Semester - II

I7MBP205C **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

SCOPE

It deals with the design and development of equipment and processes for the manufacturing of products such as agriculture, food, and pharmaceuticals. It also deals with studying various biotechnological processes used in industries for large scale production of biological product for optimization of yield in the end product and the quality of end product.

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.

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 - 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.

KARPAGAM ACADEMY OF HIGHER EDUCATION

(Deemed to be University Established Under Section 3 of UGC Act, 1956)
Coimbatore -641 021.

LECTURE PLAN
DEPARTMENT OF MICROBIOLOGY

STAFF NAME : Dr. P. SRINIVASAN
SUBJECT NAME: BIOPROCESS ENGINEERING SUB.CODE:17MBP205C
SEMESTER : II CLASS: I M.Sc (MB)

S. No	Duration	Topic to be covered	Reference
		UNIT-I	
1.	1	Design of a basic fermenter	T1:43-57
2.	1	Bioreactor configuration	T1: 56-69
3.	2	Design features	T1:97-104
4.	1	Computer control of fermentation process	T2:188-215
5.	1	Measurement and control of process	T2:188-215
6.	1	Reactors for specialized applications: Tube reactors	R1:765-773
7.	1	Packed bed reactors	R2:223-225
8.	1	Fluidized bed reactors	R2:375-395
9.	1	Cyclone reactors	R2:375-395
10.	1	Trickle flow reactors	R2:375-395
11.	1	Video presentation on working principle of fermentors	W1
12.	1	Class test I	
		Total Hours planned for Unit I	**13**
1.	2	Transport phenomena in fermentation: Gas- liquid exchange	R1: 85-88
2.	2	Mass transfer in fermentation	R2: 231-244
3.	2	Oxygen transfer and critical oxygen concentration in fermentation	R2: 231-244
4.	1	Heat transfer in fermentation	R2: 231-244
5.	1	Aeration/agitation, its importance.	R3:333-351
6.	1	Sterilization of Bioreactors, nutrients	R3:379-430
7.	2	Air supply, products and effluents	R3:823-830
8.	1	Process variables and control	R3:823-830
9.	1	Scale-up of bioreactors	R4:318-322
10.	2	Power point presentation on different types of bioreactors	W1
11.	1	Video presentation on fermentation and types	W1
12.	1	Class test I	
		Total Hours planned for Unit II	**17**

1.	1	Growth of cultures in the fermenter.	R1: 315-317
2.	1	Importance of media in fermentation.	R1: 315-317
3.	1	Media formulation and modification.	R1: 315-317
4.	1	Kinetics of growth in batch culture with respect to substrate utilization.	R1: 315-317
5.	1	Kinetics of growth in continuous culture with respect to substrate utilization.	R1: 315-317
6.	1	Steady state in a chemostat, fed-batch fermentation.	R2:24-41
7.	1	Steady state in a chemostat, fed-batch fermentation.	R2:24-41
8.	1	Yield of biomass, product, and calculation for productivity.	R2:24-41
9.	1	Storage of cultures for repeated fermentations	R2:24-41
10.	1	Scaling up of process form shake flask to industrial fermentation.	R2:24-41
11.	1	Power point presentation on growth kinetics in a fermentor	W1
12.	1	Video presentation on media formulation	W1
13.	1	Class test III	
		Total Hours planned for Unit III	**13**
1.	2	Biomass separation by centrifugation.	T1: 64-86
2.	2	Biomass separation by filtration, flocculation and other recent developments.	R2: 111-123
3.	2	Cell disintegration: Physical, chemical and enzymatic methods.	R2: 111-123
4.	1	Extraction: Solvent, two phase, liquid extraction, whole broth, aqueous multiphase extraction.	R2: 111-123
5.	1	Purification of products by different methods.	R3:445-450
6.	1	Concentration of products by precipitation	R3:445-450
7.	2	Concentration of products by ultra-filtration, reverse osmosis.	R3:452-460
8.	1	Drying and crystallization.	R3:438-563
9.	2	Power point presentation on down streaming process	W1
10.	1	Video presentation on different types of down streaming process	W1
11.	1	Class test IV	
		Total Hours planned for Unit IV	**16**
	1	Isolation of microbial cultures	R1: 9-50
	1	Selection of microbial cultures	R1: 9-50
	1	Strain improvement for the selected organism	R2: 71-73
	2	Use of recombinant DNA technology	R2: 71-73
	2	Protoplast fusion techniques for strain improvement.	R3:178-187
	1	Improvement of characters other than products and its	R3:178-187
	1	Application in the industrial important strains	R3:178-187

	2	Preservation of cultures after strain improvement programme	R3:178-187
	2	Preservation of cultures after strain improvement programme	R4:244-293
	1	Class test V	-
	2	Revision of previous year ESE question papers	-
		Total Hours planned for Unit V	**16**
Total planned hours	**75**		

TEXT BOOKS

T1: Kalaichelvan and Arulpandi, 2009. Bioprocess Technology, MJP Publishers.
T2: Umesh Kumar, 2014. Industrial Microbiology, MJP Publishers.

REFERENCE BOOK

R1: Doran, 2013. Bioprocess engineering principles, Academic Press.
R2: Peppler and Pearlman, 1979. Microbial Biotechnology. Academic Press.
R3: Umesh kumar, 2014. Industrial Microbiology, SBW Publishers.
R4: Wulf Cruegar & A. Cruegar, 2000. A Textbook of Industrial Microbiology, Panima Publishing Corp, Newdelhi.

WEBSITES

W1: www.youtube.com/basicworkingprincipleofafermentor/index.php.
W2: www.shomusbiology.com/index1.bioreactors.html
W3: www.shomusbiology.com/index1.bioreactorsandgrowthkinetics.html.
W4:www.youtube.com/industrialmicrobiologyprocess.index.php.

