircitric acid interests to Arthur Daniels Midland who areoperating such vessels up to 23 m high (Burnett, 1993).

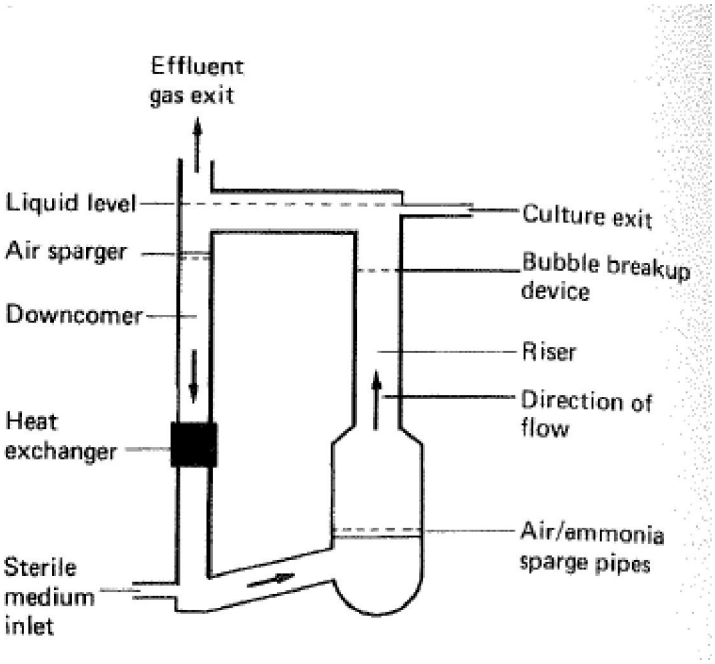
Perforated plates positioned at intervals m the tower to maintain maximum yeast production. The settling zone whichcould be of various designs, was to provide a zone freeof rising gas so that the cells could settle and return tothe main body of the tower and the clear beer could beremoved. This design must be considered as an intermediatebetween single- and multistage systems. Towersof up to 20,000 dm3 capacity and capable of producingup to 90,000 dm3 day-l have been installed. Greenshieldsand Smith (1971) commented that it was difficultto predict the upper operating limits for thesefermenters. Experiments with particular yeast strains inpilot-size towers were essential to establish optimumfull-scale operating conditions.



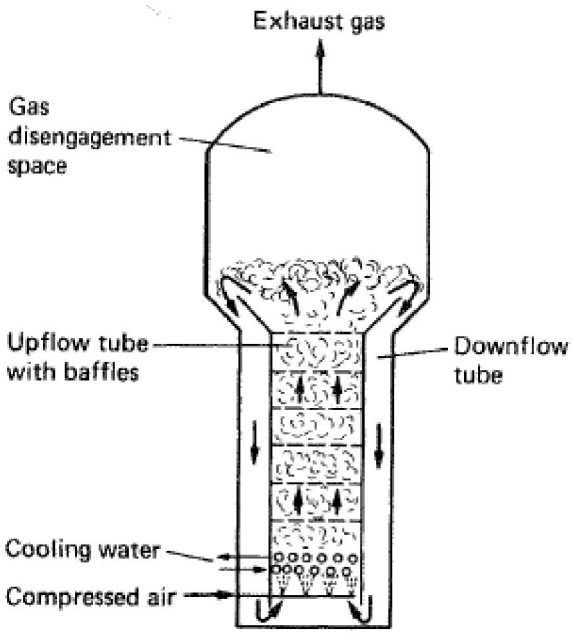
Tower Fermenter

Air-lift fermenters

An air-lift fermenter is essentially a gastight baffled riser tube (liquid ascending) connected toa downcomer tube (liquid descending). Air or gas mixtures are introduced into the base of theriser by a sparger during normal operating conditions.The driving force for circulation of medium in thevessel is produced by the difference in density betweenthe liquid column in the riser (excess air bubbles in themedium) and the liquid column in the downcomer(depleted in air bubbles after release at the top of theloop). Circulation times in loops of 45-m height may be120 seconds. More details on liquid circulation andmixing characteristics are discussed by Chen (1990).This type of vessel can be used for continuous culture.The first patent for this vessel was obtained by Schollerand Seidel (1940).It would be uneconomical to use a mechanicallystirred fermenter to produce SCP (single-cell protein)from methanol as a carbon substrate, as heat removalwould be needed in external cooling loops because ofthe high rate of aeration and agitation required tooperate the process. To overcome these problems, particularly that of cooling the medium when mechanicalagitation is used, air-lift fermenters with outer or inner loops was chosen. Development work for operational processes for SCP has been done by ICIpIc in Great Britain (Taylor and Senior, 1978; Smith,1980), Hoechst AG-Uhde GmbH in Germanyand Mitsubishi Gas Chemical Co. Inc. In Japan.



Air-lift fermenter with outer loop

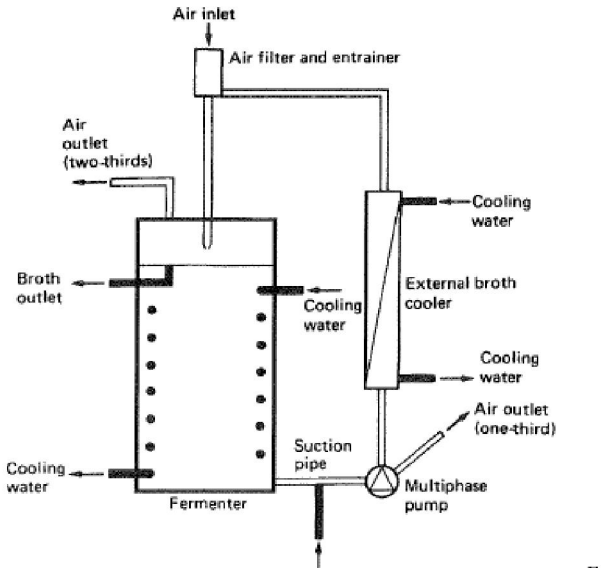


Air-lift fermenter with inner loop

The deep-jet fermenter

Some designs of continuous culture fermenterachieve the necessary mechanical power input with apump to circulate the liquid medium from the fermenterthrough a gas entrainer and back to the fermenter(Fig. 7.51; Hamer, 1979; Meyrath and Bayer,1979). Two basic construction principles have

beenused for the gas entrainer nozzles. The injector and theejector. In an injector a jet ofmedium is surrounded by a jet of compressed air.



Deep Jet fermentor

Rotating-disc fermenters

Rotating-disc contactors have been used in effluent treatment. They utilize a growing microbialfilm on slow rotating discs to oxidize the effluent.nderson and Blain (1980) have used the sameprinciple to construct small fermenters of up to 40-dm3 working volume. A wrange of filamentous fungi, includingspecies of Aspergillus, Rhizopus, Mucor and Penicillium,could be grown on the polypropylene discs. It hasbeen possible to obtain yields of 80 g dm-3 of citricacid from A. niger using this design of fermenter.

The success of a fermentation depends upon the existence of defined environmental conditions for biomass and product formation. To achieve this goal it is important to understand what is happening to a fermentation process and how to control it to obtain optimal operating conditions. Thus, temperature, pH, degree of agitation, oxygen concentration in the medium and other factors may have to be kept constant during the process. The provision of such conditions requires careful monitoring (data acquisition and analysis) of the fermentation so that any deviation from the specified optimum might be corrected by a control system. Criteria which are monitored frequently are listed in Table, along with the control processes with which they are associated. As well as aiding the maintenance of constant conditions, the monitoring of a process may provide information on the progress of the fermentation. Such information may indicate the optimum time to harvest or that the fermentation is progressing abnormally which may be indicative of

contamination or strain degeneration. Thus, monitoring equipment producesin formation indicating fermentation progress as well as being linked to a suitable control system. In initial studies the number of functions which are to be controlled may be restricted in order to gain more knowledge about a particular fermentation. Thus, the pH may be measured and recorded but not maintained at a specified pH or the dissolved oxygen concentration may be determined but no attempt will be made to prevent oxygen depletion. Also, it is important to consider the need for a sensor and its associated control system to interface with a computer (to be discussed in a later section).This chapter will consider the general types of control systems which are available, specific monitoring and control systems and the role of computers. It is apparent from the Table that a considerable number of process variables may need to be monitored during fermentation. Methods for measuring these variables, the sensors or other equipment available andpossible control procedures are outlined below.

There are three main classes of sensor:

1. Sensors which penetrate into the interior of the fermenter, e.g. pH electrodes, dissolved- oxygen electrodes.
2. Sensors which operate on samples which arecontinuously withdrawn from the fermenter, e.g. exhaust-gas analysers.
3. Sensors which do not come into contact with the fermentation broth or gases, e.g. tachometers, load cells. It is also possible to characterize a sensor in relation to its application for process control:

1. In-line sensor. The sensor is an integrated part of the fermentation equipment and the measured value obtained from it is used directly for process control.
2. On-line sensor. Although the sensor is an integral part of the fermentation equipment, the measured value cannot be used directly for control.

Category	Sensor	Possible control function
Physical	Temperature	Heat/cool
	Pressure	
	Agitator shaft power	
	rpm	
	Foam	Foam control
	Weight	Change flow rate
	Flow rate	Change flow rate
Chemical	pH	Acid or alkali addition, carbon source feed rate
	Redox	Additives to change redox potential
	Oxygen	Change feed rate
	Exit-gas analysis	Change feed rate
	Medium analysis	Change in medium composition

Table: Process sensors and their possible control functions

An operator must enter measured values in to the control system if the data is to be used in process control.

3. Off-line sensor. The sensor is not part of the fermentation equipment. The measured value cannot be used directly for process control. Anoperator is needed for the actual measurement (e.g. medium analysis or dry weight sample) and for entering the measured values into the control system for process control.

When evaluating sensors to use in measurement and control it is important to consider response time, gain, sensitivity, accuracy, ease and speed of calibration, stability, reliability, output signal (continuous or discontinuous),materials of construction, robustness, sterilization, maintenance, availability to purchase and cost.

Possible Questions

Two Marks

1. Define fermentation
2. What is meant by sparger?
3. What are impellers
4. Draw the basic schema of a fermentor.
5. What is the criteria for fermentation.

Eight Marks

1. Write about aeration and agitation in a bioreactor.
2. Comment on packed bed and trickle flow reactors.
3. Explain the basic design of a fermenter with neat diagram
4. Discuss on cyclone and photo bioreactors.
5. Explain about the computer control of fermentation process
6. Notes on tube and fluidized bed reactors.
7. Comment on configuration of bioreactor.

Questions	Option A	Option B	Option C	Option D	Answer Key
Microbes are grown in especially designed vessels called----------, containing special media for its growth.	Fermentors	Batch cookers	swap medium	conical flasks	Fermentors
Fermentation tank should be provided with ports for ---------- addition.	contamination	medium	inoculums	foam	inoculums
----------are used in side of fermentors to avoid vortex formation	spargers	bearing glands	rotameter	baffles	baffles
----------tanks are used in production of all and lactic acid fermentation	stainless steel	glass	copper	wooden	wooden
The ----------in stainless steel fermentor gives resistance to halogen salts, lodine and sea water	chromium	molybdenum	nickel	tungsten	molybdenum
-----are used to regulate the addition of medium,nutrients, defoamers.	syringe pumps	peristaltic pumps	feed pumps	pressure pumps	feed pumps
The fermentor vessel ----------should be increased with scale.	diameter	thickness	height	design	thickness
-------between top plate and vessel is very important to maintain airtight / aseptic condition.	baffles	sealing	sparger	clamp	sealing
---------device is used for giving air into fermentor	sparger	baffles	shaft	bearings box	sparger
----------type of bubbles facilitate high oxygen transfer than ----------bubbles	larger, smaller	smaller, larger	medium, large	very small, very medium	smaller, larger
The ideal aspect ratio for a fermentor is	3:01	4:01	5:01	6:01	5:01

The number of baffles used in fermentor of diameter 3dm3 is	2 TO 4	4 TO6	6 TO 8^2	8 TO 10	6 TO 8
----------spargers are used widely large scale fermentation process	porous	orifice	nozzle	combined sparger agitator	nozzle
---------removes enough moisture from the gas leaving fermentor and prevent excess fluid loss.	baffles	heat exchange	cooler	exit gas cooler	exit gas cooler
In high quality bioreactor, all the processes in fermentation are controlled by	agitator	aeration	process controller	cooler	process controller
In-line, on-line and off-line are types of ----------	foamers	agitator glands	shaft	sensors	sensors
Example of In-line sensors are	Ion-specific sensors	mass spectrophotometer	antifoam probe	medium addition probe	antifoam probe
----------sensors don't form integral part of fermentor.	in-line	on-line	off-line	fermentor	off-line
----------is generated due to ^{3}mixing by agitator and microbes action on substrates during fermentation process.	energy	heat	resistance	current	heat
--------are semiconductors of Iron, Michel oxides exhibiting large change in resistance with small change of temperature.	mercury in-glass thermometers	electrical resistance	thermistors	electrical impedance	thermistors
Stainless steel ---------- sensors are used for temperature measurements in fermentation system	Pt 100	Pl 100	Pb 100	Ps 100	Pt 100
Gas flow rate is measured by ----------	thermometers	rotameters	pistonmeters	torsion dynamometer	rotameters

The liquid flow rate is measured by using	thermometers	thermal mass flowmeter	pistonmeters	torsion dynamometer	thermal mass flowmeter
Which gauge is used for measuring pressure under aseptic condition	peristaltic	diaphragm	diaguls	bourbon tube	diaphragm
-------is used for measuring the speed of agitator	wattmeter	torsion dynamometer		tachometer	All
Peristaltic pump is mainly used for addition of ----------and--------	medium and inoculums	acid and base	buffers and antifoamers	salts and growth factors	acid and base
Dissolved oxygen in fermentation process medium measured by using ----------	galvanic electrode	pH electrode	thermometers	thermistors	galvanic electrode
If initially foam has started forming interior ----------has been used to control foam	antifoamers	mechanical foam breaker	water	controlling agitator/aeration speed	mechanical foam breaker
Oxygen diffuses from tubing into medium is measured by	galvanic electrode	paramagnetic gas analyzer	platinum electrode	thermistors	paramagnetic gas analyzer
pH denotes the presence of ----------in aqueous solution	hydrogen ion	hydroxyl ion	carboxyl ions	carbonyl ion	hydrogen ion
The voltage difference between two electrodes is used to determine ----------of unknown solution	temperature	pH	moisture	dissolved oxygen	pH
----------,---------- and---------- are three distinct areas of computer function.	logging of process data	data analysis	process control	all	All
----------system controls the addition of liquid from reservoir to fermentator	analog control	direct control	direct digital control	human control	direct digital control
Computers were employed in fermentation early ----------	1940	1950	1960	1970	1960
Computers were initially restricted in fermentation	high cost	complexity	reduction of man	high power	high cost

industry because of			power		
The computer functions in fermentation process were postulated by ---------- in 1972	Neeri	Nyili	Needham	Natel	Nyili
The signals produced during fermentation process is converted to ---------- form.	analogue	digital	data	process	analogue
------serves as junction point for inputs from computers and output signals from computer to fermentor controls such as pump.	addition reservoir	interface	tele-type	virtual display unit	interface
The ----------signal from fermentors are not understood by the computer.	voltage	current	pulse	meter	voltage
The interface converts the ----------signal to ---------	analogue to digital	digital to analogue	voltage to pulse	pulse to voltage	analogue to digital
The accuracy of computer control depends upon the number of ----------it sends to the computer	units	alarms	bits	data	bits
For variables which are not measureable, concept of ----------are used in fermentation industry	indirect sensors	gateway sensors	by pass sensors	direct sensors	gateway sensors
In fermentation, it is very important to find the ----------or---------- of product from the given carbon source.	gases or distribution	transport or energy	productivity or conversion yield	concentration or consumable	productivity or conversion yield
---------and---------- developed methods to analyse the biomass and product concentration during fermentation process.	Hump and Honey	Humphery and Cooney	Hughes and Humphery	Hyhes and Cooney	Humphery and Cooney
The capacity of the batch fermentors _____	10 – 12 litre	12 – 15 litre	20 -40 litre	6 – 8 litre	10 – 12 litre

Large fermentors range from _____	2000 – 5,000 gallons	5000 – 10,000 gallons	10,000 gallons	none of the above.	2000 – 5,000 gallons
Clogging problems occur in _____	Bacteria	Algae	Mycebial	Mycorhiza	Bacteria
Sparger size ranges from	1/64 – 1/32 inch	1/32 – 1/18 inch	1/48 – 1/32 inch	1/24 – 1/12 inch	1/64 – 1/32 inch
The first pilot fermenter was erected in India at Hindustan Antibiotic ltd, Pune in the --------- year.	1920	1930	1940	1950	1950
-------- type of bioreactor is used for vinegar production.	packed tower	photo bioreactor	pulsed column	bubble column	packed tower
---------- is used for sep and other algal protein production.	packed tower	photo bioreactor	pulsed column	bubble column	photo bioreactor
---------- is used as a enzyme bioreactor.	packed tower	photo bioreactor	pulsed column	bubble column	pulsed column
---------- are provided to maintain constant temperature inside the bioreactor	baffles	cooling coils	stirrer gland	sparger	cooling coils
The impeller should be ---------- of the vessel diameter.	1\1	none of the above	1\4	1\3	1\3
Range of fermentation tank used in enzyme production.	1500 30,000	1000 – 30,000 c	gallons	none of the above	1500 30,000
-------- fermenter is called as elongated non-mechanically stirred fermenter	Tower	Airlift	Cylindraconical	Deep jet	Tower
_____ fermentor is a gas tight baffled rise tube connected to a down comer tube.	Tower	Air lift	Cylinder conical	Deep jet	Air lift
Multiple air lift fermenter Is designed by	Bakker etal	Okabe etal	Bacon etal	Dawsa	Bakker etal
The inoculum level introduced into a production tank is usually _____.	0.5-5%	5 – 25%	20 – 40%	50%	5 – 25%

-------- fermenter is called as elongated non-mechanically stirred fermenter	Tower	Airlift	Cylindraconical	Deep jet	Tower
_____ fermentor is a gas tight baffled rise tube connected to a down comer tube.	Tower	Air lift	Cylinder conical	Deep jet	Air lift

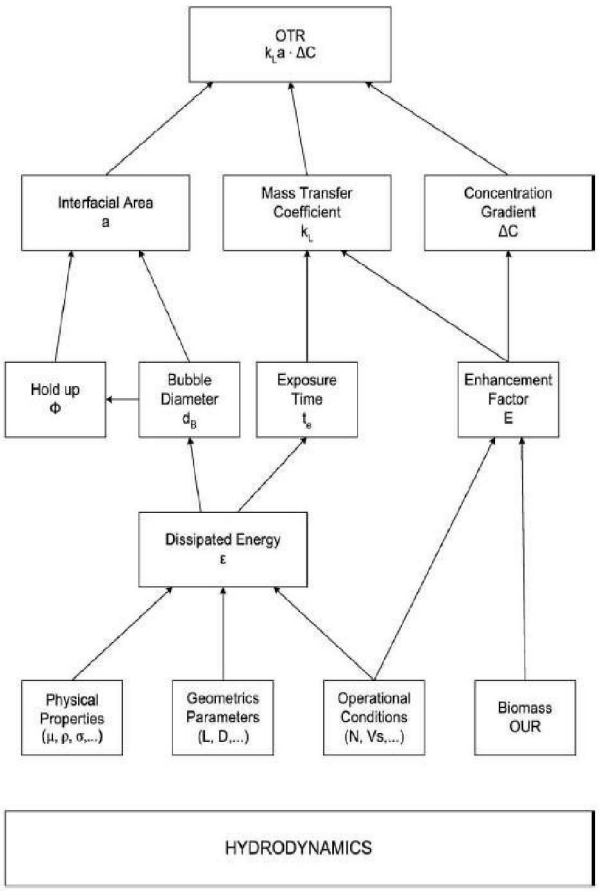
Unit II

Most industrial microbial processes are aerobic, and are mostly carried out in aqueous medium containing salts and organic substances; usually these broths are viscous, showing a non- Newtonian behaviour. In these processes, oxygen is an important nutrient thatis used by microorganisms for growth, maintenance and metabolite production, and scarcity of oxygen affects the process performance. Therefore, it is important to ensure an adequate delivery of oxygen from a gas stream to the culture broth. Consequently, accurate estimation of the oxygen transfer rate (OTR) at different scales and under different operational conditions has a relevant role for the prediction of the metabolic pathway for both growth and production of any wished metabolite in the aerobic cultus it is of critical importance for the selection, design and scale-up of bioreactors. Extensive literature on the oxygen transfer rate in bioreactors is nowadays available and a considerable part of it has been published in the last years. Substantial results on different aspects of oxygen transport have been reviewed in different works.

The oxygen mass transfer rate can be described as proportional to the concentration gradient, being the volumetric mass transfer coefficient, kLa (Eq. (4)) the proportionality constant. The maximum value of the concentration gradient is limited due to the low solubility of most gases associated to aerobic fermentation, notably oxygen. Therefore, the maximum mass transfer rate from the gas to the liquid in the bioreactor can be estimated by the product kLa•C□, being C□ the satuation concentration in the liquid phase. There are a great number of empirical equations to determine kLa, and efforts have recently been made for theoretical prediction of kLa values; most of these works having been developed for bubble columns and airlifts and a lesser number dealing with the transport in stirred tanks bioreactors. These prediction methods successfully predict the transport coefficient for bioreactors of different sizes and under different operational conditions. The bioprocesses are usually conducted under previously optimized conditions (temperature, pH, pressure, mixing, concentrations of biomass and nutrients), with an operational mode previously chosen (batch, fed-batch, resting cell, continuous).

The overall mass transfer rate is not easy to measure, because different phenomena are simultaneously taking place; also the relative importance of these phenomena changes with the scale, the type of bioreactor, etc. Therefore, the OTR is influenced by a high number of parameters (physical properties of gas and liquid, operational conditions, geometrical parameters of the bioreactor) and also by the presence of biomass, that is, the consumption of oxygen by the cells. Bioprocesses involve simultaneous transport and biochemicalreactions of several chemical species. Sometimes, the transport of substrates to cells occurs at a rate considerably higher than the rate ofthe metabolic biochemical reactions; in this case,

the overall rate of substrate conversion is governed only by the kinetics of the biochemical reactions. However, if mass transfer rate is lower than reaction rate, transport rate can be the step controlling the overall process rate and, moreover, the mass transfer rate may be influenced by the chemical rate of the bioprocess. When a species in gas phase is absorbed into a liquid and reacts there, the concentration profiles of the absorbed species change due to the chemical reaction and the absorption rate may be enhance). Oxygen absorption into a fermentation broth can be considered as the absorption of a gas into a liquid where it reacts, oxygen is consumed by the suspended microorganism, and therefore an enhancement of oxygen mass transfer rate can take place. The increase of the specific gas absorption rate per driving force unit and per interfacial area unit, due to the presence of the dispersed phase, can be characterized by an enhancement factor, E.



Oxygen transfer rate (OTR) description During aerobic bioprocess, the oxygen is transferred from a rising gas bubble into a liquid phase and ultimately to the site of oxidative phosphorylation inside the cell, which can be considered as a solid particle. The transport of oxygen from air bubbles to the cells can be represented by a number of steps and resistances, as schematized; the liquid film resistances around bubbles usually control the overall transfer rate. The simplest theory on gas–liquid mass transfer is the two film

model (Whitman,1923) and usually the gas–liquid mass transfer rate is modeled according to this theory, describing the fluxthrough each film as the product of the driving force by the mass transfer coefficient, according to:

$$J0 = kG \_ \eth pG - pi\Þ = kL \_ \eth Ci - CL\Þ \eth 1\Þ$$

being J0 the molar flux of oxygen (mol•m−2 s−1) through the gas–liquid interface; kG and kL, are the local mass transfer coefficients; pG is the oxygen partial pressure in the gas bubble; and CL, the dissolved oxygen concentration in the bulk liquid; index i refers to values at the gas– liquid interface. Since the interfacial concentrations are not directly measurable and considering the overall mass transfer coefficient, it can be rewritten:

$$J0 = KG\ pG - p\square$$

$$= KL\ C\square - CL$$

ð2Þ

where p□ is the oxygen pressure in equilibrium with liquid phase;

C□ is the oxygen saturation concentration in the bulk liquid in equilibrium to the bulk gas phase, according to Henry's law (p□=HC□); KG and KL are the overall mass transfer coefficients. Combining Eqs. (1) and (2), the following relationship is obtained:

$$KL = 1\ HkG + 1\ kL$$

ð3Þ

Taking into account that oxygen is only slightly soluble inwater (H is very large), it is commonly accepted that the greatest resistance for mass transfer is on the liquid side of the interface and the gas phase resistance can usually be neglected and thus the overall mass transport coefficient is equal to the local coefficient: KL=kL. The oxygen mass transfer rate per unit of reactor volume, NO2, is obtained multiplying the overall flux by the gas–liquid interfacial area per unit of liquid volume, a: NO2 = ad J0 = kLa _ C□− CL _ _ ð4Þ

Due to the difficulty of measuring kL and a separately, usually the product kLa is measured and this parameter – called volumetric mass transfer coefficient – characterizes the transport from gas to liquid.

MASS TRANSFER

The determination of kLa in bioreactors is essential in order to establish aeration efficiency and to quantify the effects of the operating variables on the provision of dissolved oxygen. A number of methods have been developed to determine the oxygen transfer rate in bioreactors (Van't Riet, 1979). Some of these methods are applied to others compounds as well, but others are specific for oxygen transfer measurement. When selecting a method, several factors must be taken into account (Novak and Klekner, 1988).

i. the aeration and homogenization systems used,

ii. the bioreactor type and its mechanical design,

iii. the composition of the fermentation medium and

iv. the possible effect of the presence of microorganism.

The mass balance for the dissolved oxygen in the well-mixed liquid phase can be established as: dC / dt = OTR− OUR ð5Þ

where dC/dt is the accumulation oxygen rate in the liquid phase, OTR represents the oxygen transfer rate from the gas to the liquid, described according to Eq. (4), and OUR is the oxygen uptake rate by the microorganisms; this last term can be expresed by the product qO2•CX, being qO2 the specific oxygen uptake rate of the microorganism employed and CX the biomass concentration. The most common methods applied to measuring the oxygen transfer rate in a microbial bioprocess can be classified depending on whether the measurement is realized in the absence of microorganisms or with dead cells or in the presence of biomass that consumes oxygen at the time of measurement.

THE COMPONENT PARTS OF A FERMENTATION PROCESS

Regardless of the type of fermentation (with the possible exception of some transformation processes) an established process may be divided into six basic component parts:

(i) The formulation of media to be used in culturing the process organism during the development of the inoculum and in the production fermenter.

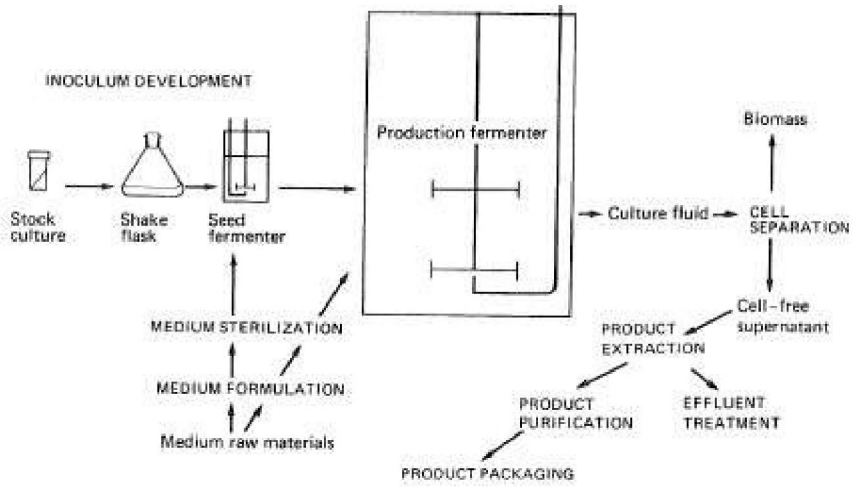
(ii) The sterilization of the medium, fermenters and ancillary equipment.

(iii) The production of an active, pure culture in sufficient quantity to inoculate the production vessel.

(iv) The growth of the organism in the production fermenter under optimum conditions for product formation.

(v) The extraction of the product and its purification.

(vi) The disposal of effluents produced by the process.



Schematics of general fermentation process

However, one must also visualize the research and development programme which is designed to gradually improve the overall efficiency of the fermentation. Before a fermentation process is stablished a producer organism has to be isolated, modified such that it produces the desired product in commercial quantities, its cultural requirements determined and the plant esigned accordingly. Also, the extraction process has to be established. The development programme would involve the continual improvement of the process organism, the culture medium and the extraction process.

STERILIZATION

A fermentation product is produced by the culture of certain organism, or organisms, in a nutrient medium. The fermentation is invaded by a foreign microbe then the following consequences may occur:

(i) The medium would have to support the growthof both the production organism and the contaminant, resulting in a loss of productivity.

(ii) If the fermentation is a continuous one then the contaminant may 'outgrow' the production organism and displace it from the fermentation.

(iii) The foreign organism may contaminate the final product, e.g. single-cell protein where the cells, separated from the broth, constitute the product.

(iv) The contaminant may produce compounds which make subsequent extraction of the final product difficult.

(v) The contaminant may degrade the desired product; this is common in bacterial contamination of antibiotic fermentations where the contaminant would have to be resistant to the normal inhibitory effects of the antibiotic and degradation of the antibiotic is a common resistance mechanism, e.g. the degradation of f3lactam antibiotics by f3-lactamase- producing bacteria.

(vi) Contamination of a bacterial fermentation with phage could result in the lysis of the culture. Avoidance of contamination may be achieved by:

(i) Using a pure inoculum to start the fermentation

(ii) Sterilizing the medium to be employed.

(iii) Sterilizing the fermenter vessel.

(iv) Sterilizing all materials to be added to the fermentation during the process.

(v) Maintaining aseptic conditions during the fermentation.

The extent to which these procedures are adopted is determined by the likely probability of contamination and the nature of its consequences. Some fermentation are described as 'protected'- that is, the medium may be utilized by only a very limited range of microorganisms, or the growth of the process organism may result in the development of selective growth conditions, such as a reduction in pH. The brewing of beer falls into this category; hop resins tend to inhibit the growth of many micro-organisms and the growth ofbrewing yeasts tends to decrease the pH of the medium. Thus, brewing worts are boiled, but not necessarily sterilized, and the fermenters are thoroughly cleaned with disinfectant solution but are not necessarily sterile. Also, the precautions used in the development of noculum for brewing are far less stringent than, for example, in an antibiotic fermentation. However, the vast majority of fermentations are not 'protected' and,if contaminated, would suffer some of the consequences previously listed.

MEDIUM STERILIZATION

Media may be sterilized by filtration, radiation, ultrasonic treatment, chemical treatment or heat. However, for practical reasons, steam is used almost universally for the sterilization of fermentation media. The major exception is the use of filtration for the sterilization of media for animal-cell culture - such media are completely soluble and contain heat labile components making filtration the method of choice. Filtration

techniques will be considered later in this chapter. Before the techniques _f\J,_which are used for the steam sterilization of culture No media are discussed it is necessary to discuss the kinetics of sterilization. The destruction of micro-organisms by steam (moist heat) may be described as a first-order chemical reaction and, thus, may be represented by the following equation:

$$-dN/dt = kN$$

N is the number of viable organisms present, t is the time of the sterilization treatment,
k is the reaction rate constant of the reaction, or the specific death rate.

It is important at this stage to appreciate that we are considering the total number of organisms present in the volume of medium to be sterilized, not the concentration - the minimum number of organisms to contaminate a batch is one, regardless of the volume of the batch. On integration of equation (5.1) the following expression is obtained:

where No is the number of viable organisms present at the start of the sterilization treatment,

Nt is the number of viable organisms present after a treatment period, t.

On taking natural logarithms, equation is reduced to:

The graphical representations of equations (5.1) and (5.3) are illustrated in Fig. 5.1, from which it may beseen that viable organism number declines exponentially over the treatment period. A plot of the naturall ogarithm of N,.INo against time yields a straight line, the slope of which equals - k. This kinetic description makes two predictions which appear anomalous:

(i) An infinite time is required to achieve sterileconditions (i.e. N,. = 0). (ij) After a certain time there will be less than one viable cell present.

Thus, in this context, a value of Nt of less than one is considered in terms of the probability of an organism surviving the treatment. For example, if it were pre-dieted that a particular treatment period reduced the population to 0.1 of a viable organism, this implies that the probability of one organism surviving the treatment is one in ten. This may be better expressed in practical terms as a risk of one batch in ten becoming contaminated. This aspect of contamination will be on sidered later. The relationship displayed in Fig. 5.1 would be observed only with the sterilization of a pure culture in one physiological form, under ideal sterilization conditions.

The value of k is not only species dependent, but dependent on the physiological form of the cell; for example, the endospores of the genus Bacillus are far more heat resistant than the vegetative cells. Richards (1968) produced a series of graphs illustrating the deviation from theory which may be experienced in practice. Figures 5.2a, 5.2b and 5.2c illustrate the effect of the time of eat treatment on the survival of a population of bacterial endospores. The deviation from an immediate exponential decline in viable spore number is due to the heat activation of the spores, hat is the induction of spore germination by the heat and moisture of the initial period of the sterilization process. In Fig. 5.2a the activation of spores is significantly more than their destruction during the early stages of thep rocess and, therefore, viable numbers increase before the observation of exponential decline. In Fig. 5.2b activation is balanced by spore death and in Fig. 5.2c activation is less than spore death.

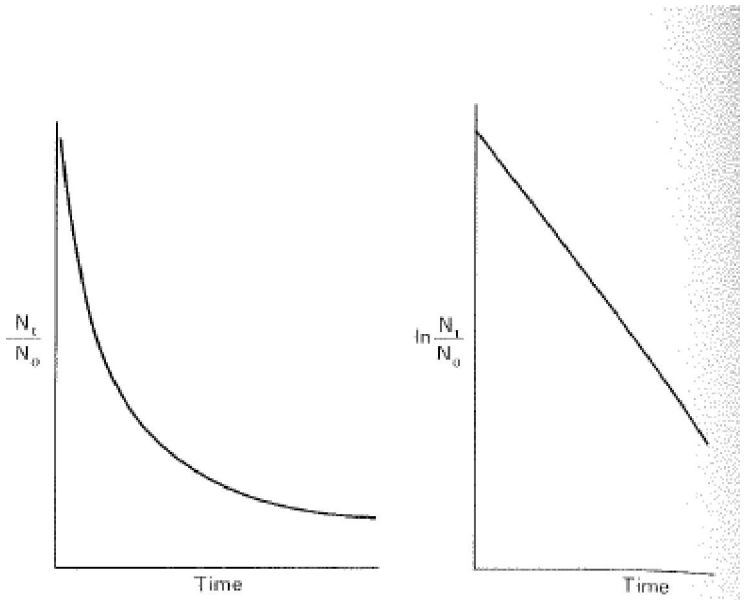


FIG. 5.1. Plots of the proportion of survivors and the natural logarithm of the proportion of survivors in a population of micro-organisms subjected to a lethal temperature over a time period.

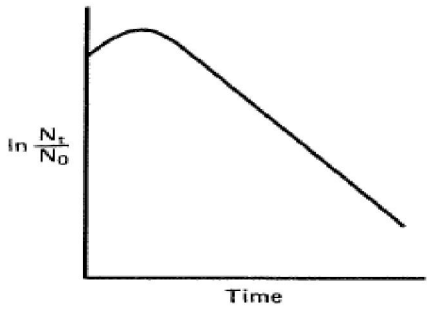


FIG. 5.2a. Initial population increase resulting from the heat activation of spores in the early stages of a sterilization process (Richards, 1968).

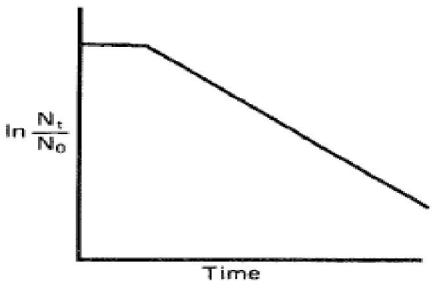


FIG. 5.2b. An initial stationary period observed during a sterilization treatment due to the death of spores being completly compensated by the heat activation of spores (Richards, 1968).

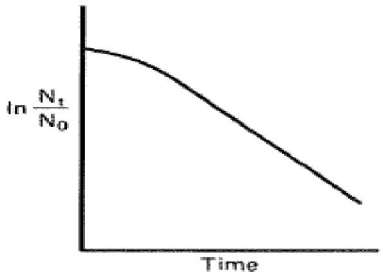


FIG. 5.2c. Initial population decline at a sub-maximum rate during a sterilization treatment due to the death of spores being compensated by the heat activation of spores (Richards, 1968).

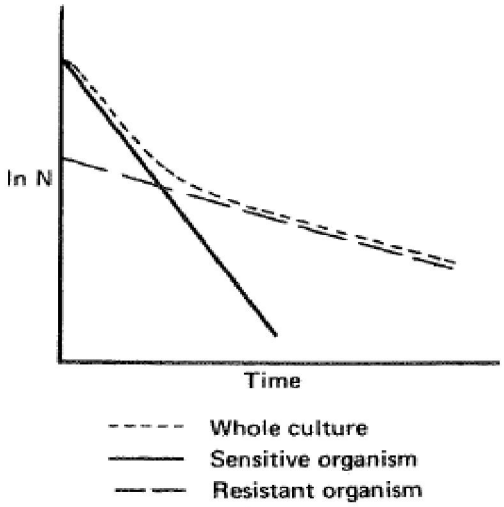


FIG. 5.3a. The effect of a sterilization treatment on a mixed culture consisting of a high proportion of a very sensitive organism (Richards, 1968).

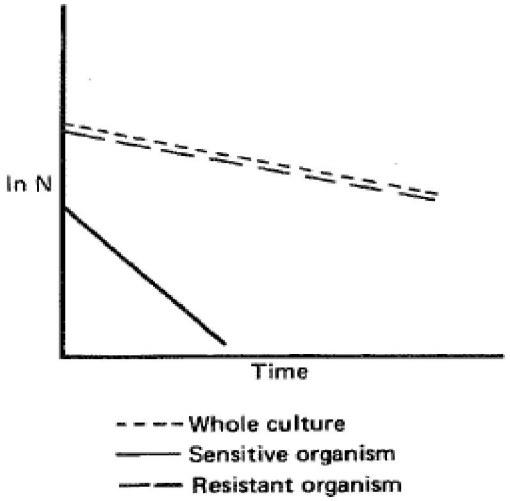


FIG. 5.3b. The effect of a sterilization treatment on a mixed culture consisting of a high proportion of a relatively resistant organism (Richards, 1968).

Possible Questions

Two marks

1. Define heat and mass transfer
2. Give the formula for transfer of heat in a fermentor
3. What is the mode of oxygen transfer in a media?
4. Comment on the relationship between critical oxygen concentration and microbial growth.
5. How is meant by scale up process? Give one example.

Eight marks

1. Discuss on the measurement and control of fermentation process.
2. What are the salient features of fermentor?
3. Give an account on oxygen transfer and critical oxygen concentration during fermentation.
4. Describe the sterilization process of fermentors.
5. Describe the sterilization process of medium.
6. Detailed notes on how oxygen is transferred from medium to the microbial cell.
7. Notes on gas-liquid exchange in fermentor.
8. How scale-up process is done in industrial fermentation?