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

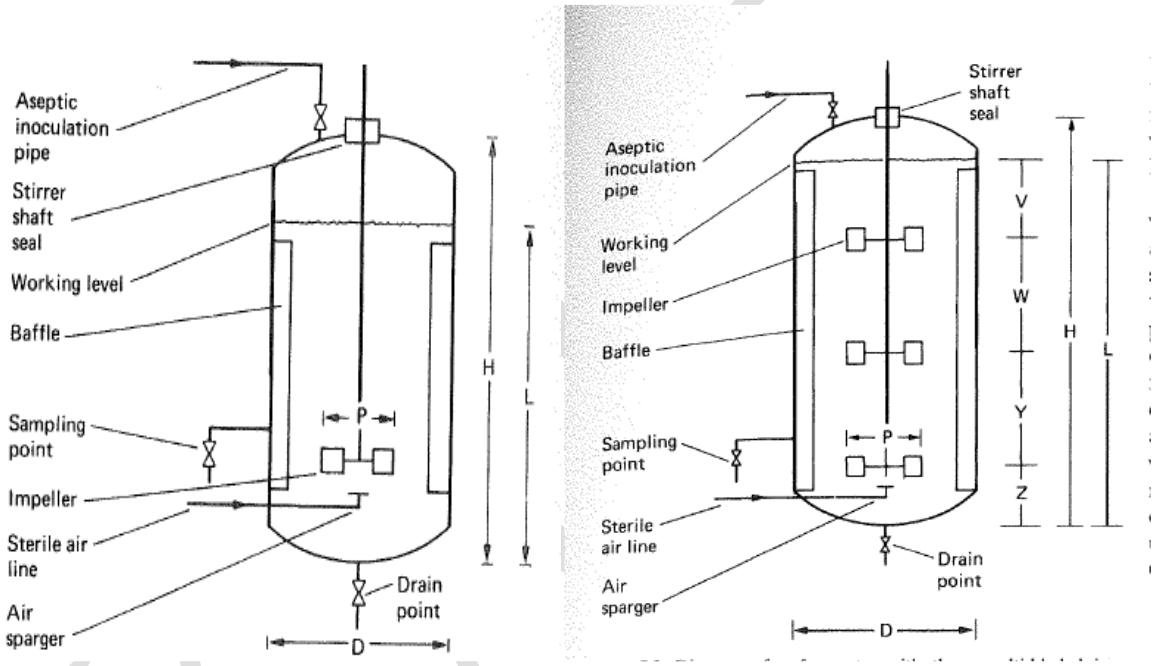
flange joints whenever possible.

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 forindividual 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, mechanicalengineering and costing. Although many different types of fermenter have been described in theliterature, 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.



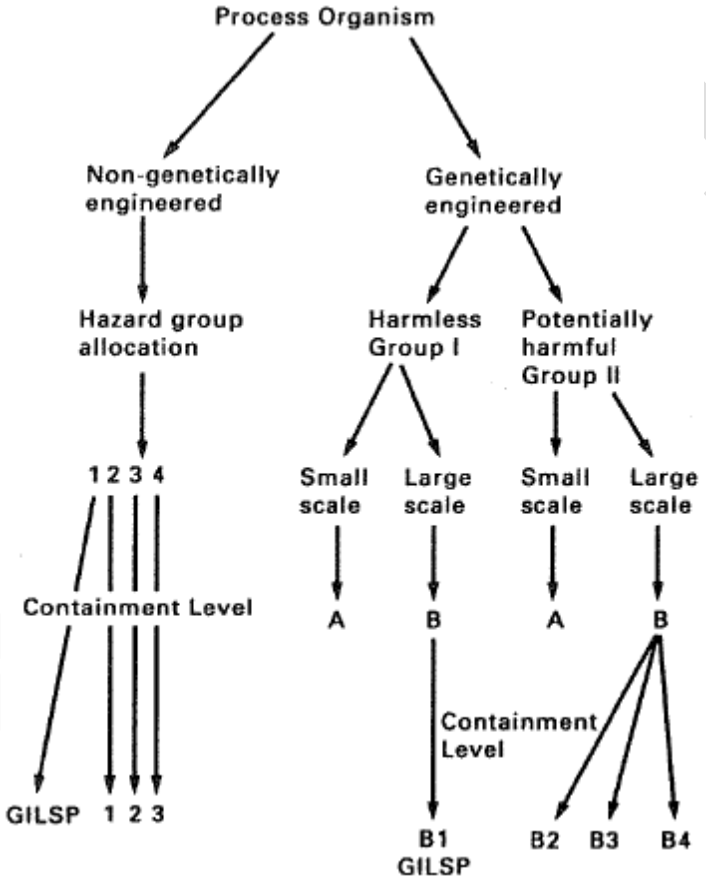
Schematics of a fermentor design (Single and multi bladed impellers)

ASEPTIC OPERATION AND CONTAINMENT

Aseptic operation involves protection against contaminationand it is a well-established and understoodconcept in the fermentation industries, whereas containment involves prevention of escape of viable cellsfrom a fermenter or downstream equipment and ismuch more recent in origin. Containment guidelineswere initiated during the 1970sTo establish the appropriate degree of containmentwhich 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 shouldthere be accidental release. Different assessmentprocedures are used depending on whether or not theorganism contains foreign DNA (genetically engineered). Once the hazards are assessed, an organismcan be classified into a hazard group for which there isan appropriate level of containment. The procedurewhich has been adopted within the European Community

is outlined. Non-genetically engineeredorganisms may be placed into a hazard group (1 to 4)using criteria to assess. risk such as those given byCollins (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 beobtained 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). Thereforelarge 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 nongeneticallyengineered 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