UNIT II	Option A	Option B	Option C	Option D	Answer Key
-------method is the most preferred method compared to other agents for mass sterilization.	steam	UV light	ethidium bromide	chlorine	steam
----------is the least expensive and reliable method for fermentor design and operation	chemicals	passing of fire flames	moist heat	dry heat	moist heat
Sterilization is necessary in fermentation as it may	contaminants are not affected	affect the yield of fermentation products	not remove pathogens	value the fermentation	affect the yield of fermentation products
The methods for sterilization involves both ----------and----------	destruction and encourages microbes	destruction and removal of microbes	alters pH and growth inactivation	removes and microbes growth	destruction and removal of microbes
Sterilization also help in ---------- and----------of complex substrates used in fermentation.	breakdown and solubility	buildup and toxicity	precipitation and agglomeration	charring and precipitation	breakdown and solubility
Sterilization has disadvantage of destruction of heat ---------compounds in medium.	sensitive	resistant	soluble	insoluble	sensitive
There is always a ---------- relationship between time and temperature in sterilization of production medium and fermentor	linear	inverse	non-linear	reverse	linear
During sterilization of fermentor tanks, it is very important to sterilize the---------- ----------attached to it	raw material	vitamins	ancillary equipments	valves	ancillary equipments

Ancillary equipments in fermentors means the ----------	seed tank	fermentation medium	extra connection	antifoamers	extra connection
The sterilization temperature of the fermentation equirement is	120°C – 15 min	120°C – 20 min	115°C – 15min	115°C – 20min	120°C – 20 min
After sterilization, all parts of fermentor are kept sterile by maintaining at ----------pressure	positive	negative	no	zero	positive
There should be no permanent direct connection below ----------and----------parts of the fementor system	medium and air	sterile and non-sterile	mixing and air	probes and medium	sterile and non-sterile
High quality valves such as---------- should be used where joints are needed connecting to fermentor	plastic	β-hydroxybutyrate	silica gel	rubber	rubber
Sugar containing medium can't be sterilized by prolonged heating because sugars undergo	reaction with contaminants	charring	caramelization	reaction with phosphates	caramelization
-----media gives lower yields and poor growth of microorganisms in fermentation	cooled	overcooled	undercooled	contaminant	overcooled
In continous sterilization, the fermentation medium is passed through ----------aiding in sterilization.	heat exchange	holding coil	cooler	heat exchange, holding coil and cooler	heat exchange, holding coil and cooler

----------are simplest method employed for sterilizing production medium.	continous fermentors	batch cookers	filtration	radiation	batch cookers
Jackets are used around fermentors to aid in ---------- and-------of production media.	batch and continuous sterilization	maintaining the pH and foam	heating and cooling	circulation of air and steering	heating and cooling
In ----------, the medium to be sterilized to maintained at particular temperature and time.	heat exchange	holding coil	cooler	heat exchange, holding coil and cooler	holding coil
By steam injection method the high temperature steam is passed into the production medium where holding time is ----------	1-3 minutes	2-4 minutes	1-5 minutes	2-5 minutes	1-5 minutes
Continuous sterilization is highly advantageous	saves production time and plant space	poor quality of medium	high steam costs	high sterilizing temperature and longer holding time	saves production time and plant space
----------media require very long sterilization time period.	synthetic	complex	cruder	semi-synthetic	cruder
The technique of air filtration in fermentation industries was developed by----------	Bourbon et al	Bourdillon et al	Billy et al	Cherey et al	Bourdillon et al
The technique developed by Bourdillon et al was studied by -----	and Cherry	Terjesen and Cherry	Stanbury and Cherry	Wahsman and Cherry	Terjesen and Cherry

----------and----------					
In air filtration technique slab of slag wool used was of --------inches thick,--------lb/ft3 thick and less than---------in diameter.	3,15,4	3,16,6	3,17,6	3,17,7	3,17,6
Cobalt increases the growth of _____.	Streptomyes olivaceus	S. griseus	S. oryzae	P. notatum	Streptomyes olivaceus
Temperature of _____ in the production tank is satisfactory during fermentation.	75° F	80° F	85° F	63° F	80° F
Stabilization of mask is practiced by reducing the pH and adding reducing agents _____	Sodium citrate	Sodium sulphite	Ammonium sulphate	Sodium thiosulphate	Sodium sulphite
Sterilization of air is done by passing it through columns filled with _____	Activated charcoal	Dry charcoal	Liquid paraffin	Melted charcoal	Activated charcoal
_____ are the antifoam agents used to suppress the foam formation.	Soyabean oil	Cord-linee oil	Palm oil	Cedar-wood oil	Soyabean oil
Mass transfer occurs in mixtures containing ------	local concentration variation	same concentration	different solute	different solvent	local concentration variation
Mass is transferred from one location to another under the influence of a ---------- in the system.	concentration gradient	concentration defecient	concentration reference	concentration base	concentration gradient
Concentration of oxygen at the surface of air	high	low	very low	medium	high

bubbles is----- compared with the rest of the fluid,					
---------- is the movement of component molecules in a mixture under the influence of a concentration difference in the system	Molecular diffusion	passive diffusion	active diffusion	microbial diffusion	Molecular diffusion
----------occurs in the direction required to destroy the concentration difference.	Molecular diffusion	passive diffusion	active diffusion	microbial diffusion	Molecular diffusion
According to ---------- molecules A will diffuse away from the region of high concentration until eventually the whole system acquires uniform composition	Diffusion theory	passive diffusion	active diffusion	Molecular diffusion	Diffusion theory
According to ----------, mass flux is proportional to the concentration gradient.	Diffusion theory	Molecular diffusion	Fick's Law of diffusion	Molecular diffusion	Fick's Law of diffusion
The only mechanism for intra particle mass transfer is ----------	passive diffusion	molecular diffusion	microbial diffusion	active diffusion	molecular diffusion
The ----------is a useful model for mass transfer between phase.	single film theory	two film theory	no film theory	multiple film theory	two film theory
The -------- of solute from one phase to another involves transport	Mass transfer	liquid transfer	oxygen transfer	ion transfer	Mass transfer

from bulk of one phase to the interface, and then from the interface to the bulk of the second phase.					
The majority of fermentation processes are ----------	aerobic	anaerobic	facultative anaerobic	microaerophilic	aerobic
The solubility of oxygen in pure water is -------at 4°C	2 mg/L	8 mg/L	4mg/L	1 mg/L	8 mg/L
The solubility of sucrose in pure water is -------at 4°C	200 g/L	400 g/L	600 g/L	800 g/L	600 g/L
The---------- of an industrial fermentation process is normally satisfied by aerating and agitating the fermentation broth.	Mass transfer	oxygen demand	nutrient availibility	nitrogen availability	oxygen demand
The productivity of many fermentations is limited by ----------.	Mass transfer	nitrogen availability	oxygen availability	oxygen demand	oxygen availability
which law describes the solubility of O_2 in nutrient solution in relation to the O_2 partial pressure in the gas phase	Ford's law	Henry's Law	Raman's law	Libert's law	Henry's Law
The effect of dissolved oxygen concentration on the specific oxygen uptake rate follows the --------- type	Diffusion theory	Henry's Law	Michaelis-Menten	film theory	Michaelis-Menten

curve					
The specific oxygen uptake rate increases with increase in the dissolved oxygen concentration up to a certain point called as ---------- above which no further increase in oxygen uptake rate occurs	specific oxygen concentration	critical oxygen concentration	microbial oxygen concentration	integrated oxygen concentration	critical oxygen concentration
Critical dissolved oxygen concentrations for *E.coli* is ------ mMoles/dm3	0.004	0.008	0.022	0.018	0.008
Critical dissolved oxygen concentrations for *Saccharomyces* sp is ------ mMoles/dm3	0.004	0.008	0.022	0.018	0.004
Critical dissolved oxygen concentrations for *Pencillium chrysogenum* is ----mMoles/dm3	0.004	0.008	0.022	0.018	0.022
At ---------- cultures may be aerated by means of the *shake-flask technique* where the culture is grown in a conical flask shaken on a platform contained in a controlled environment of chamber.	pilot scale	laboratory-scale	industrial scale	semi-industrial scale	laboratory-scale
In ---------- fermentations broth	Pilot- and industrial-	pilot scale	industrial scale	laboratory-scale	Pilot- and industrial-scale

or culture is aerated by *stirrers or agitators.*	scale				
In ---------- bioreactor are so designed that adequate supply of oxygen is obtained without agitation	*bubble columns*	CSTR	PACKED BED	FLUIDISED BED	*bubble columns*
In ---------- bioreactor are so designed that adequate supply of oxygen is obtained without agitation	AIR LIFT	CSTR	PACKED BED	FLUIDISED BED	AIR LIFT
In 1950, ---------- represented the transfer of oxygen from air to the cell, during a fermentation, as occurring in a number of steps	Bartholomew et al	Belquiren et al	Barbitol et al	Batingulo et al	Bartholomew et al
The transfer of oxygen from an air bubble into solution is the ----- step in the oxygen transfer process	first	second	third	fourth	first
The transfer of the dissolved oxygen through the fermentation medium to the microbial cell is the -----step in the oxygen transfer process.	first	second	third	fourth	second
The uptake of the dissolved oxygen by the cell is the ----step in the oxygen transfer process.	first	second	third	fourth	third

Maximum ----------production may be achieved by satisfying the organism's maximum specific oxygen demand by maintaining the dissolved oxygen concentration greater than the critical level.	waste	biomass	toxic	fourth	biomass
Critical dissolved oxygen concentrations for *Azotobacter sp* is ----------mMoles/dm3	0.004	0.008	0.022	0.018	0.018

Unit III

MEDIA FOR INDUSTRIAL FERMENTATIONS

All micro-organisms require water, sources of energy, carbon, nitrogen, mineral elements and possibly vitamins plus oxygen if aerobic. On a small scale it is relatively simple to devise a medium containing pure compounds, but the resulting medium, although supporting satisfactory growth, may be unsuitable for use in a large scale process. On a large scale one must normally use sources ofnutrients to create a medium which will meet as many as possible of the following criteria:

1. It will produce the maximum yield of product orbiomass per gram of substrate used.
2. It will produce the maximum concentration ofproduct or biomass.
3. It will permit the maximum rate of product formation.
4. There will be the minimum yield of undesired products.
5. It will be of a consistent quality and be readily available throughout the year.
6. It will cause minimal problems during media making and sterilization.
7. It will cause minimal problems in other aspect sof the production process particularly aerationand agitation, extraction, purification and waste treatment.

The use of cane molasses, beet molasses, cereal grains, starch, glucose, sucrose and lactose as carbon sources, and ammonium salts, urea, nitrates, com steep liquor, soya bean meal, slaughter-house waste and fermentation residues as nitrogen sources, have tended to meet most of the above criteria for production media because they are cheap substrates. However, othermore expensive pure substrates may be chosen if theove rall cost of the complete process can be reduced because it is possible to use simpler procedures.

It must be remembered that the medium selected will affect the design of fermenter to be used. For example, the decision to use methanol and ammonia in the single cell protein process developed by ICI picnec essitated the design of a novel fermenter design. The microbial oxidation of hydrocarbons is a highly aerobic and exothermic cprocess. Thus, the production fermenter had to have a very high oxygen transfer capacity coupled with excellent cooling facilities. ICI pic solved these problems by developing an air lift fermenter. Equally, if a fermenter is already available thiswill obviously influence the composition of the medium. The optimum concentrations of available nitrogen for griseofulvin production showed some variation with the type of fermenter used.

The problem of developing a process from the laboratory to the pilot scale, and subsequently to the industrial scale, must also be considered. A laboratory medium may not be ideal in a large fermenter with alow gas-transfer pattern. A medium with a high viscosity will also need a higher power input for effective stirring. Besides meeting requirements for growth and product formation, the medium may also influence pH variation, foam formation, the oxidation-reduction potential, and the morphological form of the organism. It may also be necessary to provide precursors or metabolic inhibitors. The medium will also affect product recovery and effluent treatment. Historically, undefined complex natural materials have been used in fermentation processes because they are much cheaper than pure substrates. However, there is often considerable batch variation because of variable concentrations of the component parts and impurities in natural materials which cause unpredictable biomass and/or product yields. As a consequence of these variations in composition small yield improvements are difficult to detect. Undefined media often make product recovery and effluent treatment more problematical because not all the components of a complex nutrient source will be consumed by the organism.

TABLE 4.2. *Element composition of bacteria, yeasts and fungi (% by dry weight)*

Element	Bacteria (Luria, 1960; Herbert, 1976; Aiba *et al.*, 1973	Yeasts (Aiba *et al.*, 1973; Herbert, 1976)	Fungi (Lilly, 1965; Aiba *et al.*, 1973)
Carbon	50–53	45–50	40–63
Hydrogen	7	7	
Nitrogen	12–15	7.5–11	7–10
Phosphorus	2.0–3.0	0.8–2.6	0.4–4.5
Sulphur	0.2–1.0	0.01–0.24	0.1–0.5
Potassium	1.0–4.5	1.0–4.0	0.2–2.5
Sodium	0.5–1.0	0.01–0.1	0.02–0.5
Calcium	0.01–1.1	0.1–0.3	0.1–1.4
Magnesium	0.1–0.5	0.1–0.5	0.1–0.5
Chloride	0.5	—	—
Iron	0.02–0.2	0.01–0.5	0.1–0.2

TABLE 4.1. *Some examples of fermentation media*

Itaconic acid (Nubel and Ratajak, 1962)	
Cane molasses (as sugar)	150 g dm^{-3}
$ZnSO_4$	1.0 g dm^{-3}
$ZnSO_4 \cdot 7H_2O$	3.0 g dm^{-3}
$CuSO_4 \cdot 5H_2O$	0.01 g gm^{-3}
Amylase (Underkofler, 1966)	
Ground soybean meal	1.85%
Autolysed Brewers yeast fractions	1.50%
Distillers dried solubles	0.76%
NZ-amine (enzymatic casein hydrolysate)	0.65%
Lactose	4.75%
$MgSO_4 \cdot 7H_2O$	0.04%
Hodag KG-1 antifoam	0.05%
Avermectin (Stapley and Woodruff, 1982)	
Cerelose	45 g
Peptonized milk	24 g
Autolysed yeast	2.5 g
Polyglycol P-2000	2.5 cm^3
Distilled water	1 dm^3
pH	7.0
Endotoxin from *Bacillus thuringiensis* (Holmberg *et al.*, 1980)	
Molasses	0–4%
Soy flour	2–6%
KH_2PO_4	0.5%
KH_2PO_4	0.5%
$MgSO_4.7H_2O$	0.005%
$MnSO_4.4H_2O$	0.003%
$FeSO_4.7H_2O$	0.001%
$CaCl_2$	0.005%
$Na(NH_4)_2PO_4.4H_2O$	0.15%
Lysine (Nakayama, 1972a)	
Cane blackstrap molasses	20%
Soybean meal hydrosylate (as weight of meal before hydrolysis with 6N H_2SO_4 and neutralized with ammonia water)	1.8%
$CaCO_3$ or $MgSO_4$ added to buffer medium	
Antifoam agent	

Clavulanic acid (Box, 1980)	
Glycerol	1%
Soybean flour	1.5%
KH_2PO_4	0.1%
10% Pluronic L81 antifoam in soya bean oil	0.2%(v/v)
Oxytetracycline (Anonymous, 1980)	
Starch	12% + 4% (Additional feeding)
Technical amylase	0.1%
Yeast (dry wt.)	1.5%
$CaCO_3$	2%
Ammonium sulphate	1.5%
Lactic acid	0.13%
Lard oil	2%
Total inorganic salts	0.01%
Gibberellic acid (Calam and Nixon, 1960)	
Glucose monohydrate	20 g dm^{-3}
$MgSO_4$	1 g dm^{-3}
$NH_4H_2HPO_4$	2 g dm^{-3}
KH_2PO_4	5 g dm^{-4}
$FeSO_4 \cdot 7H_2O$	0.01 g dm^{-3}
$MnSO_4 \cdot 4H_2O$	0.01 g dm^{-3}
$ZnSO_4 \cdot 7H_2O$	0.01 g dm^{-3}
$CuSO_4 \cdot 5H_2O$	0.01 g dm^{-3}
Corn steep liquor (as dry solids)	7.5 g dm^{-3}
Glutamic acid (Gore *et al.*, 1968)	
Dextrose	270 g dm^{-3}
$NH_4H_2PO_4$	2 g dm^{-3}
$(NH_4)_2HPO_4$	2 g dm^{-3}
K_2SO_4	2 g dm^{-3}
$MgSO_4 \cdot 7H_2O$	0.5 g dm^{-3}
$MnSO_4 \cdot H_2O$	0.04 g dm^{-3}
$FeSO_4 \cdot 7H_2O$	0.02 g dm^{-3}
Polyglycol 2000	0.3 g dm^{-3}
Biotin	12 μg dm^{-3}
Penicillin	11 μg dm^{-3}
Penicillin (Perlman, 1970)	
Glucose or molasses (by continuous feed)	10% of total
Corn-steep liquor	4–5% of total
Phenylacetic acid (by continuous feed)	0.5–0.8% of total
Lard oil (or vegetable oil) antifoam by continuous addition pH to 6.5 to 7.5 by acid or alkali addition	0.5% of total

Some nutrients are frequently added in substantial excess of that required, e.g. P, K; however, others are often near limiting values, e.g. Zn, Cu. The concentration of P is deliberately raised in many media to increase the buffering capacity. These points emphasize the need for considerable attention to be given to medium design. Some micro-organisms cannot synthesize specific nutrients, e.g. amino acids, vitamins or nucleotides. Once a specific growth factor has been identified it can be incorporated into a medium in adequate amounts asa pure compound or as a component of a complex mixture.

The carbon substrate has a dual role in biosynthesis and energy generation. The carbon requirement for biomass production under aerobic conditions may be estimated from the cellular yield coefficient (Y) which is defined as:

Quantity of cell dry matter produced / Quantity of carbon substrate utilized

An adequate supply of the carbon source is essential for a product-forming fermentation process. In a critical study, analyses are made to determine the observed conversion of the carbon source to product compares with the theoretical maximum yield. This may be difficult because of limited knowledge ofthe

biosynthetic pathways. Theoretical yields for penicillin G biosynthesis on the basis of material and energy balances using a biosynthetic pathway based on reaction stoichiometry. The other major nutrient which will be required isoxygen which is provided by aerating the culture. The design of a medium will influence the oxygen demand of a culture in that the more reduced carbon sources will result in a higher oxygen demand. The amount ofoxygen required may be determined stoichiometrically.

WATER

Water is the major component of all fermentation media, and is needed in many of the ancillary services such as heating, cooling, cleaning and rinsing. Clean water of consistent composition is therefore required in large quantities from reliable permanent sources. When assessing the suitability of a water supply it is important to consider pH, dissolved salts and effluent contamination. The mineral content of the water is very important in brewing, and most critical in the mashing process, and historically influenced the siting of breweries and the types of beer produced. Hard waters containing high CaS04 concentrations are better for the English Burton bitter beers and Pilsen type lagers, while waters with a high carbonate content are better for the darker beers such as stouts. Nowadays, the water may be treated by deionization or other techniques and salts added, or the pH adjusted; to favour different beers sot hat breweries are not so dependent on the local water source.

ENERGY SOURCES

Energy for growth comes from either the oxidation of medium components or from light. Most industrial micro-organisms are chemo-organotrophs, therefore the commonest source of energy will be the carbonsource such as carbohydrates, lipids and proteins. Some micro- organisms can also use hydrocarbons or methanol as carbon and energy sources.