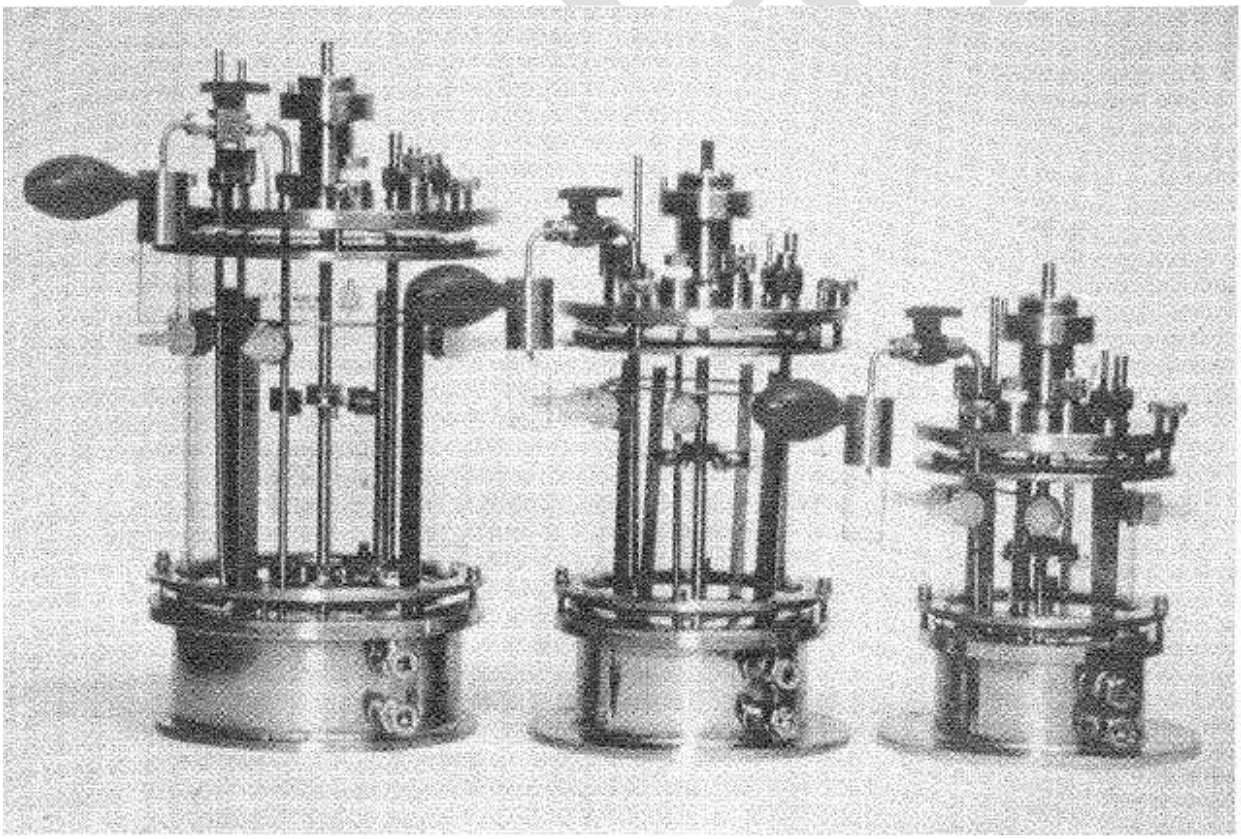
In fermentations with strict aseptic requirements itis important to select materials that can withstandrepeated steam sterilization cycles. On a small scale (1to 30 dm3) it is possible to use glass and/or stainlesssteel. Glass is useful because it gives smooth surfaces,is non-toxic, corrosion proof and it is usually easy toexamine the interior of the vessel. Two basic types offermenter 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 bottomplates. 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 thosewith just a top plate.

At pilot and large scale, when allfermenters are sterilized *in situ*, any materials used willhave to be assessed on their ability to withstand pressuresterilization and corrosion and on their potentialtoxicity and cost. Walker and Holdsworth (1958), Solomons(1969) and Cowan and Thomas (1988) have discussedthe suitability of various materials used in theconstruction of fermenters. Pilot-scale and industrialscale 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% chromiumare classified as steel alloys and those containing morethan 4% are classified as stainless steels. Mild steelcoated with glass or phenolic epoxy materials has occasionallybeen used.

Walker and Holdsworth (1958) stated that the extentof vessel corrosion varied considerably and did notappear to be entirely predictable. Athough stainlesssteel is often quoted as the only satisfactory material, ithas been reported that mild-steel vessels were verysatisfactory after 12-years use for penicillin fermentations(Walker and Holdsworth, 1958) and mild steelclad 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-steelfermenter 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 differentmanufacturing process treatments such as rolling, picklingor heat treatment. The film is stabilized bychromium 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 fermentel'there must be adequate provision for temperaturecontrol which will affect the design of the vesselbody. Heat will be produced by microbial activity andmechanical agitation and if the heat generated by thesetwo processes is not ideal for the particular manufacturingprocess then heat may have to be added to, orremoved from, the system. On a laboratory scale littleheat is normally generated and extra heat has to beprovided by placing the fermenter in a thermostaticallycontrolled bath, or by the use of internal heating coilsor a heating jacket through which water is circulated orby a silicone heating jacket. The silicone jacket consistsof a double silicone rubber mat with heating wiresbetween the two mats; it is wrapped around the vesseland held in place by Velcro strips (Applikon, 1989).

Once a certain size has been exceeded, the surfacearea covered by the jacket becomes too small to removethe heat produced by the fermentation. Whenthis situation occurs internal coils must be used andcold water is circulated to achieve the correct temperatureJackson, 1990). Different types of fermentationwill influence the maximum size of vessel that can beused with jackets alone.

AERATION AND AGITATION

Theprimary purpose of aeration is to provide microorganismsin submerged culture with sufficient oxygenfor metabolic requirements, while agitation should ensurethat a uniform suspension of microbial cells isachieved 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 brothslow viscosity and low total solids are used (ArnoldSteel, 1958). Thus, mechanical agitation is usually acquired in fungal and actinomyceteNon-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 requireenergy 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.

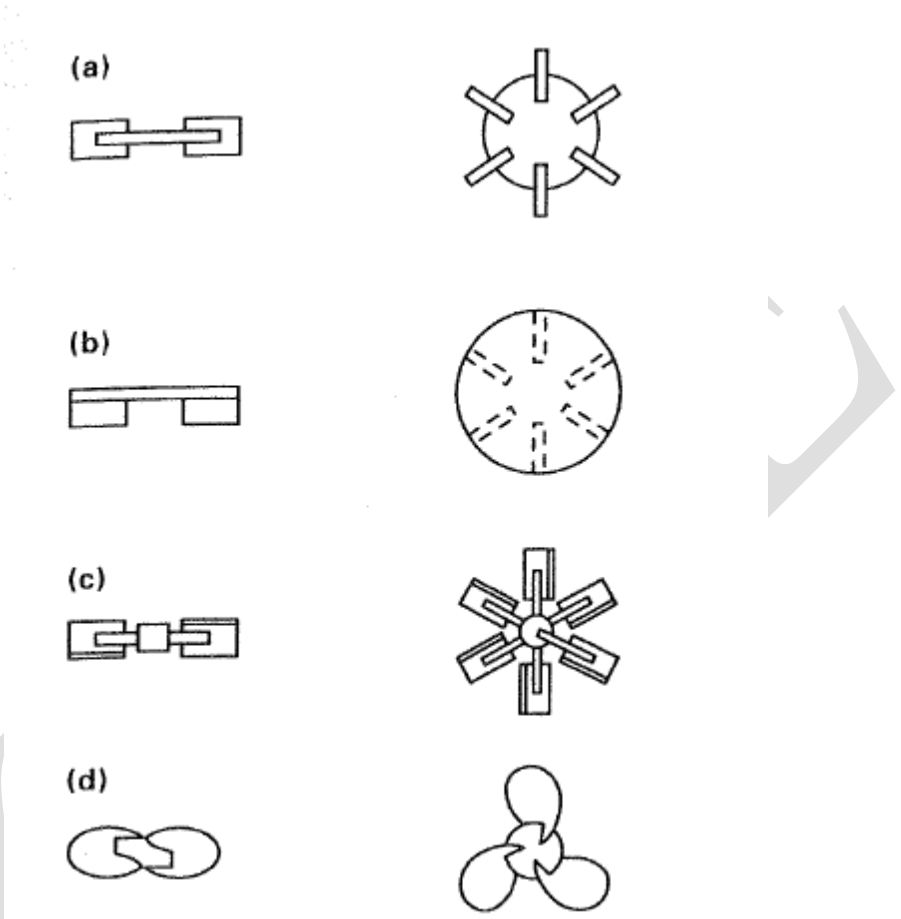
(d) The aeration system (sparger).