CARBON SOURCES

Factors influencing the choice of carbon source It is now recognized that the rate at which thecarbon source is metabolized can often influence the formation of biomass or production of primary or secondarymetabolites. Fast growth due to high concentrations of rapidly metabolized sugars is often associatedwith low productivity of secondary metabolites.At one time the problem was overcome byusing the less readily metabolized sugars such as lactose, but many processes now usesemi-continuous or continuous feed of glucose or sucrose, Alternatively, carbon catabolite regulation might be overcome by genetic modification of the producer organism

TABLE 4.4. *Carbon catabolite regulation of metabolite biosynthesis*

Metabolite	Micro-organism	Interfering carbon source	Reference
Griseofulvin	*Penicillium griseofulvin*	Glucose	Rhodes (1963); Rhodes *et al.* (1955)
Penicillin	*P. chrysogenum*	Glucose	Pirt and Rhigelato (1967)
Cephalosporin	*Cephalosporium acremonium*	Glucose	Matsumura *et al.* (1978)
Aurantin	*Bacillus aurantinus*	Glycerol	Nishikiori *et al.* (1978)
α-Amylase	*B. licheniformis*	Glucose	Priest and Sharp (1989)
Bacitracin	*B. licheniformis*	Glucose	Weinberg (1967)
Puromycin	*Streptomyces albouiger*	Glucose	Sankaran and Pogell (1975)
Actinomycin	*S. antibioticus*	Glucose	Marshall *et al.* (1968)
Cephamycin C	*S. clavuligerus*	Glycerol	Aharonowitz and Demain (1978)
Neomycin	*S. fradiae*	Glucose	Majumdar and Majumdar (1965)
Cycloserine	*S. graphalus*	Glycerol	Svensson *et al.* (1983)
Streptomycin	*S. griseus*	Glucose	Inamine *et al.* (1969)
Kanamycin	*S. kanamyceticus*	Glucose	Busek and Majumdar (1973)
Novobiocin	*S. niveus*	Citrate	Kominek (1972)
Siomycin	*S. sioyaensis*	Glucose	Kimura (1967)

The main product of a fermentation process will often determine the choice of carbon source, particularly if the product results from the direct dissimilation of it. In fermentations such as ethanol or single-cell protein production where raw materials are 60 to 77%of the production cost, the selling price of the product will be determined largely by the cost of the carbon source. It is often part of a company development programme to test arange of alternative carbon sources to determine the yield of product and its influence on the process and the cost of producing biomass and/or metabolite. This enables a company to use alternative substrates, depending on price and availability in different locations, and remain competitive. The purity of the carbon source may also affect the choice of substrate. For example, metallic ions must be removed from carbohydrate sources used in some citricacid processes.

The method of media preparation, particularly sterilization, may affect the suitability of carbohydrates for individual fermentation processes. It is often best to sterilize sugars separately because they may react with ammonium ions and amino acids to form black nitrogen containing compounds which will partially inhibit the growth of many micro-organisms. Starch suffer srom the handicap that when heated in the sterilization process it gelatinizes, giving rise to very viscous liquids, so that only concentrations of up to 2% can be used without modification.

The choice of substrate may also be influenced by government legislation. Within the European Economic Community (EEC), the use of beet sugar and molasses is encouraged, and the minimum price controlled. The quantity of imported cane sugar and molasses is carefully monitored and their imported

prices set so thatthey will not be competitive with beet sugar. If theworld market sugar price is very low then the EEC fermentation industry will be at a disadvantage unless it receives realistic subsidies.

EXAMPLES OF COMMONLY USED CARBON SOURCES CARBOHYDRATES

It is common practice to use carbohydrates as the carbon source in microbial fermentation processes. The most widely available carbohydrate is starch obtained rom maize grain. It is also obtained from other cereals, potatoes and cassava. Maize and other cereals may also be used directly in a partially ground state, e.g. maize chips. Starch may also be readily hydrolysed by dilute acids and enzymes to give a variety of glucose preparations (solids and syrups). Hydrolysed cassava starch is used as a major carbon source for glutamic acid production in Japan. Syrups produced by acid hydrolysis may also contain toxic products which maymake them unsuitable for particular processes. Barley grains may be partially germinated and heat treated to give the material known as malt, which contains a variety of sugars besides starch.

Malt is the main substrate for brewing beer and lagerin many countries. Malt extracts may also be preparedfrom malted grain. Sucrose is obtained from sugar cane and sugar beet.It is commonly used in fermentation media in a very impure form as beet or cane molasses which are the residues left after crystallization of sugar solutions in sugar refining. Molasses is used in the production of high-volume flow-value products such as ethanol, SCP, organic and amino acids and some microbial gums. The use of lactose and crude lactose (milk whey powder) in media formulations is now extremely limited since the introduction of continuous-feeding processes utilizing glucose.

Corn steep liquor is a by-product after starch extraction from maize. Although primarily usedas a nitrogen source, it does contain lactic acid, smalla mounts of reducing sugars and complex polysaccharides. Certain other materials of plant origin, usually included as nitrogen sources, such as soyabean meal and Pharmamedia, contain small but significant amounts of carbohydrate.

OILS AND FATS

Oils were first used as carriers for antifoams in antibiotic processes. Vegetable oils(olive, maize, cotton seed, linseed, soya bean, etc.) may also be used as carbon substrates, particularly for their content of the fatty acids, oleic, linoleic and linolenic acid, because costs are competitive with those of carbohydrates. In an analysis of commodity prices forsugar, soya bean oil and tallow between 1978 and 1985,it would have been cheaper on an available energybasis to use sugar during 1978 to mid 1979 and late1983 to 1985, whereas oil would have been the chosen substrate in the intervening period. A typical oil containsapproximately 2.4 times the energy of glucose ona per weight basis. Oils also have a volume advantage as it would take 1.24 dm3 of soya bean oil to add 10kcal of energy to a fermenter, whereas it would take 5dm3 of glucose or sucrose assuming that they are being added as 50% w/w solutions. Ideally, in

any fermentationprocess, the maximum working capacity of a vessel should be used. Oil based fed- batch fermentation spermit this procedure to operate more successfully than those using carbohydrate feeds where a larger spare capacity must be catered for to allow for responses to a sudden reduction in the residual nutrient level. Oils also have antifoam properties which may make downstream processing simpler, but normally they are not used solely for this purpose.

HYDROCARBONS AND THEIR DERIVATIVES

There has been considerable interest in hydrocarbons. Development work has been done using n-alkanes for production of organic acids, amino acids, vitamins and co-factors, nucleic acids, antibiotics, enzymes and proteins. Methane, methanol and n-alkanes have all been used as substrates for biomass production.On a weight basis n-alkanes have approximately twice the carbon and three times the energy content of the same weight of sugar. Although petroleum-type products are initially impure they canbe refined to obtain very pure products in bulk quantities which would reduce the amount of effluent treatment and downstream processing. At this time the view was also held that hydrocarbons would not besubject to the same fluctuations in cost as agriculturally derived feed stocks because it would be a stable priced commodity and might be used to provide a substrate.

NITROGEN SOURCES

Most industrially used micro-organisms can utilizein organic or organic sources of nitrogen. Inorganic nitrogen may be supplied as ammonia gas, ammonium salts or nitrates. Ammonia has been used for pH control and as the major nitrogen sourcein a defined medium for the commercial production of human serum albumin by Saccharomyces ceriuisiae. Ammonium salts such as ammonium sulphate will usually produce acid conditions as the ammonium ion is utilized and the free acid will beliberated. On the other hand nitrates will normally cause an alkaline drift as they are metabolized. Ammoniumnitrate will first cause an acid drift as the ammonium ion is utilized, and nitrate assimilation isrepressed.Organic nitrogen may be supplied as amino acid, protein or urea. In many instances growth will be faster with a supply of organic nitrogen, and a few microorganism have an absolute requirement for amino acids. It might be thought that the main industrial needfor pure amino acids would be in the deliberate addition to amino acid requiring mutants used in aminoacid production. However, amino acids are more commonly added as complex organic nitrogen sources which are non-homogeneous, cheaper and readily available. In lysine production, methionine and threonine are obtained from soybean hydrolysate since it would be too expensive to use the pure amino acids.

FACTORS INFLUENCING THE CHOICE OF NITROGEN SOURCE

Control mechanisms exist by which nitrate reductase,an enzyme involved in the conversion of nitrate to ammonium ion, is repressed in the presence of ammonia. For this reason ammonia or ammonium ion is the preferred nitrogen source. In fungi that have been investigated, ammonium ion represses uptake of amino acids by general and specific amino acid permeases. In Aspergillus nidulans, ammonia also regulates the production of alkaline and neutral proteases).

Therefore, in mixtures of nitrogen sources, individual nitrogencomponents may influence metabolic regulation sothat there is preferential assimilation of one componentuntil its concentration has diminished.

MINERALS

All micro-organisms require certain mineral elements for growth and metabolism. In many media, magnesium, phosphorus, potassium, sulphur, calcium and chlorine are essential components, and because of the concentrations required,they must be added as distinct components. Others such as cobalt, copper, iron, manganese, molybdenum and zinc are also essential but are usually present as impurities in other major ingredients. There is obviously a need for batch analysis of media components to ensure that this assumption can be justified, otherwise there may be deficiencies or excesses indifferent batches of media. As a consequence of product composition analysis, as outlined earlier in this chapter, it is possible to estimate the amount of a specific mineral for medium design, e.g. sulphur in penicillins and cephalosporins, chlorine in chlortetracycline.

TABLE 4.10. *The range of typical concentrations of mineral components* (g dm^{-3})

Component	Range
*KH_2PO_4	1.0–4.0 (part may be as buffer)
$MgSO_4 \cdot 7H_2O$	0.25–3.0
KCl	0.5–12.0
$CaCO_3$	5.0–17.0
$FeSO_4 \cdot 4H_2O$	0.01–0.1
$ZnSO_4 \cdot 8H_2O$	0.1–1.0
$MnSO_4 \cdot H_2O$	0.01–0.1
$CuSO_4 \cdot 5H_2O$	0.003–0.01
$Na_2MoO_4 \cdot 2H_2O$	0.01–0.1

*Complex media derived from plant and animal materials normally contain a considerable concentration of inorganic phosphate.

CHELATORS

Many media cannot be prepared or autoclaved withoutthe formation of a visible precipitate of insolublemetal phosphates. When the medium was autoclaved, a white precipitate of metal formed, containing all the iron and most of the calcium, manganese and zinc present in the medium. The problem of insoluble metal phosphate(s) may eliminated by incorporating low concentration schelating agents such as ethylene diaminetetra acetic acid (EDTA), citric acid, polyphosphates, etc., into medium. These chelating agents preferentially complexes with the metal ions in a medium. The ions then may be gradually utilized by the organism. The precipitate was eliminated from Mandel and Weber's medium by the addition EDTA at 25 mg dm-3. It is important to check that achelating agent does not cause inhibition of growth the micro-organism which is being cultured. In many media, particularly those commonly used inlarge scale processes, there may not be a need to add a chelating agent as complex ingredients such as yeast extracts or proteose peptones will complex with metal ions and ensure gradual release of them during growth.

GROWTH FACTORS

Some micro-organisms cannot synthesize full complementof cell components and therefore require preformed compounds called growth factors. The growth factors most commonly required are vitamins, but theremay also be a need for specific amino acids, fatty acids or sterols. Many of the natural carbon and nitrogen sources used in media formulations contain all or some of the required growth factors. When there is a vitamin deficiency it can oftenbe eliminated by careful blending of materials. It is important to remember that ifonly one vitamin is required it may be occasionally more economical to add the pure vitamin, instead ofusing a larger bulk of a cheaper multiple vitamin source. Calcium pantothenate has been used in one medium formulation for vinegar production. In processes used for the production of glutamic acid, limited concentrations of biotin must be present in the medium.

Possible questions

Two marks

1. How are media sterilized for fermentation?
2. Mention the essential carbon and nitrogen sources for the microbial growth.
3. Write short notes on the role of metals and minerals for fermentation process.
4. Use of oils and fats acts as antifoaming agent. Justify.
5. What are the advantages and disadvantages in using molasses for media formulation.

Eight marks

1. How is air sterilized in fermentation process?
2. What is the criteria for selecting a media for fermentation?
3. Define chelators and their importance in the media fermentation?
4. Explain in detail about different types of raw materials used for formulating media?
5. Explain about microbial growth kinetics according to the media used.

UNIT III	Option A	Option B	Option C	Option D	Answers
In Pencillin fementation the precursor added is ----------	phenyl acetic acid	benzyl alcohol	Benzene	pyridine	phenyl acetic acid
Buffering agents used in media formulation is	mono and dihydropotassium phosphates	triiodosodium phosphates	Tetraiodiosodium phosphates	monophosphates	mono and dihydropotassium phosphates
Foaming during fermentation process creates	oxidation	reduction	contamination	production	contamination
The antifoaming agent used in pencillin fermentation is	lard oil	lard oil with ocetadecanol	decanol	mustard oil with decanol	lard oil with ocetadecanol
The citric acid fermentation *Aspergillus niger* culture is grown at ----------pH values prevent contamination	low	high	medium	very high	low
----------media is mainly used in fermentation process.	synthetic	semi-synthetic	non-synthetic	differential	non-synthetic
Which is the common raw material source used in fermentation process	food waste	agricultural waste	industrial toxic waste	Biofuel waste	agricultural waste
---------- is rich in biotin, panthothenic acid , thiamine, phorphorus and sulphur.	cane molasses	beet molasses	fruit molasses	cheese molasses	cane molasses
In Beet molasses ----------is limiting	biotin	pyridoxine	thiamine	pantothenic acid	biotin

compared to cane molasses					
---------- require biotin for growth in production.	Bacteria	yeasts	viruses	phages	yeasts
In India there is very large utilization of cane blackstrap molasses in ---------- industry	textile	animal fodder	alcohol	dyes	alcohol
The ---------- contain 17% sugar, 1%acid and 0.3%ash	rust	just	must	bust	must
In grapes the nitrogen content should be ---------- as it may result in underisable fermentation.	high	medium	low	no	low
----------is 6.6-7.1% in cheddar whey	total solids	protein	lactose	fat	total solids
Cheese whey is an important raw material in ---------- production	lactic acid	aspartic acid	glutamic acid	citric acid	lactic acid
----------and-----------are the main sources of starch	molasses and cereals	cheese whey and tubers	cereals, roots and tubers	cereals and cornsteep liquor	cereals, roots and tubers
Wheat, maize and rice are example of ----------	molasses	cereals	roots	tubers	cereals
Cellulose are carbohydrates made of repeating units of --------	α-glucose	β-glucose	α-galactose	β-galactose	β-glucose
In ---------- industry the	cheese, cheese whey	starch, starch liquor	paper pulp, sulfite	wood, molasses	paper pulp, sulfite waste

digestion process of wood with calcium bisulfate under heat and pressure to give spent liquid called as ----------			waste liquor		liquor
Sulfite waste liquor contains ---------sugars	1%	2%	3%	4%	2%
Wood molasses syrup contain -------of fermentable sugars	60-80%	65-85%	70-90%	75-95%	65-85%
The untreated cellulosic wastes have been used in production of	ethanol	single cell protein	fuel	vitamins	single cell protein
During manufacture of starch, gluten from corn--------- is formed by steeping of corn.	sulfite waste liquor	corn steep liquor	wood molasses syrup	distillers soluble	corn steep liquor
The clean, yellow, fine powder prepared from embryo of cotton seed is called as ----------	corn-steep liquor	soya bean meal	Pharmamedia	distiller's soluble	Pharmamedia
Pharmamedia is used as production media for --------- production.	penicillin	streptomycin	tetracycline	griseofulvin	tetracycline
Initially fermentation industries used corn steep liquor for ------- production.	mushroom	penicillin	vitamin	organic acid	penicillin
Soya bean meal is used as	penicillin	streptomycin	tetracycline	griseofulvin	streptomycin

production medium for ----------					
Vegetable oils are used as	animal feed	antifoams	mushroom production media	pH adjustment	antifoams
ATCC is ---------- founded in 1925	American type culture collection	African type culture collection	Auxenic type culture collection	Australian type culture collection	American type culture collection
The specific gravity of oil used in presence of cultures is	0.821-0.860	)0.865-0.890	0.752-0.812	0.718-0.835	0.865-0.890
----------is the simplest and common method of maintaining microbial cultures.	serial subculture	lyophilisation	cryopreservation	dessication	serial subculture
The Drying-up of medium encourages good ---------of *Streptomyces sp*	growth	sporulation	storage	collection	sporulation
----------and----------are usually maintained in liquid medium	Bacteriophages and *Actinomycetes*	Bacteriophages and *clostridium*	Viruses and *Acetobacter*	Bacteriophages and *Streptomyces*	Bacteriophages and *clostridium*
The mineral oil overlay method was firstused by Bwell and Weston in ----------	1945	1947	1949	1950	1947
Temperature of liquid nitrogen is	-130˚C	-150˚C	-176˚C	-196˚C	-196˚C
The first commercial production of lactic acid in the US was in _____	1881	1882	1880	1883	1881
Lyophilization is the most	long term	short term	both a and b	none of the above	long term

satisfactory method of long term preservation of microorganisms					
---------- is perhaps the most popular form of suspended metabolism.	overlaying cultures with mineral oil	lyophilization	nitrogen storage	none of the above	lyophilization
Hwang in ---------- year recommends precooling to 7 degree Celsius.	1966	1969	1974	1980	1966
---------- stressed the importance of the elimination of air and moisture from lyophilized cultures prior to sealing of ampoules.	Meryman	Dewald	leogetring	Louis Pasteur.	Dewald
The catabolism of sugars is an ---------- process	Oxidative	Non oxidative	Reoxidised	Deoxidation	Oxidative
The largest mechanical stirred fermentation vessels developed during stage 3 were in the range of _____.	75000-80000dm3	80,000-150,000dm3	150,000-175,000dm3	10,000-50,000dm3	80,000-150,000dm3
----------culture where a portion of the culture is harvested at regular intervals and replaced by an equal volume of medium	Fed batch	Batch	Semi continuous	Continuous	Semi continuous
---------- culture	Batch	Fed batch	Continuous	Semi	Fed batch

where medium is fed to the culture resulting in an increases in volume				continuous	
-------- is a culture system which contain a initial limited amount of nutrient	Fed batch	Batch	Semi continuous	Continuous	Batch
_____ properties are temperature, concentration, pressure and specific heat.	Intensive	Extensive	Physical	Chemical	Intensive
_____ properties are mass, volume, entropy and energy.	Intensive	Extensive	Physical	Chemical	Extensive
----------is performed in order to mix the three phases with in the fermenter	Aeration	Agitation	Reaction	Fermentation	Agitation
----------phase dissolved nutrients and metabolism are present	Gaseous	Liquid	Solid	Semisolid	Liquid
In --------- phase o2 and co2 are present	Gaseous	Liquid	Aeration	Agitation	Gaseous
In --------- phase cells and solid substrates are present	Gaseous	Solid	Liquid	Semisolid	Solid
Transfer in to liquid from the gaseous phase is enhanced by ------	Agitation	Aeration	Fermentation	Precipitation	Agitation

---------- culture is a open system where fresh medium is continuously added	Batch	Continuous	Fed batch	Airlift	Continuous
Aim of ATCC is to_____ _	Prevent contaminatio n	To maintain antibiotics	Preserve cultures	None of the above	Preserve cultures
Oil should be autoclaved at _____	15 lb/in^2 for 2 hr	30 lb/in^2 for 2 hr	45 lb/in^2 for 2 hr	60 lb/in^2 for 2 hr	15 lb/in^2 for 2 hr
Lyophilization refers to _____	Short term preservation of microorganis ms	long term preservation of Microorganis ms	killing of Microorgani sms	None of the above	long term preservation of Microorganism s
Dessicant used in lyophilizer are	Phosphorus	silica gel	Magnesium	Copper	silica gel
_____ is used in storage at very low temp	glycerol	mineral oil	Paraffin wax	All the above	glycerol
The soil culture tubes are kept in refrigerator at above _____ temperature	5-8^0 C	4-6^0 C	2-4^0 C	6-8^0 C	5-8^0 C
De Becze and Liebmann in the year _____ used the first large scale fermentor for the production of yeast.	1941	1944	1948	1940	1944

Unit IV

DOWNSTREAMING PROCESS

The choice of recovery process is based on the following criteria:

1. The intracellular or extracellular location of the product.
2. The concentration of the product in the fermentation broth.
3. The physical and chemical properties of the desired product (as an aid to selecting separation procedures).
4. The intended use of the product.
5. The minimal acceptable standard of purity.
6. The magnitude of bio-hazard of the product orbroth.
7. The impurities in the fermenter broth.
8. The marketable price for the product.