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 antifoamaddition.

Agitators may be classified as disc turbines, vaneddiscs, open turbines of variable pitch and propellersThe 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 verticallyto the underside. Air from the sparger hits theunderside of the disc and is displaced

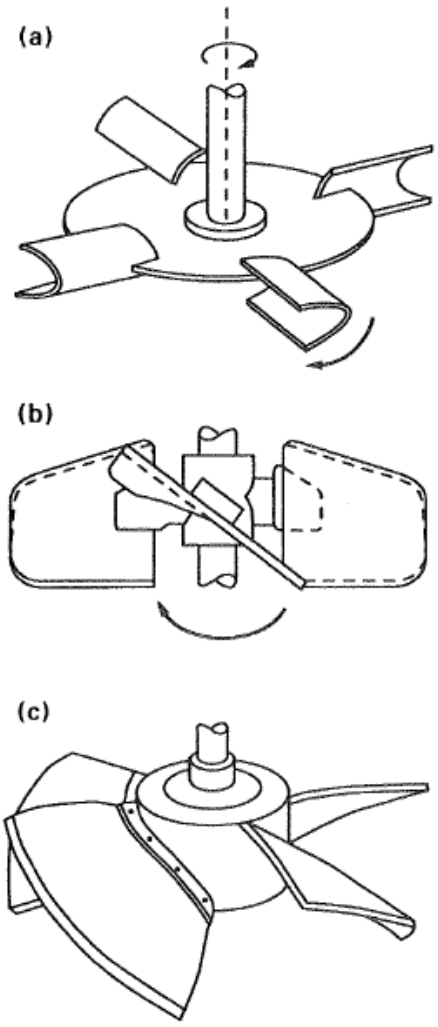
towards thevanes where the air bubbles are broken up into smallerubbles. The vanes of a variable pitch open turbine andthe blades of a marine propeller are attached directlyto a boss on the agitator shaft. In this case the airbubbles do not initially hit any surface before dispersionby the vanes or blades.



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 annulusThe 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 ratherthan radial (Cooper *et at.,* 1944). The disc turbine wasthought to be essential for forcing the sparged air intothe agitator tip zone where bubble break up wouldoccur.



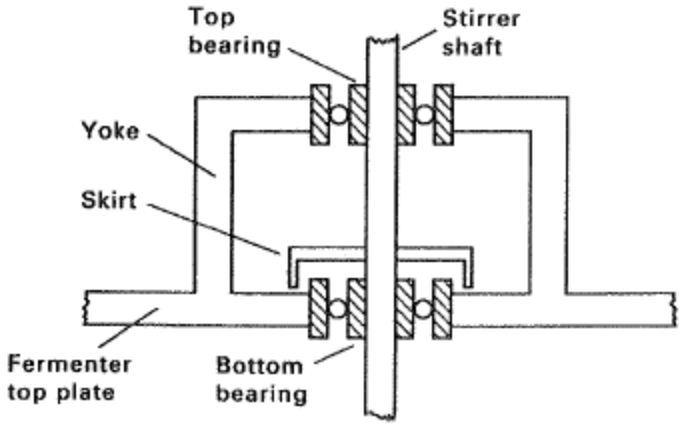
Different types of Agitator (a) Scaba agitator; (b) Lightnin' A315agitator (four blades) and (c) Prochem Maxflo T agitator

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 andprovided the agitator speed is high enough, good gasdispersion will occur in low-viscosity broths (Smith,1985). It has been also shown that similar oxygen-transferefficiencies are obtained at the same power inputper unit volume, regardless of the agitator type.

STIRRER GLANDS AND BEARINGS

The satisfactory sealing of the stirrer shaft assembly top platehas been one of the most difficult problems to overcomein the construction of fermentation equipmentwhich can be operated aseptically for long periods. Anumber of different designs have been developed toobtain aseptic seals. The stirrer shaft can enter thevessel from the top, side (Richards, 1968) or bottom ofthe vessel. Top entry is most commonly used, butbottom entry may be advantageous if more space isneeded on the top plate for entry ports, and the shortershaft permits higher stirrer speeds to be used by eliminatingthe problem of the shaft whipping at high speeds.Originally, bottom entry stirrers were considered undesirableas the bearings would be submerged. Chain *etal.* (1952) successfully operated vessels of this type, andthey have since been used by many other workers.Mechanical seals can be used for bottom entry providedthat 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 agitatedvessels of all sizes to prevent a vortex and to improveaeration efficiency. In vessels over 3-dm3 diameter sixor eight baffles may be used (Scragg, 1991). Baffles aremetal strips roughly one-tenth of the vessel diameterand attached radially to the wall. The agitation effect is onlyslightly increased with wider baffles, but drops

sharplywith narrower baffles (Winkler, 1990). Walker andHoldsworth (1958) recommended that baffles should beinstalled 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 coolingcoils may be attached to baffles to improve the coolingcapacity of a fermenter without unduly affecting thegeometry.