The main objective of the first stage for the recovery of an extracellular product is the removal of large solid particles and microbial cells usually by centrifugation or filtration. In the next stage, the broth is fractionated or extracted into major fractions using ultrafiltration, reverse osmosis, adsorption/ion-exchange/gel filtration or affinity chromatography, liquid-liquid extraction, two phase aqueous extraction or precipitation. Afterwards, the product-containing fraction is purified by fractional precipitation, further more precise chromatographic techniques and crystallization to obtain a product which is highly concentrated and essentially free from impurities. Other products are isolated using modifications of this flow-stream. Attempts to simplify this outline extraction procedure for antibiotic recovery using 'whole broth' processing have met with limited success. The technique of 'whole broth' processing involves initial removal of large particles, which is then followed by passage of thebroth (including cells) through, for example, well mixed ion-exchange columns or counter-current liquid- liquid extraction units to extract the product directly.

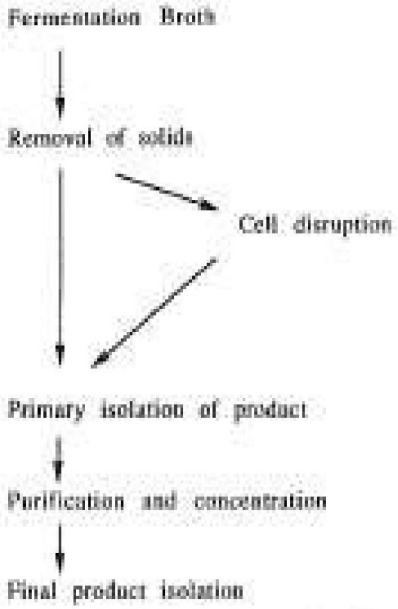


FIG. 10.1. Stages in the recovery of product from a harvested fermentation broth.

1. Harvest broth from fermenter
2. Chill to 5–10°C
3. Filter off *P. chrysogenum* mycelium using rotary vacuum filter
4. Acidify filtrate to pH 2.0-2.5 with H_2SO_4
5. Extract penicillin from aqueous filtrate into butyl acetate in a centrifugal counter-current extractor (treat/dispose aqueous phase)
6. Extract penicillin from butyl acetate into aqueous buffer (pH 7.0) in a centrifugal counter-current extractor (recover and recycle butyl acetate)
7. Acidify the aqueous fraction to pH 2.0-2.5 with H_2SO_4 and re-extract penicillin into butyl acetate as in stage 5
8. Add potassium acetate to the organic extract in a crystallization tank to crystallize the penicillin as the potassium salt
9. Recover crystals in a filter centrifuge (recover and recycle butyl acetate)
10. Further processing of penicillin salt

FIG. 10.2. Recovery and partial purification of penicillin G.

It may be possible to modify the handling characteristics of the broth so that it can be handled simpler equipment making use of a number of techniques:

1. Selection of a micro-organism which produce pigments or undesirable metatlolites.
2. Modification of the fermentation reduce the production of metabolites.
3. Precise timing of harvesting.
4. pH control after harvesting.
5. Temperature treatment after harvesting.
6. Addition of flocculating agents.
7. Use of enzymes to attack cell walls.

It must be remembered that the fermentation product recovery are integral parts of an overall Because of the interactions between the two,stage should be developed independently, as thisresult in problems and unnecessary expense. The parameters to consider included time of harvest, pigment production, ionic strength and culture medium constituents. Large volumes of supernatants containing extracellular enzymes need immediate processing while harvesting times and enzyme yields might not be predictable. This can make recovery programmes difficult to plan. Pigment production might make some recovery procedures difficult, when the pigment binds to the same resin as the enzyme. Changes in fermentation conditions may reduce pigment formation. Certain antifoams remain in the supernatant and affect ultrafiltration orion-exchange resins used in recovery stages. Trials maybe needed to find the most suitable antifoam. The ionic strength of the production medium may be too high, resulting in the harvested supernatant needing dilution with demineralised water before it can be processed. Such a negative procedure should be avoided if possible by unified research and development programmes. Media formulation is dominated by production requirements, but the protein content of complex media should be critically examined in view of subsequent enzyme recovery. When considering water recycle in biomass production. Theystated that the interaction between the different unit operations in a recycle process made it imperative that commercial plant design and operation should be viewed in an integrated fashion.

Flow sheets for recovery of penicillin, cephamycin C, citric acid and micrococcal nuclease are given in Figures to illustrate the range of techniques used in microbiological recovery processes. A series of comprehensive flow sheets for alcohols, organic acids, antibiotics, carotenoids, polysaccharides, intra- and extra-cellular enzymes, single-cell proteins and vitamin. Other reviews on separation and purification are

available for penicillin, amino acids, enzymes, single-cell protein and polysaccharides. In the selection of processes for the recovery of biological products it should always be understood that recovery and production are inter linked, and that good recovery starts in the fermentation by the selection of, amongst other factors, the correct media and time of harvesting.

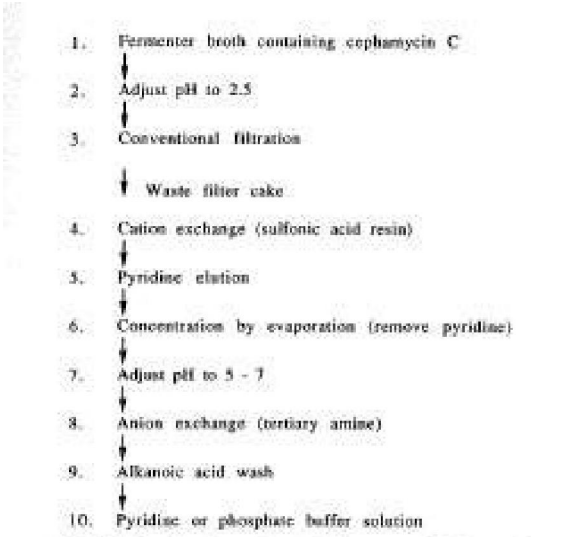


FIG. 10.3. Purification of cephamycin C: sequential ion exchange process (Omstead *et al.*, 1985).

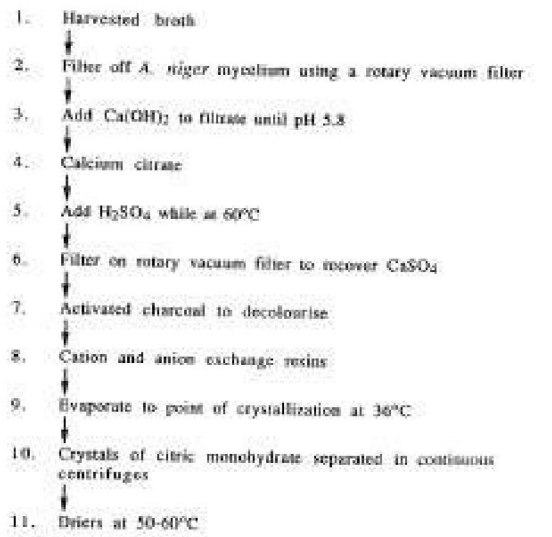


FIG. 10.4. Recovery and purification of citric acid (Sodesk *et al.*, 1981).

The recovery and purification of many compounds may be achieved by a number of alternative routes. The decision to follow a particular route involves comparing the following factors to determine the most appropriate under a given set of circumstances:

- Capital costs.
- Processing costs.
- Throughput requirements.
- Yield potential.
- Product quality.
- Technical expertise available.
- Conformance to regulatory requirements.
- Waste treatment needs.
- Continuous or batch processing.
- Automation.
- Personnel health and safety

The major problem currently faced in product recovery is the large-scale purification of biologically active molecules. For a process to be economically viable large-scale production is required and therefore large scale separation, recovery, and purification. This then requires the transfer of small-scale preparative/analytical technologies (e.g. chromatographic techniques)to the production scale whilst maintaining efficiency ofthe process, bio-activity of the product and purity of the product so that it conforms with safety legislation and regulatory requirements.

REMOVAL OF MICROBIAL CELLS AND OTHER SOLID MATTER

Microbial cells and other insoluble materials are normally separated from the harvested broth by filtration or centrifugation. Because of the small size of many microbial cells it will be necessary to consider the use of filter aids to improve filtration rates, while heat and flocculation treatments are employed as techniques for increasing sedimentation rates in centrifugation. The methods of cell and cell debris separation described in the following sections have been practiced for many years. Some potential developments in cell recovery. These include the use of electrophoresis and di-electrophoresis to exploit the charged properties of microbial cells, ultrasonic treatment to improve flocculation characteristic and magnetic separations. All these techniques suffer high cost and scale-up difficulties and currently are not appropriate technologies. Of more current interest is the use of two-phase liquid extraction. Though still most appropriately used for separation of selected soluble components, it is easy to scale up and use conditions which are gentle on the product.

FOAM SEPARATION

Foam separation depends on using methods which exploit differences in surface activity of materials. The material may be whole cells or molecular such as a protein or colloidal, and is selectively adsorbed or attached to the surface of gas bubbles rising through a liquid, to be concentrated or separated and finallyremoved by skimming. It may be possible tomake some materials surface active by the application of surfactants such as long-chain fatty acids,and quaternary ammonium compounds. Materials surface active and collected are termed whereas the surfactants are termed collectors when developing this method of separation, the variables which may need experimental investigation are pH, air-flow rates, surfactants collagen collector ratios. It was shown that up90% of the cells were removed in 1 minute and 99%10 minutes. The technique also proved successful Chiarellasp. and Chlamydomonassp. In other with E. coli, Grieves and Wang (1966) were able to achieve cell enrichment ratios of between 10 and 1 X10 6 using ethyl-hexadecyl-dimethyl ammonium bromide.

PRECIPITATION

Precipitation may be conducted at various stages of the product recovery process. It is a particularly useful process in that it allows enrichment and concentration in one step, thereby reducing the volume of material for further processing. It is possible to obtain some products (or to remove certain impurities) directly from the broth by precipitation, or to use the technique after a crude cell lysate has been obtained. Typical agents used in precipitation render the compound of interest insoluble and these include

(a) Acids and bases to change the pH of a solution until the isoelectric point of the compound is reached and pH equals pI, when there is then no overall charge on the molecule and its solubility is decreased.

(b) Salts such as ammonium and sodium sulphate are used for the recovery and fractionation of proteins. The salt removes water from the surface of the protein revealing hydrophobic patches which come together causing the proteinto precipitate. The most hydrophobic proteinswill precipitate first, thus allowing fractionation to take place.

(c) Organic solvents. Dextrans can be precipitated out of a broth by the addition of methanol. Chilled ethanol and acetone can be used in the precipitation of proteins mainly due to changes in the dielectric properties of the solution.

(d) Non-ionic polymers such as polyethylene glycol (PEG) can be used in the precipitation of proteins and are similar in behaviour to organic solvents.

(e) Polyelectrolytes can be used in the precipitaton of a range of compounds, in addition to theiruse in cell aggregation.

(f) Protein binding dyes (triazine dyes) bind to and precipitate certain classes of protein.

(g) Affinity precipitants are an area of much current interest in that they are able to bind to, and precipitate, compounds selectively.

FILTRATION

Filtration is one of the most common processes used at all scales of operation to separate suspended particles from a liquid or gas, using a porous medium which retains the particles but allows the liquid or gas to pass through. Gas filtration has been discussed in detail elsewhere. It is possible to carry out filtration under a variety of conditions, but a number of factors will obviously influence the choice of the most suitable type of equipment to meet the specified requirements at minimum overall cost, including:

1. The properties of the filtrate, particularly itsviscosity and density.
2. The nature of the solid particles, particularlytheir size and shape, the size distribution and packing characteristics.

3. The solids: liquid ratio.

4. The need for recovery of the solid or liquidfraction or both.

5. The scale of operation.

6. The need for batch or continuous operation.

7. The need for aseptic conditions.

8. The need for pressure or vacuum suction toensure an adequate flow rate of the liquid.

PLATE AND FRAME FILTERS

A plate and frame filter is a pressure filter in which the simplest form consists of plates and frames arranged alternately. The plates are covered with filter cloths or filter pads. The plates and frames are assembled on a horizontal framework and held together by means of a hand screw or hydraulic ram so that there is no leakage between the plates and frames which form a series of liquid-tight compartments. The slurry is fed to the filter frame through the continuous channel formed by the holes in the corners of the plates and frames. The filtrate passes through the filter cloth or pad, runs down grooves in the filter plates and is then is charged through outlet taps to a channel. Sometimes, if aseptic conditions are required, the outlets may lead directly into a pipe. The solids are retained within the frame and filtration is stopped when the frames are completely filled or hen the flow of filtrate becomes uneconomically low.

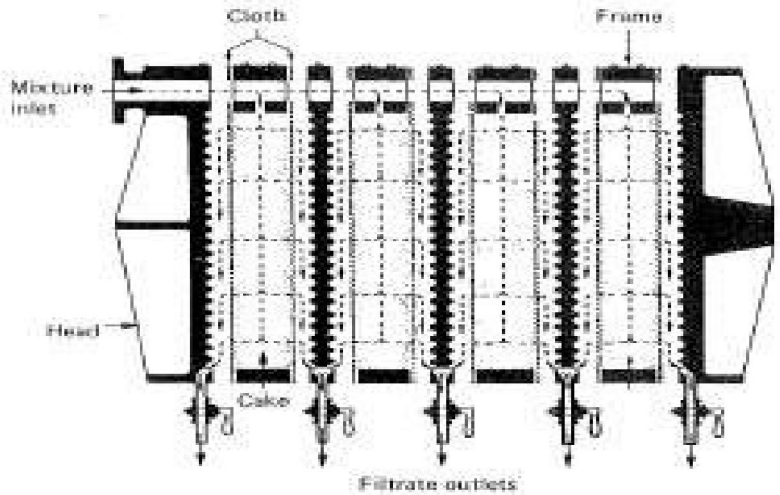


FIG. 10.8. Flush plate and frame filter assembly. The cloth is shown away from the plates to indicate flow of filtrate in the grooves between pyramids (Purchas, 1971).

PRESSURE LEAF FILTERS

There are a number of intermittent batch filter usually called by their trade names. These filters incorporate ea number of leaves, each consisting of a metal framework of grooved plates which is covered with a fine wire mesh, or occasionally a filter cloth and often pre coated with a layer of cellulose fibres. The process slurry is fed into the filter which is operated under pressure or by suction with a vacuum pump. Because the filters are totally enclosed it is possible to sterilize them with steam. This type of filter is particularly suitable for 'polishing' large volumes of liquids with low solids content or small batch filtrations of valuable solids.

(i) Vertical metal-leaf filter

This filter consists of a number of vertical porous metal leaves mounted on a hollow shaft in a cylindrical pressure vessel. The solids from the slurry gradually build up on the surface of the leaves and the filtrate is removed from the plates via the horizontal hollow shaft. In some designs the hollow shaft can be slowly rotated during filtration. Solids are normally removed at the end of a cycle by blowing air through the shaft and into the filter leaves.

(ii) Horizontal metal-leaf filter

In this filter the metal leaves are mounted on a vertical hollow shaft within a pressure vessel. Often, only the upper surfaces of the leaves are porous. Filtration is continued until the cake fills the space between the disc-shaped leaves or when the operational pressure has become excessive. At the end of a process cycle, the solid cake can be discharged by releasing the pressure and spinning the shaft with a drive motor.

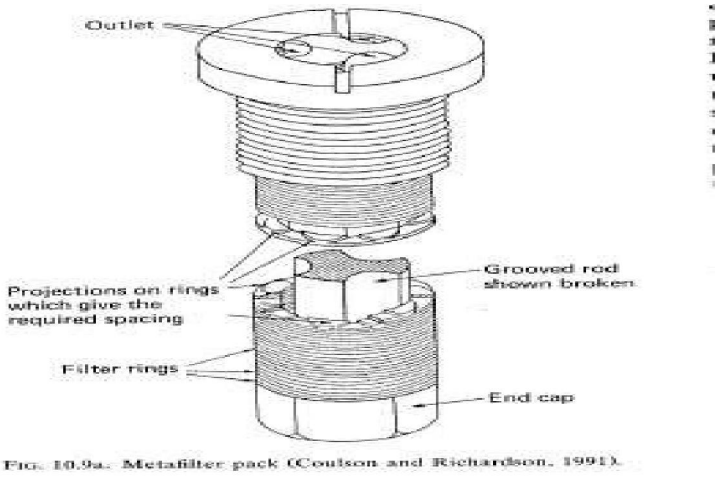


Fig. 10.9a. Metafilter pack (Coulson and Richardson, 1991).

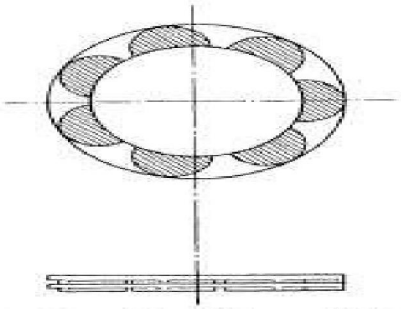


Fig. 10.9b. Rings for metafilter (Coulson and Richardson, 1991).

ROTARY VACUUM FILTERS

Large rotary vacuum filters are commonly used industries which produce large volumes of liquid need continuous processing. The filter consists rotating, hollow, segmented drum covered with aor metal filter which is partially immersed in a containing the broth to be filtered. Slurry is fed on to the outside of the revolving drum and vacuum pressure is applied internally so that the filtrate is drawn through the filter, into the drum and finally to a collecting vessel. The interior of the drum is divided into a series of compartments, to which the vacuum pressure is normally applied for most of each revolution as the drum slowly revolves (~ 1 rpm). However, just before discharge of the filter cake, air pressure may be applied internally to help ease the filter cake off the drum. A number of spray jets may be carefully positioned so that water can be applied to rinse the cake. This washing is carefully controlled so that dilution n of the filtrate is minimal.

It should be noted that the driving force for filtration (pressure differential across the filter) is limited to on eatmosphere (l00 kN m-Z) and in practice it is significantly less than this. In contrast, pressure filters can be operated at many atmospheres pressure. A number perforatory vacuum drum filters are manufactured, which differ in the mechanism of cake discharge from thedrum:

(i) String discharge.

(ii) Scraper discharge.

(iii) Scraper discharge with pre coating of the drum.