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

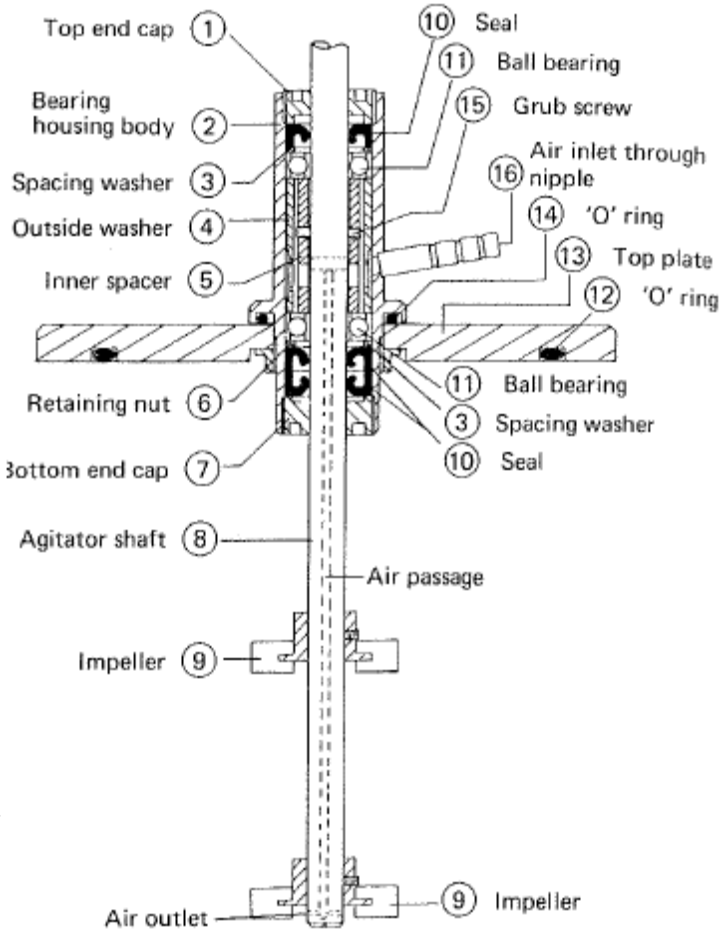
The porous sparger of sintered glass, ceramics ormetal, has been used primarily on a laboratory scale innon-agitated vessels. The bubble size produced fromsuch spargers is always 10 to 100 times larger than thepore size of the aerator block (Finn, 1954). Thethroughput of air is low because of the pressure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

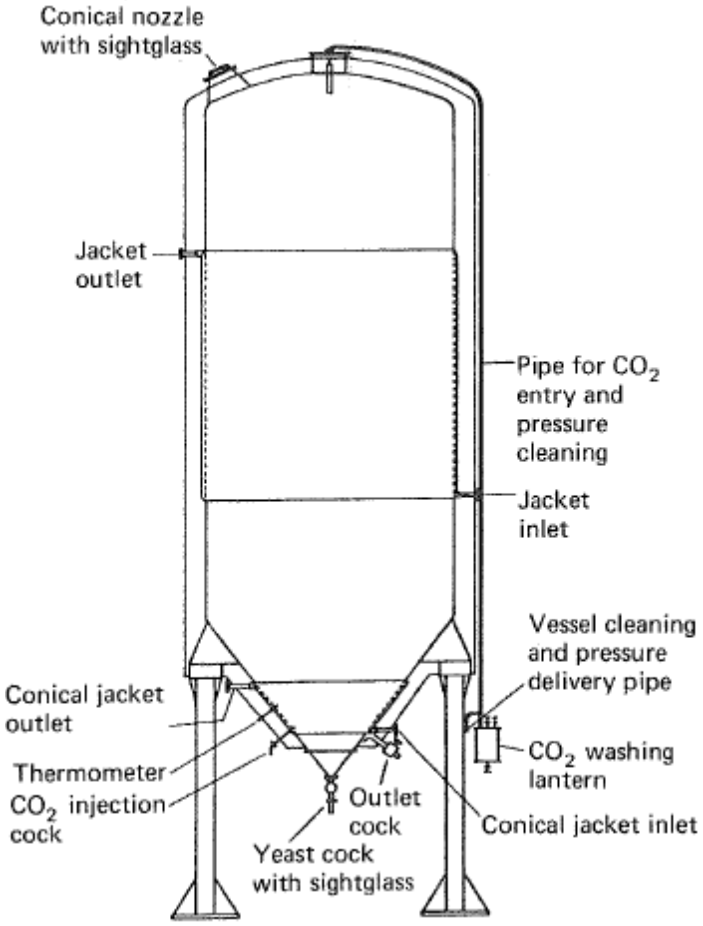
The investigations on yeast growth in SUlphite wasteliquor in Germany, Japan and the United States ofAmerica led to the development of the Waldhof-typefermenter (Inskeep *et al.*, 1951; Watanabe, 1976).Inskeep *et al.* (1951) have given a description of aproduction vessel based on a modification of the 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.