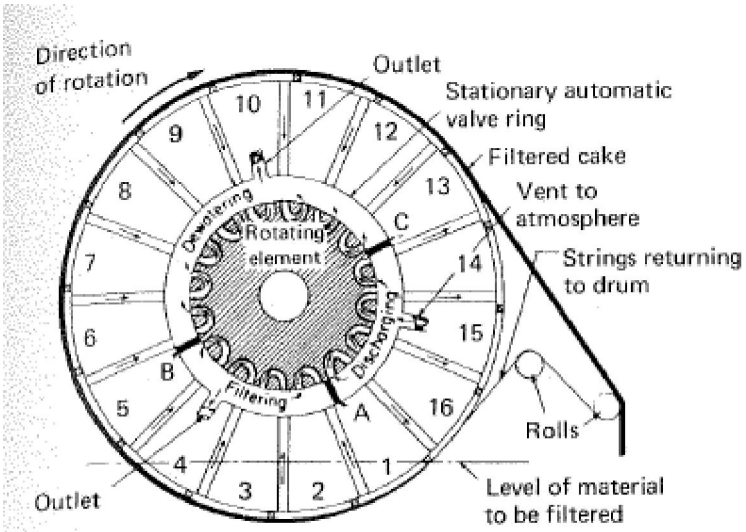


FIG. 10.10. Diagram of string-discharge filter operation. Sections 1 to 4 are filtering; sections 5 to 12 are dewatering; and section 13 is discharging the cake with the string discharge. Sections 14, 15 and 16 are ready to start a new cycle. A, B and C represent dividing members in the annular ring (Miller *et al.*, 1973).

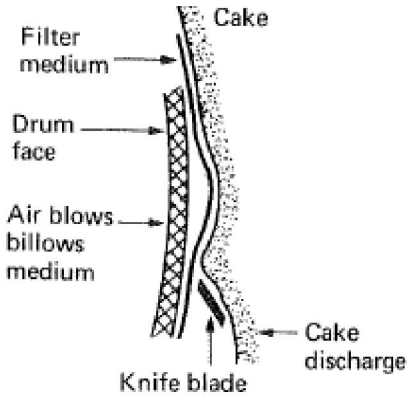


FIG. 10.12. Cake discharge on a drum using a scraper (Talcott *et al.*, 1980).

CENTRIFUGATION

Micro-organisms and other similar sized particles can be removed from a broth by using a centrifuge when filtration is not a satisfactory separation method. Although a centrifuge may be expensive when compared with a filter it may be essential when:

1. Filtration is slow and difficult.

2. The cells or other suspended matter must be obtained free of filter aids.

3. Continuous separation to a high standard of hygiene is required. Non-continuous centrifuges are of extremely limited capacity and therefore not suitable for large-scale separation. The centrifuges used in harvesting fermentation broths are all operated on a continuous or semi-continuous basis. Some centrifuges can be used for separating two immiscible liquids yielding a heavy phase and light phase liquid, as well as a solids fraction. They may also be used for the breaking of emulsions. According to Stoke's law, the rate of sedimentation of spherical particles suspended in a fluid of Newtonian viscosity characteristics is proportional to the square of the diameter of the particles, thus the rate of sedimentation of a particle under gravitational force is:

$$V_g = \frac{d^2 g\,(\rho_P - \rho_L)}{18\mu}$$

where V_g = rate of sedimentation (m s^{-1})
d = particle diameter (m)
g = gravitational constant (m s^{-2})
ρ_P = particle density (kg m^{-3})
ρ_L = liquid density (kg m^{-3})
μ = viscosity (kg m^{-1} s^{-1})

This equation can then be modified for sedimentation in a centrifuge:

$$V_c = \frac{d\omega^2 r\,(\rho_P - \rho_L)}{18\mu}$$

where V_c = rate of sedimentation in the centrifuge (m s^{-1}),
ω = angular velocity of the rotor (s^{-1}),
r = radial position of the particle (m).
Dividing equation (10.6) by equation (10.5) yields

$$\frac{\omega^2 r}{g}.$$

This is a measure of the separating power of a centrifuge compared with gravity settling. It is often referred to as the relative centrifugal force and given the symbol Z. It is evident from this formula that factors influencing the rate of sedimentation over which one has little or no control are the difference in density between the cells and the liquid (increased temperature would lower media density but is of little practical use with fermentation broths), the diameter of the cells (could be increased by coagulation/flocculation) and the viscosity of the liquid. Ideally, the cells should have a large diameter, there should be a large density difference between cell and liquid and the liquid should have a low viscosity. In practice, the cells are usually verysmall, of low density and are often suspended in viscous media. Thus it can be seen that the angular velocity and diameter of the centrifuge are the major factors to be considered when attempting to maximize the rate of sedimentation (and therefore throughput) of fermentation broths.

TYPES OF CENTRIFUGES

A number of centrifuges will be described vary in their manner of liquid and solid discharge, unloading speed and their relative maximum ca~>acitie:s. When choosing a centrifuge for a specific process it important to ensure that the centrifuge will be ableperform the separation at the planned production and operate reliably with minimum manpower. Large scaletests may therefore be necessary with fermentation broths or other materials to check that the correct centrifuge is chosen.

THE BASKET CENTRIFUGE (PERFORATED-BOWL BASKET CENTRIFUGE)

Basket centrifuges are useful for separating mouldmycelia or crystalline compounds. The centrifuge is most commonly used with a perforated bowl lined witha filter bag of nylon, cotton, etc.. A continuous eed is used, and when the basket is filled with the filter cake it is possible to wash the cake before removing it. The bowl may suffer from blinding with soft biological materials so that high centrifugal forces cannot be used. These centrifuges are normally operatedat speeds of up to 4000 rpm for feed rates of 50 to 300dm3 min -1 and have solids holding capacity of 30 to500 dm3. The basket centrifuge may be considered to be a centrifugal filter.

THE TUBULAR-BOWL CENTRIFUGE

This is a centrifuge to consider using for particle size ranges of 0.1 to 200 p.m and up to 10% solids in the in-going slurry. Figure 1O.16a shows an arrangement used in a Sharples Super- Centrifuge. The main component of the centrifuge is a cylindrical bowl (or rotor), which may be of a variable design depending on application, suspended by a flexible shaft(B), driven by an overhead motor or air turbine (C).The inlet to the bowl is via a nozzle attached to the bottom bearing (D). The feed which may consist of solids and light and heavy liquid phases is introduced by the nozzle (E). During operation solids sediment on the bowl wall

while the liquids separate into the heavy phase in zone (0) and the light phase in the centralzone (H). The two liquid phases are kept separate in their exit from the bowl by an adjustable ring, with theheavy phase flowing over the lip of the ring. Rings of various sizes may be fitted for the separation of liquids of various relative densities. Thus the centrifuge maybe altered to use for:

(a) Light-phase/heavy-phase liquid separation.

(b) Solids/light-liquid phase/heavy-liquid phase separation.

(c) Solids/liquid separation (using a different rotor)

THE SOLID-BOWL SCROLL CENTRIFUGE (DECANTER CENTRIFUGE)

This type of centrifuge is used for continuous handling of fermentation broths, cell lysates and coarse materials such as sewage sludge. The slurry is fed through the spindle of an archimedean screw within the horizontal rotating solids bowl. Typically the speed differential between the bowl and the screw is in the range 0.5 to 100 rpm. The solids settling on the walls of the bowl are scraped to the conical end of the bowl. The slope of the cone helps to remove excess liquid from the solids before discharge. The liquid phase is discharged from the opposite end of the bowl. The speed of this type of centrifuge is limited to around 5000 rpm in larger models because of the lack of balance within the bowl, with smaller models having bowl speeds of up to 10000rpm. Bowl diameters are normally between 0.2 and 1.5metres, with the length being up to five times the diameter. Feed rates range from around 200 dm3 h- 1to 200 m3 h -1 depending on scale of operation and material being processed. A number of variants on the design are available:

The Recovery and Purification of Fermentation Products

(a) Cake washing facilities (screen bowl decanters).

(b) Vertical bowl decanters.

(c) Facility for in-place cleaning.

(d) Bio-hazard containment features; steam sterilization in-situ, two or three stage mechanical seals, control of aerosols, containment casings and the use of high pressure sterile gas in seals to prevent the release of micro-organisms

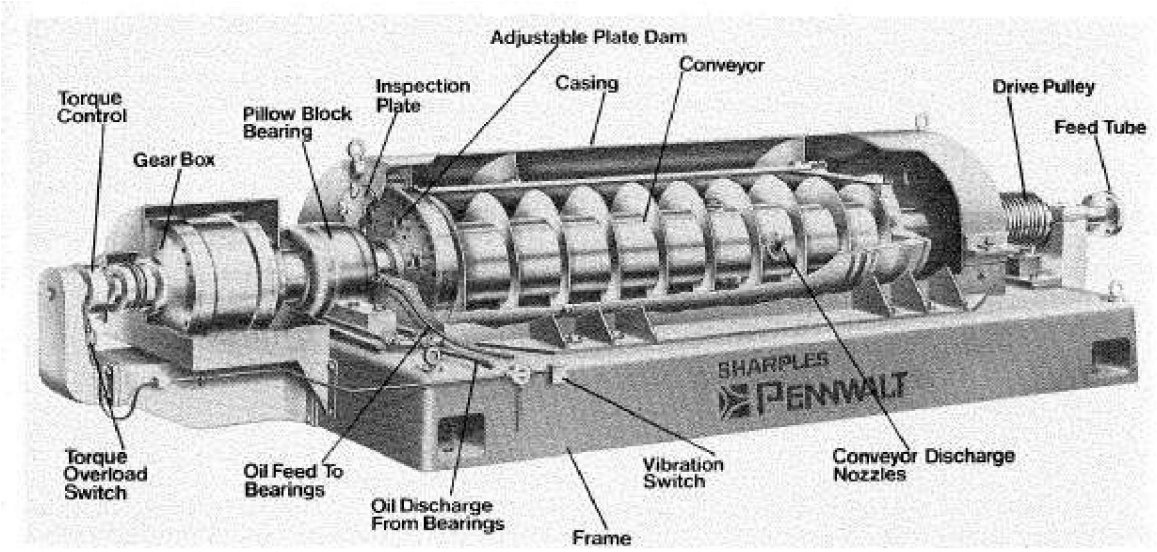


FIG. 10.17b. Cutaway view of a Sharples Super-D-Canter continuous solid-bowl centrifuge, Model P-5400 (Alfa Laval Sharples Ltd, Camberley, U.K.).

FREEZING-THAWING

Freezing and thawing of a microbial cell paste will inevitably cause ice crystals to form and their expansion followed by thawing will lead to some subsequent disruption of cells. It is slow, with limited release ofcellular materials, and has not often been used as a technique on its own, although it is often used in combination with other techniques. F3- Glucosidase has been obtained from S. Cerevisiae by this method. A sample of 360 g of frozen yeast paste was thawed at 50 for 10 hours. This cycle was repeated twice before further processing.

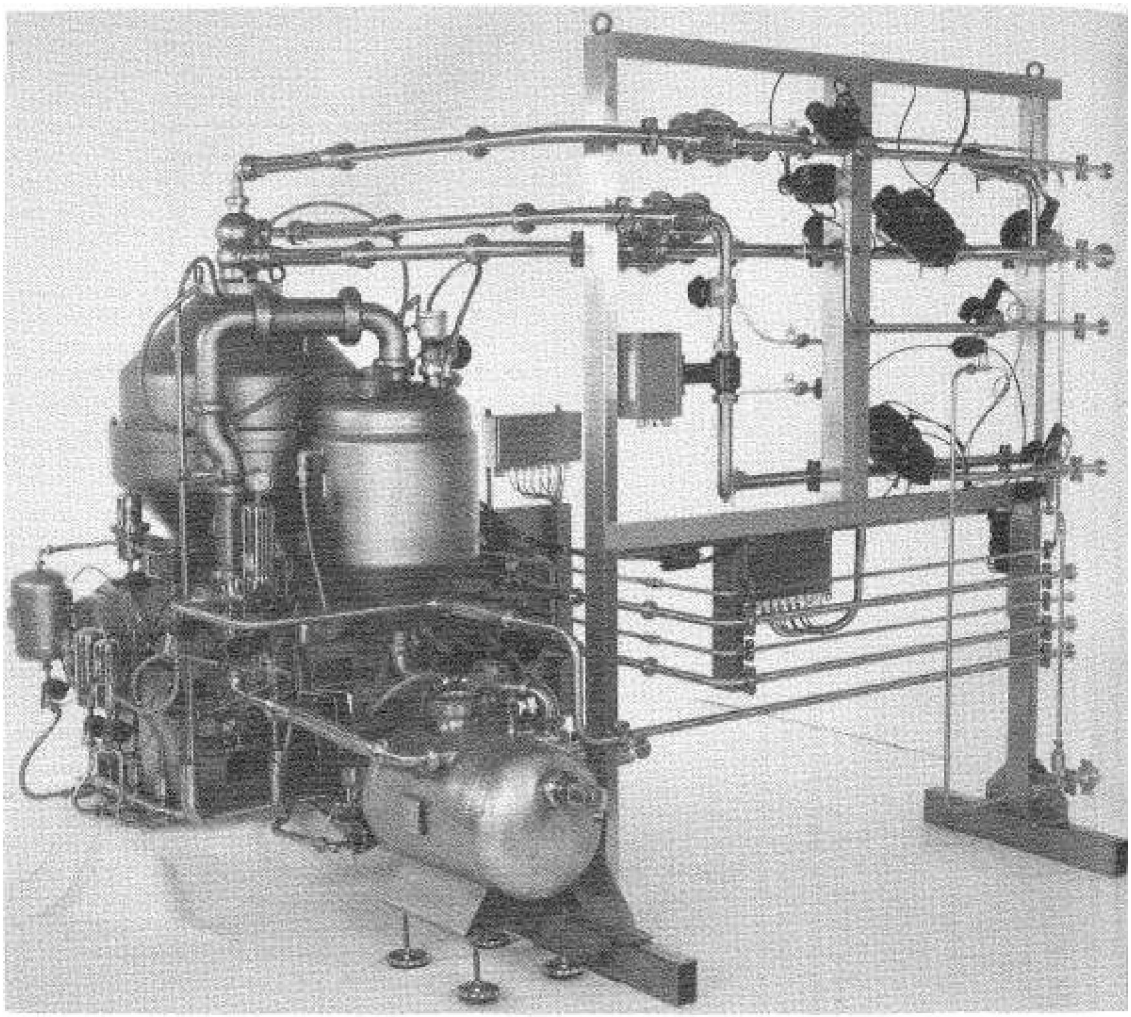


FIG. 10.20. Alfa Laval BTUX 510 disc stack centrifuge (Alfa Laval Sharples Ltd, Camberley, U.K.).

Possible Questions

Two Marks

1. Define filtration
2. What is meant by tangential cross flow filtration?
3. Write short notes on the role of centrifuge in separating the product.
4. Comment on crystallization.
5. What is meant by Freeze drying.

Eight Marks

1. Discuss about the batch and continuous fermentation.
2. Difference between solid state fermentation and submerged fermentation?
3. Brief on kinetics of batch and continuous fermentation.
4. Explain the yield of biomass and product and calculation of productivity.
5. What are the advantages and disadvantages of long term storage and culture revival?

UNIT IV	Option A	Option B	Option C	Option D	Answer Key
Fungus Mycelium should be suspended in _____ medium to maintain its structure.	Dimethyl sulphoxide	Rose Bengal medium	Sabouraud's dextrose agar medium	None of the above	Dimethyl sulphoxide
The volume of inoculum used to cultivate bacteria are _____.	0.1-2%	0.5-5%	3-5%	06-Apr	0.5-5%
Organic acids are produced in _____	Crowded plate technique	giant colony technique	Primary screening	Pour plate technique	Primary screening
Antibiotics from soil are easily isolated from_____	giant colony technique	Crowded plate technique	Primary screening	Pour plate technique	giant colony technique
Optimum Prodution of lysine takes place in medium containing _____ mg of biotin	5 mg/litre	20 mg/litre	15 mg/litre	25 mg/litre	20 mg/litre
Optimum Production of Arnithine occurs in a medium containing _____ mg of Arginine.	100 mg	200 mg	300 mg	400 mg	200 mg
-------- developed a fermentor for the production of acetone.	Liebmann	Robert Koch	Weizmann	chain weizmann	chain weizmann
In -------- year Straunch patented a system in which the aeration tubes were introduced	1930	1934	1940	1944	1934

with water and steam					
Carbon source used for production of tctracycline is _____	Molasses	Corn steep liquor	Barley	None of the above	Molasses
Mycellium undergoes autolysis with raise in _____	pH value	temp	Ionic conc	none	pH value
Example for non ionic detergents	Tween 80	Tween 20	Tween 40	none of the above	Tween 20
Example for coagulating agent	Calcium phosphate	Calcium carbonate	Caciumsulphate	All the above	Calcium phosphate
_____ is an established and final purification of a diverse range of compounds.	Drying	Crystallization	Filtration	HPLC	Crystallization
---------- filters are used to filter.	Polytetra flouro ethylene	Poly vinyl chloride	Glass and mineral fibres	All the above	All the above
_____ is the organism used in the first truly large scale aseptic fermentation vessels.	Clostridium acetobutylicum	C. perfringens	S. cereviseae	E.coli	Clostridium acetobutylicum
_____ is to provide microorganisms in submerged culture with sufficient oxygen for metabolic requirements.	Aeration	Agitation	Impeller	Baffler	Aeration
_____ ensures uniform suspension on microbial cells.	Aeration	Agitation	Sparger	Baffler	Agitation
---------device is used to introduce	Spargers	Impellers	Baffles	Turbines	Spargers

air in fermenter					
Aeration and agitation of a liquid medium may lead to the formation of	Acid	Alkali	Foam	Air	Foam
---------- are surface active agents reducing the Surface tension in the foam t.	Antifoam	Buffles	Yeast	Cell	Antifoam
-------- fermenter is called as elongated non-mechanically stirred fermenter	Tower	Airlift	Cylindraconical	Deep jet	Tower
_____ fermentor is a gas tight baffled rise tube connected to a down comer tube.	Tower	Air lift	Cylinder conical	Deep jet	Air lift
Multiple air lift fermenter Is designed by	Bakker etal	Okabe etal	Bacon etal	Dawsa	Bakker etal
Silicon compound are example are of inert ------- agent	Antibacterial	Antifoam	Anti fungal	Antiprotozal	Antifoam
---------- is the main compound in corn steep liquor	Lactic acid	Amino acid	Tartaric acid	Lactose	Lactic acid
Impeller are used in the fermentor helps in	Aeration	Antifoaming	Agitation	Absorption	Agitation
_____ or _____ is added to adjust pH if too acidic.	Ammonia	Sodium hydroxide	Both a or b	Sulphuric acid	Both a or b
_____ chromatography separates	Adsorption	Affinity	Ion exchange	Column	Adsorption

according to the affinity of the protein, for the surface of the solid matrix					
-------- chromatography is a powerful and highly selective purification technique.	Adsorption	Affinity	Ion exchange	Column	Affinity
Microbial cells and other insoluble materials are normally separated from the harvested broth by_____ or _____.	Filtration	Centrifugation	Filtration OR centrifugation	Sedimentation	Filtration OR centrifugation
Salts such as ammonium and ---------- are used for the discover of protein	NH3SO4	Na2SO4	CaCl2	Ca	Na2SO4
Dextrans can be precipitated out of a broth by the addition of _____.	Methanol	Ethanol	Butanol	Alcohol	Methanol
---------- method is used for large scale enzyme purification	Liquid shear	Solid shear	Ultrasonication	Freeze thawing	Liquid shear
Ultrasonication has frequency of ---------- khz	200	2	20	2000	20
---------- damage the cell membrane and lead to the release of intracellular components	Osmotic shock	Alkali treatment	Detergent	Enzyme	Osmotic shock

_____ caused by a sudden change in salt concentration will cause disruption of a number of cell types.	Osmotic shock	Alkali	Protease	SDS	Osmotic shock
_____ is the separation process where the solvent molecules are passed to flow through Semipermiable membrane in the opposite direction.	Ultra filtration	Reverse osmosis	Liquid membranes	pumping	Reverse osmosis
_____ of any product is often the last stage of a manufacturing process.	Filtration	Centrifugation	Drying	Packing	Drying
--------- drier is mostly used for drying of biological materials	Freeze	Spray	Drum	Tray	Spray
_____ device are the most economical available for handling large volumes.	Freeze	Spray	Drum	Tray	Spray
------- drying is an important operation in the production of biological and pharmaceuticals	Freeze	Drum	Spray	Tray	Freeze
_____ is an established and final purification of a diverse range of compounds.	Drying	Crystallization	Filtration	HPLC	Crystallization

_____ are metal strips roughly one- tenth of the vessel diameter and attached radically to the wall	Sparger	Baffler	Magnetic devices	Impellers	Baffler
A combined sparger and agitator may be used in --------- fermenter	Laboratary	Tower	Airlift	Batch	Laboratary
---------were first used as careeier for antifoam in antibiotic processes	Oils	Fats	Carbohydrate	Acids	Oils
Which is the by-product after starch extraction from maize.	Corn steep liquor	Barley	Molasses	Soybean oil	Corn steep liquor
Chemically defined amino acid media devoid of protein are used in production of _____	Acids	Vitamin	Vaccines	Antibiotics	Vaccines
_____does not appear to play a nutritional role in the metabolism of fungi.	Chlorine	Fluoride	Copper	Cadmium	Chlorine
_____ is used to carry out microbiological process on batch basis.	Batch fermentor	Continuous fermentor	Fed batch fermentor	Semi continuous fermentor	Batch fermentor
Small lab fermentor is in the size range of	1-21+	0.5-11+	1-101+	5-101+	1-21+
Larger fermentor range from _____	5000-10000	100-1000	1000-5000	10000-20000	5000-10000

gallous.					
pH control is achieved by _____ device.	Anti titrator	Aerator	Baffler	Impeller.	Anti titrator
_____ consists of circular discs to which blades are fitted with bolts.	Impellers	Sparger	Baffler	Aerator	Impellers
Size of the holes in the sparger ranges from_____	1/64-1/32	1/32- 1/64	1/32- 1/32	1/64 -1/64	1/64-1/32
Steady state condition can be achieved by operation on _____ principles.	Chemostatic	Turbidostatic	Both chemostat and turbidostat	Photostat	Both chemostat and turbidostat
CSTF is expanded as	Continuous stirred tank fermentor	Continuous solid tank fermentor	Cell suspended tank fermentor	Continuous solid type fermentor	Continuous stirred tank fermentor
The temperature of _____ in the production tanks is satisfactory during fermentation.	80°F	70°F	90°F	100°F	80°F
Industrial alcohol production can be carried out in very large fermentor upto _____ Gallous.	12500	125	25000	100000	12500
_____ chromatography separates according to the affinity of the protein, for the surface of the solid matrix	Adsorption	Affinity	Ion exchange	Column	Adsorption

-------- chromatography is a powerful and highly selective purification technique.	Adsorption	Affinity	Ion exchange	Column	Affinity
Microbial cells and other insoluble materials are normally separated from the harvested broth by_____ or _____.	Filtration	Centrifugation	filtration or centrifugation	Sedimentation	filtration or centrifugation
---------- stressed the importance of the elimination of air and moisture from lyophilized cultures prior to sealing of ampoules.	Meryman	Dewald	leogetring	Louis Pasteur.	Dewald

Unit V

THE ISOLATION, PRESERVATION AND IMPROVEMENT OF INDUSTRIALLYIMPORTANT MICRO-ORGANISMS

Introduction

Microbes are required for the production of fermentation products. They are very valuable for specific product. Not all the microbes will give one product produced efficiently by specific microbe.

The isolation of a desired organism for a fermentation process may be time consuming and very expensive procedure and it is therefore essential that it retain the desirable characteristics that led to its selection. In addition, the culture used for the fermentation process should remain viable and free from contamination. Thus, industrial cultures must be preserved and maintained in such way as to eliminate genetic change, protect against contamination, and retain viability.

Different techniques are used for maintenance and preservation of different organisms based on their properties. Selected method should also conserve the properties of the organisms.

Techniques for the Preservation of microbes broadly divided into two

1. **Methods where organisms are in Continuous metabolic active state**
2. **Methods where organisms are in Suspended metabolic state**

1. Continuous metabolic active state preservation technique

In this technique, organisms preserved on nutrient medium by repeated sub-culturing. In this technique, any organisms are stored by using general nutrient medium. Here repeated sub- culturing is required due to depletion or drying of nutrient medium. This technique includes preservation by following methods.

Periodic transfer to fresh media

Organisms grown in general media on slant, incubated for particular period at particular temperature depending on the characteristics of the selected organisms, then it is stored in refrigerator. These cultures can be stored for certain interval of time depending on the organism and its growth conditions. After that time interval, again these organisms transferred to new fresh medium and stored in refrigerator.

Overlaying culture with mineral oil

Organisms are grown on agar slant then they are covered with sterile mineral oil to a depth of 1 cm. above the tip of the surface. This method is simple; one can remove some organisms in aseptic condition with the help of sterile wire loop and still preserving the initial culture. Some species preserved satisfactorily for 15 – 20 years by this method.

Storage in sterile soil

This method is widely used for preserving spore forming bacteria and fungi. In this method, organisms will remain in dormant stage in sterile soil. Soil sterilized then spore suspension added to it aseptically, this mixture dried at room temperature and stored in refrigerator. Viability of organisms found around 70 – 80 years.

Saline suspension

Normal Saline used to provide proper osmotic pressure to organism's otherwise high salt concentration is inhibitory for organisms. Organisms kept in screw cap bottles in normal saline, stored at room temperature, wherever required transfer made on agar slats, and incubated.

THE PRESERVATION OF INDUSTRIALLY IMPORTANT MICRO-ORGANISMS

The isolation of a suitable organism for a commercial process may be a long and very expensive procedure and it is therefore essential that it retains the desirable characteristics that led to its selection. Also,the culture used to initiate an industrial fermentation must be viable and free from contamination. Thus, industrial cultures must be stored in such way as to eliminate genetic change, protect against contamination and retain viability. An organism may be keptviable by repeated sub-culture into fresh medium, but, at each cell division, there is a small probability of mutations occurring and because repeated sub-culture involves very many such divisions, there is a high probability that strain degeneration would occur. Also, repeated sub-culture carries with it the risk of contamination. Thus, preservation techniques have been developed to maintain cultures in a state of 'suspended animation' by storing either at reduced temperature or in a dehydrated form. Storage at reduced temperature

STORAGE ON AGAR SLOPES

Cultures grown on agar slopes may be stored in arefrigerator (5°) or a freezer (- 20°) and sub- culturedat approximately 6-monthly intervals. The time of subculturemay be extended to I year if the slopes arecovered with sterile medicinal grade mineral oil.

STORAGE UNDER LIQUID NITROGEN

The metabolic activities of micro-organisms may be reduced considerably by storage at the very low temperatures (-150° to -196°) which may be achieve dusing a liquid nitrogen refrigerator. Snell (1991) claimed that this aproach is the most universally applicable of all preservation methods. Fungi, bacterio phage, viruses, algae, yeasts, animal and plant cells and tissue cultures have all been successfully preserved. The technique involves growing a culture to the maximum stationary phase, resuspending the cells in a cryoprotective agent (such as 10% glycerol) and freezing the suspension insealed ampoules before storage under liquid nitrogen.ome loss of viability is suffered during the freezing and thawing stages but there is virtually no loss during the storage period. Thus, viability may be predictable even after a period of many years. Snell (1991) suggested that liquid nitrogen is the method of choice forthe preservation of valuable stock cultures and may be the only suitable method for the long term preservationof cells that do not survive freeze-drying. Although the equipment is expensive the process is economical onlabour. However, the method has the major disadvantage that liquid nitrogen evaporates an dustbe replenished regularly. If this is not done, or the apparatus fails, then the consequences are the loss of the collection.

Storage in a dehydrated form

DRIED CULTURES

Dried soil cultures have been used widely for culture preservation, particularly for sporulating mycelial organisms. Moist, sterile soil may be inoculated with aculture and incubated for several days for some growth to occur and then allowed to dry at room temperature for approximately 2 weeks. The dry soil may be storedin a dry atmosphere or, preferably, in a refrigerator.The technique has been used extensively for the storageof fungi and actinomycetes and Pridhamet al. (1973)observed that of 1800 actinomycetes dried on soil about50% were viable after 20-years storage. Malik (1991) described methods which extend theapproach using substrates other than soil. Silica gel andporcelain beads are suggested alternatives and detailed methods are given for these simple, inexpensive techniques in Malik's discussion.

LYOPHILIZATION

Lyophilization, or freeze-drying, involves the freezing of a culture followed by its drying under vacuum, technique involves growing the culture to the maximum stationary phase and resuspending the cells in a protective medium such as milk, serum or sodium glutamate. A few drops of the suspension are transferred to anampoule, which is then frozen and subjected to a high vacuum until sublimation is complete, after which the ampoule is sealed. The ampoules may be stored in a refrigerator and the cells may remain viable for 10years or more (Perlman and Kikuchi, 1977).Lyophilization is very convenient for service culture collections (Snell, 1991) because, once dried, the cultures need no further attention and the

storage equipment(a refrigerator) is cheap and reliable. Also, the freeze dried ampoules may be dispatched as such, stillin a state of 'suspended animation' whereas liquid nitrogen stored cultures begins to deteriorate. However, freeze-dried cultures are tedious to open and revitalize and several sub-cultures may be needed before the cells regain their typical characteristics. Overall, the technique appears to be second only to liquid nitrogen storage and even when liquid nitrogen is used make an excellent insurance against the possibility of the breakdown of the nitrogen freezer.

THE USE OF RECOMBINATION SYSTEMS FOR THE IMPROVEMENT OF INDUSTRIAL MICRO-ORGANISMS

Hopwood (1979) defined recombination, in its broadest sense, as "any process which helps to generate new combinations of genes that were originally present in different individuals". The use of recombination mechanisms for the improvement of industrial strains has increased significantly due to the developments in ecombinant DNA technology and the necessity to develop new methods of strain improvement as the eturns generated from mutation and selection programmes decreased. However, it should be appreciated that mutation and selection techniques are frequently used in association with recombination systems in a strain improvement programme. The parasexual cycle in the filamentous fungi has been applied to strain development as have protoplast fusion techniques in a wide range of micro-organisms.

THE APPLICATION OF THE PARASEXUAL CYCLE

Many industrially important fungi do not possess asexual stage and therefore it would appear difficult toachieve recombination in these organisms. However ,Pontecorvoet aZ.(1953) demonstrated that nuclearfusion and gene segregation could take place outside,or in the absence of, the sexual organs. The process as termed the parasexual cycle and has been demonstrated in the imperfect fungi, A. niger and P. chrysogenum,as well as the sexual fungus A. niduzans. In order for parasexual recombination to take place in animperfect fungus, nuclear fusion must occur between unlike nuclei in the vegetative hyphae of the organism.Thus, recombination may be achieved only in an organism in which at least two different types of nucleic exist, i.e. a hetero karyon. The heterozygous diploid nucleus resulting from the fusion of the two different haploid nuclei may give rise to a diploid clone and, in rare cases, a diploid nucleus in the clone may undergo an abnormal mitosis resulting in mitotic segregation and the development of recombinant clones which maybe either diploid or haploid. Recombinant clones may be detected by their displayof recessive characteristics not expressed in the heterokaryon. Analysis of the recombinants normally demonstrates them to be segregant for only one, or a few linked, markers and culture of the sergeants results in the development of clones displaying morerecessive characters than the initial segregant. The process of recombination

during the growth of the heterozygous diploid may occur in two ways: crossing over, which results in diploid recombinants and haploidization, which results in haploid recombinants. Mitotic crossing over is the result of an abnormal mitosis. The normal mitosis of a heterozygous cell is shown in Fig. 3.26. During mitosis, each pair homologous chromosomes replicate to produce pairs of chromatids and a chromatid of one pair migrates to a pole of the cell with a chromatid of other pair. Division of the cell at the equator results the production of two cells, both of which are heterozvgous for all the genes on the chromosome. Crossing over involves the exchange of distal segments between chromatids of homologous chromosomes shown in Fig. 3.27. This process may result in production of daughter nuclei homozygous formation of one pair of chromosomes and in the expressions of any recessive alleles contained in that portion.mhe clone arising from the partial homozygote will recombinant and further mitotic crossing over in recombinant will result in the expression of more recessive alleles.

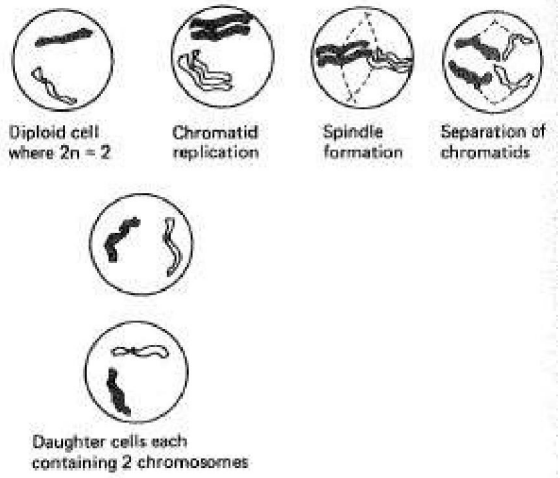


FIG. 3.26. Diagrammatic representation of the mitotic division of a eukaryotic cell containing two chromosomes. The nuclear membrane has not been portrayed in the figure.

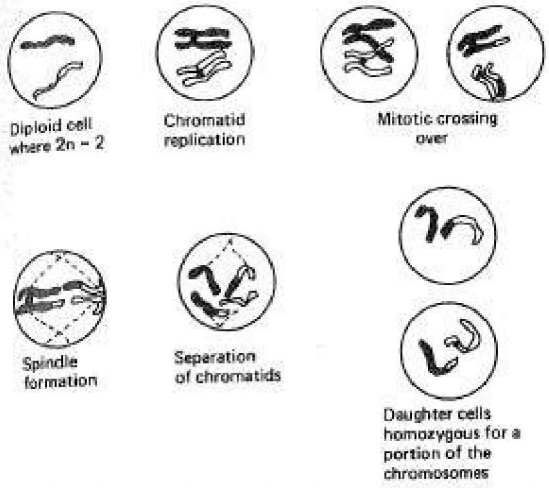


FIG. 3.27. Diagrammatic representation of mitosis including mitotic crossing over.

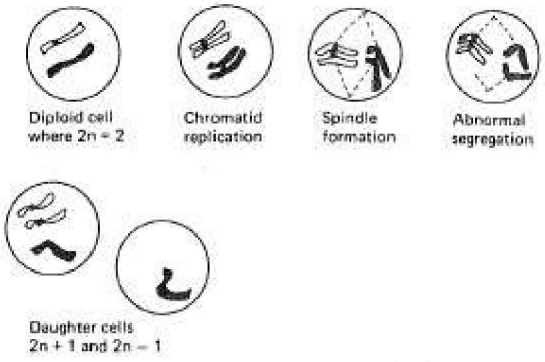


FIG. 3.28. Diagrammatic representation of mitosis involving haploidization.

THE APPLICATION OF PROTOPLAST FUSION TECHNIQUES

Protoplasts are cells devoid of their cell walls and may be prepared by subjecting cells to the action ofwall degrading enzymes in isotonic solutions. Protoplasts may regenerate their cell walls and are thencapable of growth as normal cells. Cell fusion, followed by nuclear fusion, may occur between protoplasts ofstrains which would otherwise not fuse and the resulting fused protoplast may regenerate a cell wall andgrow as a normal cell. Thus, protoplasts may be used to

Overcome some recombination barriers. Protoplast fusion has been demonstrated in a large number of industrially important organisms including Streptomycesspp. (Hopwood et al., 1977), Bacillus spp. (Fodor and Alfoldi, 1976), corynebacteria (Karasawaet al., 1986),filamentous fungi (Ferenczyet al., 1974) and yeasts (Sipiczki and Ferenczy, 1977).Fusion of fungal protoplasts appears to be an excellent technique to obtain heterokaryons between strains where conventional techniques have failed, or, indeed,as the method of choice. Thus, this approach has allowed the use of the parasexual cycle for breedingpurposes in situations where it had not been previouslypossible. This situation is illustrated by the work of Peberdyet at. (1977) who succeeded in obtaining heterokaryons between P. chrysogenumand P. cyaneofuluumand demonstrated the formation of diploids which gave rise to recombinants after treatment with p- ftuorophenylalanine or benomy Although it has been claimed that P. chrysogenumand P. Cyaneofulvum are not different species of Penicillium (Samson et al., 1977), Peberdyet al. still demonstrated that protoplast fusion could be successful where conventional techniques had failed. A demonstration of the use of protoplast fusion foran industial fungus is provided by the work of Hamlynand Ball (1979) on the cephalosporin producer, C.acremonium. These workers compared the effectivenessof conventional techniques of obtaining nuclear fusionbetween strains of C. Acremonium with the protoplast fusion technique. The results from conventional techniques suggested that nuclear fusion was difficult to achieve. Electron microscopic examination of fusedprotoplasts indicated that up to 1% underwent immediate nuclear fusion. Recombinants were obtained inboth sister and divergent crosses. A cross between an asporulating, slow-growing strain with a sporulating fast-growing strain which only produced one-third of the cephalosporin level of the first strain eventually resulted in the isolation of a recombinant which combined the desirable properties of both strains, i.e. astrain which demonstrated good sporulation, a high growth rate and produced 40% more antibiotic than the higher-yielding parent. Chang et al. (1982) utilized protoplast fusion to combine the desirable qualities of two strains of Penicillium chysogenum. Protoplasts from two strains, differing in colony morphology and the abilities to produce penicillin V.

Protoplasts are also useful in the filamentous fungi for manipulations other than cell fusion. Rowlands (1992) suggested that they may be used in mutagenesis of non-sporulating fungi. Spores are the cells of choice for the mutagenesis of filamentous fungi but this isobviously impossible for non-sporulating strains. Mycelial fragments may be used but these will be multinucleate and very high mutagen doses arerequired. Although some protoplasts will be non-nucleate or multi-nucleate at least some will be uninucleate which will express any modified genes after mutation. Also, protoplasts will take up DNA in in vitro genetic manipulation experiments.

Protoplast fusion has also been applied to the improvement of amino acid producing strains. Karasawaet al. (1986) used the technique to improve the fermentation rates of lysine producers developed using repeated mutation and directed selection. Such strains were good lysine producers but showed low glucose consumption and growth rates, undesirable features which had been inadvertently introduced during the selection programme. A protoplast fusion was performed between the lysine producer and a fast growing strain and afusant was isolated displaying the desirable characteristics of high lysine production and high glucose consumption rate resulting in a much faster fermentation.

TABLE 3.7. *The use of protoplast fusion for the improvement of a penicillin V producer* (Lein, 1986)

Characteristic	Parent A	Parent B	Best recombinant
Spores per slant ($\times 10^8$)	2.2	2.5	7.5
Germination frequency (%)	99	40	49
Colour of sporulating colonies	Green	Pale green	Deep green
Seed growth	Good	Poor	Good
Penicillin V yield (mg cm^{-3})	11.7	18.5	18.0
Phenylacetic oxidation	Yes	No	No

THE APPLICATION OF RECOMBINANT DNA TECHNIQUES

The transfer of DNA between different species of bacteria has been achieved experimentally using both in vivo and in vitro techniques (Atherton et al., 1979).Thus; genetic material derived from one species may be incorporated into another where it may be expressed. In vivo techniques make use of phage particles which will pick up genetic information from the chromosome of one bacterial species, infect another bacterial species and in so doing introduce the genetic information from the first host. The information from the first host may then be expressed in the second host. Whereas, the in vivo techniques depend on vectors collecting information from one cell and incorporating it into another, the in vitro techniques involve the insertion of the information into the vector by in vitro manipulation followed by the insertion of the carrier and its associated 'extra' DNA into the recipient cell. Because the DNA is incorporated into the vector by in

vitro methods the source of the DNA is not limited to that of the host organism of the vector. Thus, DNA from human or animal cells may be introduced into the recipient cell. Atherton et al. (1979) listed the basic requirements for the in vitro transfer and expression of foreign.