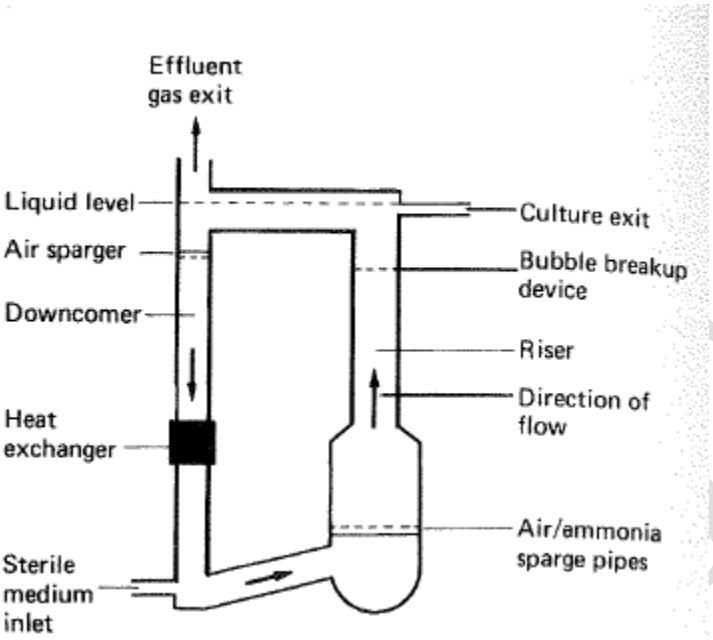


Tower Fermenter

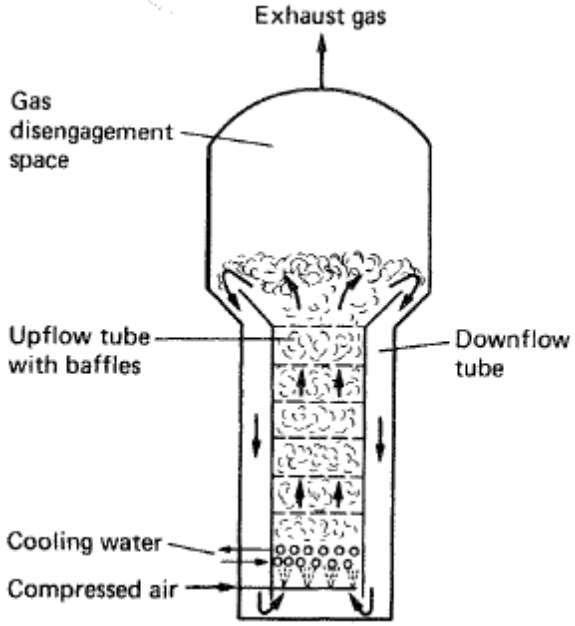
Air-lift fermenters

An air-lift fermenter is essentially a gastightbaffled 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 were chosen.

development work foroperational 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. inJapan.



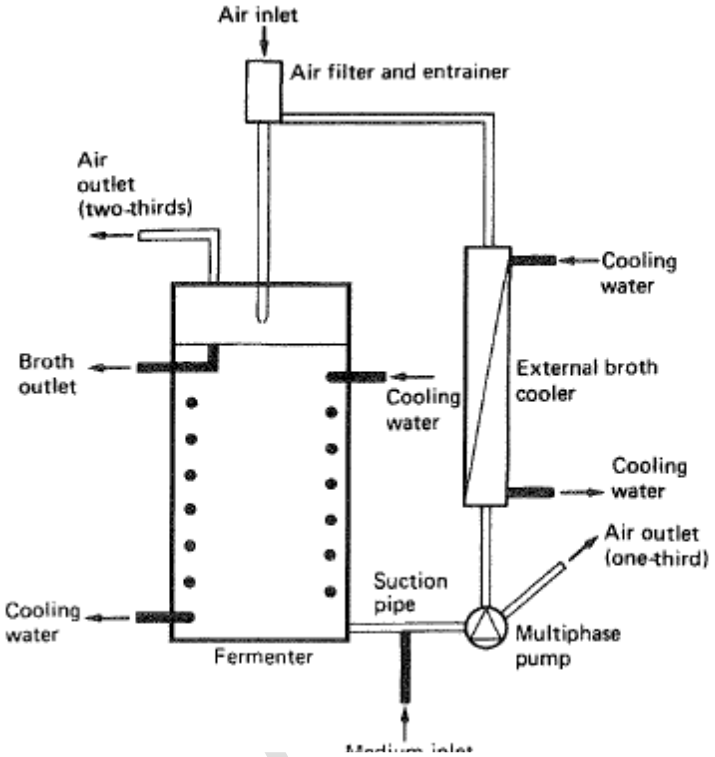
Air-lift fermenter with outer loop



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.



e Fir

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.

INSTUMENTATION AND CONTROL

The success of a fermentation depends upon the existenceof defined environmental conditions for biomassand product formation. To achieve this goal it is importantto understand what is happening to a fermentationprocess and how to control it to obtain optimal operatingconditions. Thus, temperature, pH, degree of agitation,oxygen concentration in the medium and otherfactors may have to be kept constant during the process.The provision of such conditions requires careful monitoring(data acquisition and analysis) of the fermentationso that any deviation from the specified optimummight be corrected by a control system. Criteria whichare monitored frequently are listed in Table, alongwith the control processes with which they are associated.As well as aiding the maintenance of constantconditions, the monitoring of a process may provideinformation on the progress of the fermentation. Suchinformation may indicate the optimum time to harvestor that the fermentation is progressing abnormallywhich may be indicative of contamination or straindegeneration. Thus, monitoring equipment producesinformation indicating fermentation progress as well asbeing linked to a suitable control system.In initial studies the number of functions which areto be controlled may be restricted in order to gainmore knowledge about a particular fermentation. Thus,the pH may be measured and recorded but not maintainedat a specified pH or the dissolved oxygen concentrationmay be determined but no attempt will bemade to prevent oxygen depletion.Also, it is important to consider the need for asensor and its associated control system to interfacewith a computer (to be discussed in a later section).This chapter will consider the general types of controlsystems which are available, specific monitoring andcontrol systems and the role of computers. It is apparent from the Table that a considerable number of process variables may need to be monitoredduring a fermentation. Methods for measuring thesevariables, 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 thefermenter, e.g. pH electrodes, dissolved-oxygenelectrodes.

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 thefermentation broth or gases, e.g. tachometers,load cells.It is also possible to characterize a sensor in relation toits application for process control:

1. *In-line sensor.* The sensor is an integrated part ofthe fermentation equipment and the measuredvalue obtained from it is used directly for processcontrol.

2. On-line sensor. Although the sensor is an integralpart of the fermentation equipment, the measuredvalue cannot be used directly for control.

Table: Process sensors and their possible control functions

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

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

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

When evaluating sensors to use in measurement andcontrol 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 Question

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.

DEPARTMENT OF MICROBIOLOGY, KARPAGAM ACADEMY OF HIGHER EDUCATION
BIOPROCESS ENGINEERING- 17MBP205C
UNIT I

Microbes are grown in especially designed vessels called----------, containing special media for its growth
Fermentation tank should be provided with ports for ---------- addition.
----------are used in side of fermentors to avoid vortex formation
)----------tanks are used in production of all and lactic acid fermentation
The ----------in stainless steel fermentor gives resistance to halogen salts, lodine and sea water
-----are used to regulate the addition of medium,nutrients, defoamers.
The fermentor vessel ----------should be increased with scale.
-------between top plate and vessel is very important to maintain airtight / aseptic condition.
---------device is used for giving air into fermentor
----------type of bubbles facilitate high oxygen transfer than ----------bubbles
The ideal aspect ratio for a fermentor is
The number of baffles used in fermentor of diameter 3dm3 is
)----------spargers are used widely large scale fermentation process
---------removes enough moisture from the gas leaving fermentor and prevent excess fluid loss.
In high quality bioreactor, all the processes in fermentation are controlled by
In-line, on-line and off-line are types of ----------
Example of In-line sensors are
----------sensors don't form integral part of fermentor.
)----------is generated due to mixing by agitator and microbes action on substrates during fermentation
--------are semiconductors of Iron, Michel oxides exhibiting large change in resistance with small change c
Stainless steel ---------- sensors are used for temperature measurements in fermentation system
Gas flow rate is measured by ----------
The liquid flow rate is measured by using
Which gauge is used for measuring pressure under aseptic condition
-------is used for measuring the speed of agitator
Peristaltic pump is mainly used for addition of ---------and--------
Dissolved oxygen in fermentation process medium measured by using ----------
If initially foam has started forming interior ----------has been used to control foam
Oxygen diffuses from tubing into medium is measured by
pH denotes the presence of ----------in aqueous solution
The voltage difference between two electrodes is used to determine ----------of unknown solution
----------,----------and---------- are three distinct areas of computer function.
----------system controls the addition of liquid from reservoir to fermentator
Computers were employed in fermentation early ----------
Computers were initially restricted in fermentation industry because of
The computer functions in fermentation process were postulated by ----------in 1972
The signals produced during fermentation process is converted to ----------form.
------serves as junction point for inputs from computers and output signals from computer to fermentor cor
The ----------signal from fermentors are not understood by the computer.
The interface converts the ----------signal to ----------
The accuracy of computer control depends upon the number of ----------it sends to the computer

For variables which are not measureable, concept of ----------are used in fermentation industry
In fermentation, it is very important to find the ----------or---------- of product from the given carbon
---------and----------developed methods to analyse the biomass and product concentration during ferment
The capacity of the batch fermentors _____
Large fermentors range from _____
Clogging problems occur in _____
Sparger size ranges from
The first pilot fermenter was erected in India at Hindustan Antibiotic ltd, Pune in the --------- year.
-------- type of bioreactor is used for vinegar production.
---------- is used for sep and other algal protein production.
---------- is used as a enzyme bioreactor.
---------- are provided to maintain constant temperature inside the bioreactor
The impeller should be ---------- of the vessel diameter.
Range of fermentation tank used in enzyme production.
-------- fermenter is called as elongated non-mechanically stirred fermenter
_____ fermentor is a gas tight baffled rise tube connected to a down comer tube.
Multiple air lift fermenter Is designed by
The inoculum level introduced into a production tank is usually _____.
-------- fermenter is called as elongated non-mechanically stirred fermenter
_____ fermentor is a gas tight baffled rise tube connected to a down comer tube.

Option A

Fermentors
contamination
spargers
stainless steel
chromium
syringe pumps
diameter
baffles
sparger
larger, smaller
03:01
2 TO 4
porous
baffles
agitator
foamers
Ion-specific sensors
in-line
energy
mercury in-glass thermometers
Pt 100
thermometers
thermometers
peristaltic
wattmeter
medium and inoculums
galvanic electrode
antifoamers
galvanic electrode
hydrogen ion
temperature
logging of process data
analog control
1940
high cost
Neeri
analogue
addition reservoir
voltage
analogue to digital
units

indirect sensors
gases or distribution
Hump and Honey
10 – 12 litre
2000 – 5,000 gallons
Bacteria
1/64 – 1/32 inch
1920
packed tower
packed tower
packed tower
baffles
1\1
1500 30,000
Tower
Tower
Bakker etal
0.5-5%
Tower
Tower

Option B

Batch cookers
medium
bearing glands
glass
molybdenum
peristaltic pumps
thickness
sealing
baffles
smaller, larger
04:01
4 TO6
orifice
heat exchange
)aeration
agitator glands
mass spectrophotometer
on-line
heat
electrical resistance
Pl 100
rotameters
thermal mass flowmeter
diaphragm
torsion dynamometer
acid and base
pH electrode
mechanical foam breaker
paramagnetic gas analyzer
hydroxyl ion
pH
data analysis
direct control
1950
complexity
Nyili
digital
interface
current
digital to analogue
alarms

gateway sensors
transport or energy
Humphery and Cooney
12 – 15 litre
5000 – 10,000 gallons
Algae
1/32 – 1/18 inch
1930
photo bioreactor
photo bioreactor
photo bioreactor
cooling coils
none of the above
1000 – 30,000 c
Airlift
Air lift
Okabe etal
5 – 25%
Airlift
Air lift

Option C

swap medium
inoculums
rotameter
copper
nickel
feed pumps
height
sparger
shaft
medium, large
05:01
6 TO 8
nozzle
cooler
process controller
shaft
antifoam probe
off-line
resistance
thermistors
Pb 100
pistonmeters
pistonmeters
diaguls

buffers and antifoamers
thermometers
water
platinum electrode
carboxyl ions
moisture
process control
direct digital control
1960
reduction of man power
Needham
data
tele-type
pulse
voltage to pulse
bits

by pass sensors
productivity or conversion yield
Hughes and Humphery
20 -40 litre
10,000 gallons
Mycebial
1/48 – 1/32 inch
1940
pulsed column
pulsed column
pulsed column
stirrer gland
1\4
gallons
Cylindraconical
Cylinder conical
Bacon etal
20 – 40%
Cylindraconical
Cylinder conical

Option D

conical flasks
foam
baffles
wooden
tungsten
pressure pumps
design
clamp
bearings box
very small, very medium
06:01
8 TO 10
combined sparger agitator
exit gas cooler
cooler
sensors
medium addition probe
fermentor
current
electrical impedance
Ps 100
torsion dynamometer
torsion dynamometer
bourbon tube
tachometer
salts and growth factors
thermistors
controlling agitator/aeration speed
thermistors
carbonyl ion
dissolved oxygen
all
human control
1970
high power
Natel
process
virtual display unit
meter
pulse to voltage
data

direct sensors
concentration or consumable
Hyhes and Cooney
6 – 8 litre
none of the above.
Mycorhiza
1/24 – 1/12 inch
1950
bubble column
bubble column
bubble column
sparger
1\3
none of the above
Deep jet
Deep jet
Dawsa
50%
Deep jet
Deep jet

Answer Key

Fermentors
inoculums
baffles
wooden
molybdenum
feed pumps
thickness
sealing
sparger
smaller, larger
05:01
6 TO 8
nozzle
exit gas cooler
process controller
sensors
antifoam probe
off-line
heat
thermistors
Pt 100
rotameters
thermal mass flowmeter
diaphragm
all
acid and base
galvanic electrode
mechanical foam breaker
paramagnetic gas analyzer
hydrogen ion
pH
all
direct digital control
1960
high cost
Nyili
analogue
interface
voltage
analogue to digital
bits

gateway sensors
productivity or conversion yield
Humphery and Cooney
10 – 12 litre
2000 – 5,000 gallons
Bacteria
1/64 – 1/32 inch
1950
packed tower
photo bioreactor
pulsed column
cooling coils
1\3
1500 30,000
Tower
Air lift
Bakker etal
5 – 25%
Tower
Air lift