DNA in a host micro-organism as follows:

(j) A 'vector' DNA molecule (plasmid or phage) capable of entering the host cell and replicating within it. Ideally the vector should be small, easily prepared and must contain at least on esite where integration of foreign DNA will not destroy an essential function.

(ij) A method of splicing foreign genetic informationinto the vector.

(iii) A method of introducing the vectorforeignDNA recombinants into the host cell and selectingfor their presence. Commonly used simple characteristics include drug resistance, immunity, plaque formation, or an inserted gene recognizable by its ability to complement a known auxotroph.

(iv) A method of assaying for the 'foreign' gene product of choice from the population of recombinant screated.

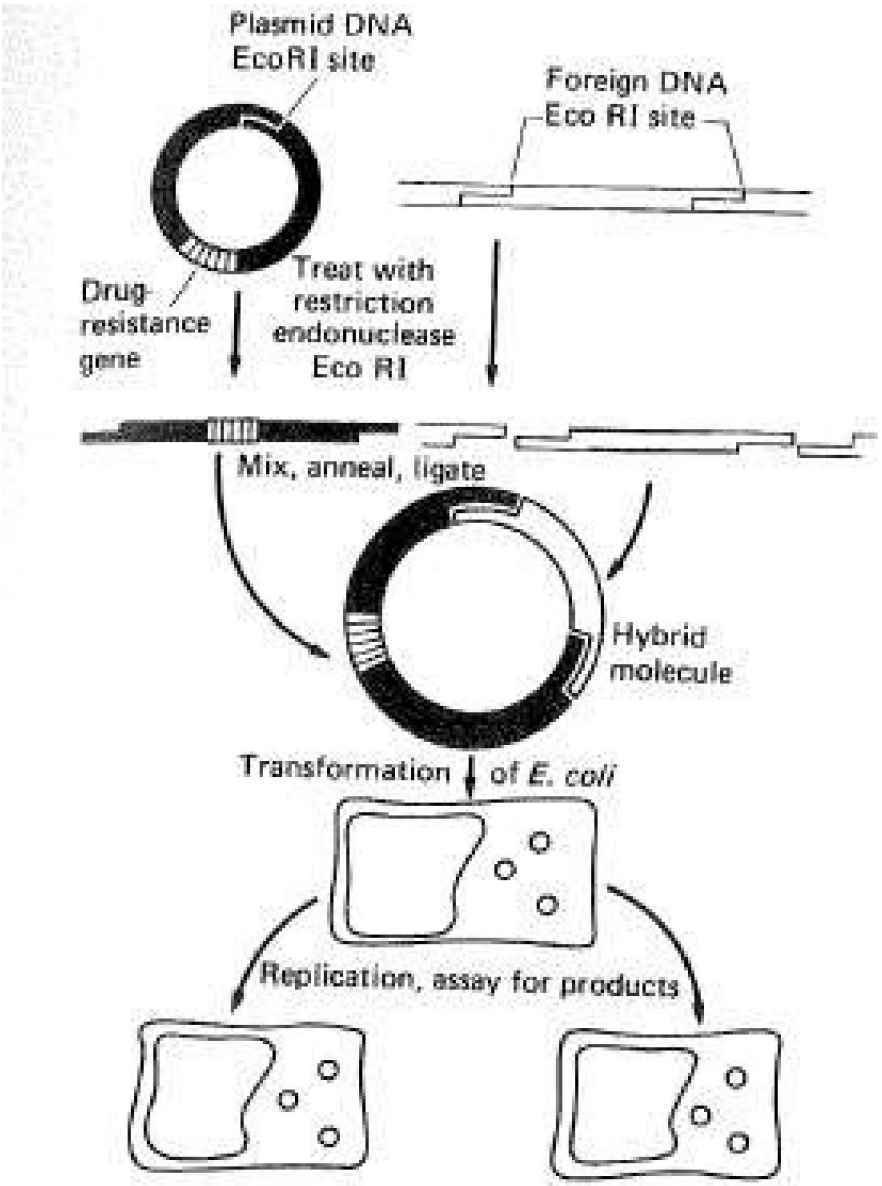


FIG. 3.29. A summary of the steps in *in vitro* genetic recombination. Both plasmid vector and foreign DNA are cut by the restriction endonuclease, EcoRI, producing linear double-stranded DNA fragments with single-stranded cohesive projections. EcoRI recognizes the oligonucleotide sequence $\overset{GAATTC}{CTTAAG}$ and will cut any double-stranded DNA molecule to yield fragments with the same cohesive ends $\overset{GAATT}{C}$, $\overset{C}{TTAAG}$. On mixing vector and foreign DNA, hybrids form into circular molecules which can be covalently joined using DNA ligase. Transformation of *E. coli* results in the low-frequency uptake of hybrid molecules whose presence can be detected by the ability of the plasmid to confer drug resistance on the host (Atherton *et al.*, 1979).

The use of recombinant DNA technology for the improvement of native microbial products Recombinant DNA technology has been used widelyfor the improvement of native microbial products. Frequently,this has involved 'self cloning' work where achromosomal gene is inserted into a plasmid and theplasmid incorporated into the original strain and maintainedat a high copy number. Thus, this is not anexample of recombination because the engineered strain is altered only in the number of copies of thegene and does not contain genes which were present originally in a different organism. However, the techniques employed in the construction of these strains are the same as those used in the construction of chimeric strains, so it is logical to consider this aspec there. The first application of gene amplification to industrial strains was for the improvement of enzyme production. Indeed, some regulatory mutants isolated by conventional means owed their productivity to their containing multiple copies of the relevant gene as well as the regulatory lesion.

Possible Questions

Two marks

1. Write short notes on isolation of pure culture.
2. Write about the criteria for selecting industrial culture.
3. How are cultures preserved using oil.
4. What is the advantage of freeze drying?
5. How are industrial strains propagated?

Eight marks

1. Discuss about the batch and continuous fermentation.
2. Difference between solid state fermentation and submerged fermentation?
3. Brief on kinetics of batch and continuous fermentation.
4. Explain the yield of biomass and product and calculation of productivity.
5. How cultures are preserved?
6. Write about the role of rDNA technology in preserving culture.

UNIT V	Option A	Option B	Option C	Option D	Answer Key
Industrial microbiology deals with areas of microbiology involving economic aspects, where valuable parts are prepared	from costly substrates	from cheaper and disposable substrates	from unavailable substrates	from foreign countries	from cheaper and disposable substrates
In history of industrial microbiology, the period of ignorance is	pre-1800	1800-1900	post-1900	post-2000	pre-1800
The period of discovery in history of industrial microbiology is from	pre-1800	1800-1900	post-1900	post-2000	1800-1900
The period of industrial development in history of industrial microbiology is	pre-1800	1800-1900	post-1900	post-2000	post-1900
Bread was 1st baked around ----------B.C	1000	2000	3000	4000	4000
Wine is produced from	malt	molasses	grapes	sugarcane	grapes
The compound Microscope produced by Zaccharies Jensen had no provision for	resolution power	focusing	light facility	specimen holding	focusing
Anton von Leewanhoek was able to obtain magnification upto ---------- diameters.	150-300	160-270	140-250	150-200	160-270
In middle of last century fermentation was	biological	physical	chemical	electrical	chemical

consider to be a ----process.					
Fermentation was first described as chemical process by	Pasteur	Robert Koch	Liebig	Anton van Leewanhoek	Liebig
In 1873,----------described that yeastsare involved in fermentation process for alcohol production	Pasteur	Schwann	Robert Koch	Berzelius	Schwann
In fermentation, yeast converts ----------to----------and -----	carbohydrate, alcohol, carbonic acid	fatty acids, alcohol, carbonic acid	sugar, alcohol, carbonic acid	starch, alcohol, carbonic acid	sugar, alcohol, carbonic acid
who isolated the microbes associated fementation.	Schwann	Bertholet	Pasteur	Koch	Pasteur
The optimum temperature condition for fermentation process was in range from-----°C	20-40	30-50	40-60	20-30	30-50
Pasteur identified the organisms involved in the transformation of sugar to ----------	pyruvic acid	lactic acid	citric acid	stearic acid	lactic acid
Lactic acid organism is a ----------	fungi	bacteria	virus	protozoa	bacteria
who made an important discovery that fermentation takes place in absence of oxygen.	Schwann	Pasteur	Koch	Bertholet	Pasteur
In 1861 pasteur did experiments on ----------and---------- fermentation	butyric acid and acetic acid	acetone and butanol	lactic acid and acetic acid	acetic acid and citric acid	butyric acid and acetic acid

----------and----------requested Pasteur to study the problem of sowing wine that thereatened French wine industry.	Dumas and Napoleon 111	Dumas and Flemming	Napoleon111 and Schwann	Duman and Leewanhoek	Dumas and Napoleon 111
The process of sterilization of wine introduced by Pasteur is called	pasteurization	ultrafiltration	low temperature,high holding time	high temperature ,low holding time	pasteurization
which method is most satisfory method for long time preservation of microbes	mineral oil overlay	lyophilisation	cryopreservation	periodic transfer	lyophilisation
The major antibiotics such as streptomycin and neomycin etc were isolated from----------	*Bacillus sp*	*Staphylococcus sp*	*Streptococcus sp*	*Streptomyces sp*	*Streptomyces sp*
During 1910-1920,----------and---------were produced by Industrial fermentation	ethanol and glycerol	lactic acid and amylases	acetone and n-butanol	acetone and lactic acid	acetone and n-butanol
During 1920-1930,---------- was produced by industrial fermentation	lactic acid	acetic acid	citric acid	glutanic acid	citric acid
During 1930-1940, first vitamin to be produced by industrial fermentation is------	riboflavin	vitamin B12	vitamin C	vitamin D	riboflavin
The acetone-butanol fermentation is also called as ----------	Watsmann	Walksman	Websmann	Weizmann	Weizmann

----process					
The important quality of production strain is	should be a high yielding strain	unstable biochemical characteristics	produce underisable substances	not easily cultivate	should be a high yielding strain
The screening techniques involves ----------and----------	primary and secondary	secondary and tertiary	primary and quartenary	secondary and quartenary	primary and secondary
Primary screening technology involves the isolation of new microbial species exhibiting the	desined color	desired shape	desired quality	desired property	desired property
Crowded plate technique is an example of ----------screening	primary	secondary	tertiary	quartenary	primary
The primary screening technique which is employed for a detect and isolating antibiotic producing strain is	crowded plate technique	auxanography	enrichment culture technique	use of indicator dye	crowded plate technique
Enrichment culture technology was designed by soil microbiologist----------	Pasteur	Koch	Ehrlich	Beijerinck	Beijerinck
which technique is largely employed to identify the growth factor producing strain extracellularly	crowded plate technique	auxanography	enrichment culture technique	use of indicator dye	auxanography
Neutral red, bromothymol blue dyes are added to partly buffered nutrient agar media to detect microorganisms	vitamins	growth factors	organic acids	amines	organic acids

capable of producing					
Example of enrichment substrate used is	nutrient broth	cellulose powder	peptone	minimal media	cellulose powder
which screening helps in segregation microbe that have real potential in fermentation industry.	primary	secondary	tertiary	quartenary	secondary
The suitable protective medium used at the ----------is 10% inositol in dissolved water	Commonwealth Mycological Institute	American type culture collection	African type culture collection	Indian Mycological Institute	Commonwealth Mycological Institute
The fermentation product produced by the identified industrial strain should be ----------	old	novel	gold	critical	novel
The selected industrial strain is----------by secondary screening.	optimized	priotized	compared	deselected	optimized
The process of lyophilization was first applied to microfungi on layers scale by ----------and ----------in 1942	Raper and Alexander	Thomas and Alexander	Koch and Alexander	Koch and Thomas	Raper and Alexander
The important criteria in handling the industrially productive strain is to prevent	stability	contamination	oxidation	reduction	contamination
Mutation is done by----------and----------methods	physical and chemical	chemical and political	physical and botanical	chemical and zoological	physical and chemical

Phosphorous pentoxide silica gel/freezing trap are examples of----------	crypreservation	desiccant	preservants	mineral oil overlay	desiccant
In multivalent regulatory mechanism of a branched biosynthetic pathway, ----------end products inhibit the enzyme.	single	double	triple	all	all
The two categories of mutants are----------and----------	autotropic mutants and mutants resistant to analogues	phototropic mutants and mutants resistant to analogues	auxenic mutants and mutants resistant to analogues	auxotropic mutants and mutants sensitive to analogues	autotropic mutants and mutants resistant to analogues
The wild strain of *Coryrebacterium glutamicus* secretes both -------and----------	lysine and threonine	lysine and methionine	threonine and methionine	threonine and pectin	lysine and threonine
The mutant strain of *Coryrebacterium glutamicus* produces upto ----------g of lysine litre in medium	50	60	70	80	60
The oil used in oil overlay method is	British Pharmacopoeia Medicinal Paraffin oil.	American Pharmacopoeia Medicinal Paraffin oil.	Australian Pharmacopoeia Medicinal Paraffin oil.	African Pharmacopoeia Medicinal Paraffin oil.	British Pharmacopoeia Medicinal Paraffin oil.
The lysine biosynthesis, the end products lysine and threonine inhibit the enzyme.	aspartate kinase	homoserine phosphatase	serine kinase	tryptophan synthase	aspartate kinase
An analogue of threonine is ----------	α-amino,β-hydroxyvaleric acid	β-amino,β-hydroxyvaleric acid	ч-amino,β-hydroxyvaleric acid	)£-amino,β-hydroxyvaleric acid	α-amino,β-hydroxyvaleric acid
The analogue is ----	ecofriendly	toxic	nutrient	non-toxic	toxic

----------to sensitive mutant cells in the population.					
An example of analogue resistant mutant is ----------capable of excreating threomine upto 12.6g/l.	*Brevibacterium flavum*	*Brevibacterium lactum*	*Brevibacterium aseptum*	*Brevibacterium glutans*	*Brevibacterium flavum*
An example of revertant mutant is----------for the enzyme threonine deaminase.	*Hydrophiles*	*Hydromonas*	*Hydrogenomonas*	*Hydromonothrobis*	*Hydrogenomonas*
---------- mutants are important in fermentation industry as produce high yields of particular enzymes in absence of inducing substrates.	auxotrophic	mutants resistant to analogue	constitutive	mutants sensitive to analogues.	constitutive
ATCC is ---------- founded in 1925	American type culture collection	African type culture collection	Auxenic type culture collection	Australian type culture collection	American type culture collection
Secondary metabolites are produced during _____.	Lag phase	Log phase	Trophophase	Idiophase	Trophophase
Extra chromosomal elements which carry information for synthesis of products is called _____.	Protoplast	Chloroplast	Plasmid	Spheroplast	Plasmid
_____ is the industrially used promoter.	CaMV	TMV	BMV	HMV	CaMV
----------and----------are usually maintained in liquid medium	Bacteriophages and *Actinomycetes*	Bacteriophages and *clostridium*	Viruses and *Acetobacter*	Bacteriophages and *Streptomyces*	Bacteriophages and *clostridium*

Lyophilization is the most satisfactory method of long term preservation of microorganisms	long term	short term	both a and b	none of the above	long term
---------- is perhaps the most popular form of suspended metabolism.	overlaying cultures with mineral oil	lyophilization	nitrogen storage	none of the above	lyophilization
Hwang in ---------- year recommends precooling to 7 degree Celsius.	1966	1969	1974	1980	1966
---------- stressed the importance of the elimination of air and moisture from lyophilized cultures prior to sealing of ampoules.	Meryman	Dewald	leogetring	Louis Pasteur.	Dewald

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KARPAGAM ACADEMY OF HIGHER EDUCATION

(Deemed to be University Established Under Section 3 of UGC Act 1956)

COIMBATORE – 641 021.

I M.Sc. CIA I, February 2019

MICROBIOLOGY

SECOND SEMESTER

BIOPROCESS ENGINEERING

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

PART-A **(20x 1=20 marks)**

(Multiple Choice Question No. 1 to 20)

1. _____is a culture system which contain a initial limited amount of nutrient
   (a) Fed batch (b)Batch
   (c) Semi continuous (d)Continuous
2. CSTF is expanded as _____.
   (a)Continuous Stirred Tank Fermentor (b)Continuous Solid Tank Fermentor
   (c) Cell Suspended Tank Fermentor (d)Continuous Solid Type Fermentor
3. _____.are used in side of fermentors to avoid vortex formation
   (a)Spargers (b)Bearing glands
   (c)Rotameter (d)Baffles
4. In 1861 Pasteur did experiments on _____and_____fermentation.
   (a)Butyric acid and acetic acid (b)Acetone and butanol
   (c)Lactic acid and acetic acid (d)Acetic acid and citric acid
5. Soya bean meal is used as production medium for _____.
   (a)Penicillin (b)Streptomycin
   (c)Griseofulvin (d)Vitamin B complex
6. The clean, yellow, fine powder prepared from embryo of cotton seed is called as _____.
   (a)Corn-steep liquor (b)Soya bean meal
   (c)Pharmamedia (d)Distiller's soluble
7. Ancillary equipments in fermentors means the _____.
   (a)Seed tank (b)Fermentation medium
   (c)Extra connection (d)Antifoamers
8. Fragile and heat sensitive equipments are sterilized by_____.
   (a)Ethylene oxide (b)autoclave
   (c)Radiation (d)Filtration
9. Enrichment technique was designed by _____.
   (a)Beijernick (b)Pasteur
   (c)Bacon (d)Hooke
10. The medium which doesn't contain particular essential nutrient is called as _____.
    (a)Enriched media (b)Minimal media
    (c)Molten agar (d)Selective media
12. 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.
13. The primary screening technique which is employed for a detect and isolating antibiotic producing strain is_____
    (a) Crowded plate technique (b) Auxanography
    (c) Enrichment culture technique (d) Use of indicator dye

14. The transfer of oxygen from an air bubble into solution is the _____ step in the oxygen transfer process.
    (a)First (b)Second
    (c)Third (d)Fourth
15. Critical dissolved oxygen concentrations for *E.coli* is _____ mM/dm^3
    (a)0.004 (b)0.008
    (c)0.022 (d)0.018
16. Antibiotics are produced by_____ method.
    (a)Submerged cultured (b)Disc diffusion
    (c)Fermentation (d)Tissue culture
17. Penicillin antibiotic is produced from
    (a)*Penicillium Sp* (b)*AspergillusSp*
    (c)*Fusarium Sp* (d)*Staphylococcus Sp*
18. In fermentation, yeast converts _____to_____and _____
    (a)Carbohydrate, alcohol, carbonic acid (b)Fatty acids, alcohol, carbonic acid
    (c)Sugar, alcohol, carbonic acid (d)Starch, alcohol, carbonic acid
19. In oil overlaying method paraffin oil is used in specific gravity of _____.
    (a)0.743 -0.780 (b)0.801-0.825
    (c)0.901-0.925 (d) 0.865-0.890
20. The process of sterilization of wine introduced by Pasteur is called_____.
    (a)Pasteurization (b)Ultrafiltration
    (c)LTHT (d)HTLT

PART-B **(3x 2=6 marks)**

(Answer all questions)

21. Define bioprocess engineering
22. Define batch cultivation
23. Define enzyme.

PART-C **(3x 8=24 marks)**

(Compulsory question)

24. a) Explain the different types of fermentation processes.

(Or)

b) Write a brief notes on aeration, agitation and body construction materials used in fermentor.

25. a) What is meant by scaling up of industrial process?

(Or)

b) Explain the mass transfer mechanism.

26. a) Discuss on the types of carbon sources in fermentation medium.

(Or)

b) Write about the growth kinetics of batch fermentation.

******* (All the Best) *******