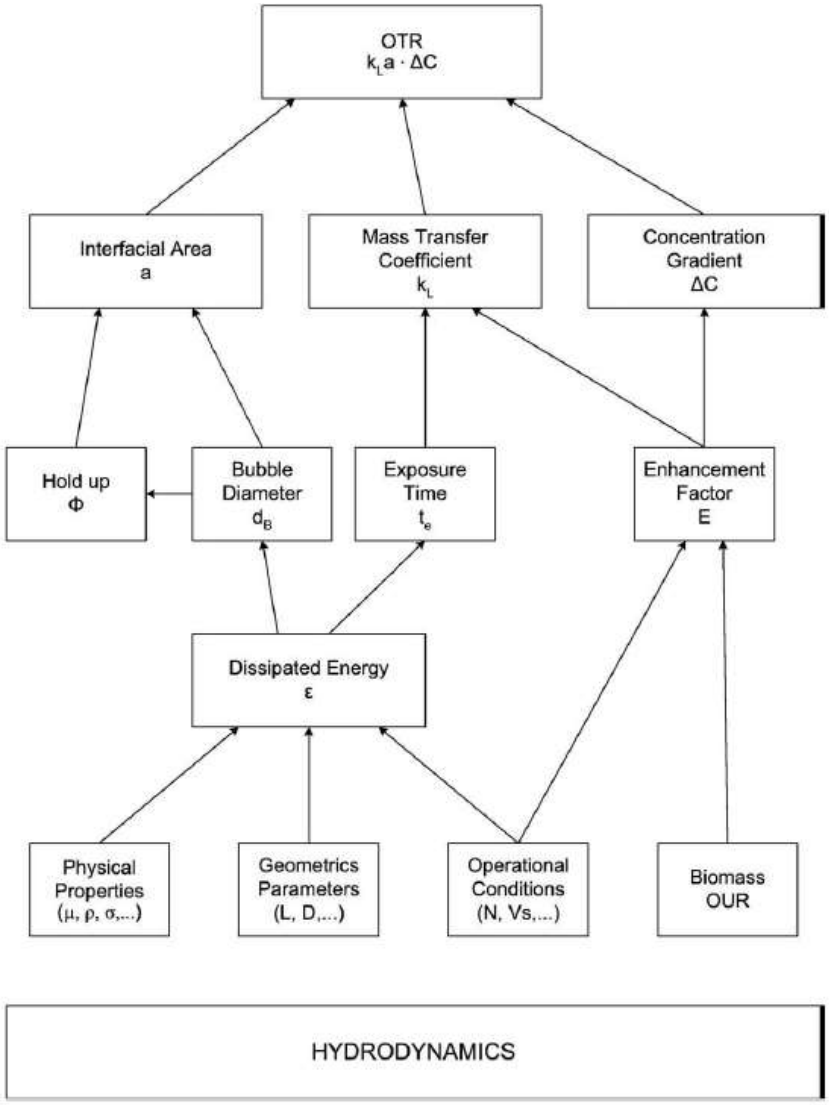
Unit II

Most industrial microbial processes are aerobic, and are mostly carried out in aqueous medium containing salts and organic subtances;usually these broths are viscous, showing a non-Newtonian behavior. 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 $kLa \cdot C^*$, 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 ofsubstrates to cells occurs at a rate considerably higher than the rate ofthe metabolic biochemical reactions; in this case, the overall rate ofsubstrate conversion is governed only by the kinetics of thebiochemical reactions. However, if mass transfer rate is lower thanreaction rate, transport rate can be the step controlling the overallprocess rate and, moreover, the mass transfer ratemay be influenced bythe chemical rate of the bioprocess. When a species in gas phase isabsorbed into a liquid and reacts there, the

concentration profiles of theabsorbed species change due to the chemical reaction and the absorption rate may be enhance). Oxygen absorption intoa fermentation broth can be considered as the absorption of a gas into aliquid where it reacts, oxygen is consumed by the suspended microorganism,and therefore an enhancement of oxygen mass transferrate can take place. The increase of the specificgas absorption rate per driving force unit and per interfacial areaunit, 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 _ ðpG−piÞ = kL _ ðCi−CLÞ ð1Þ

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⋆

= KL C⋆−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.

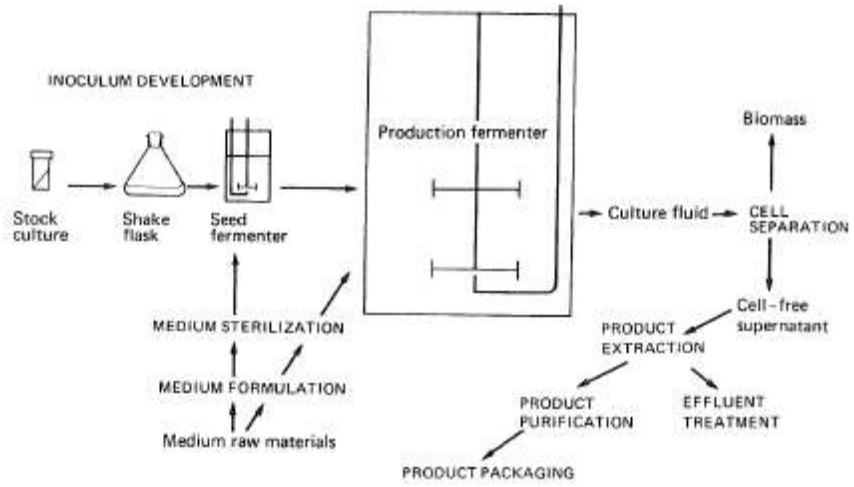
(iii) The production of an active, pure culture in sufficient quantity to inoculate the production

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 ofcertain 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 thenthe contaminant may 'outgrow' the productionorganism and displace it from the fermentation.

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

(iv) The contaminant may produce compoundswhich make subsequent extraction of the finalproduct difficult.

(v) The contaminant may degrade the desiredproduct; this is common in bacterial contaminationof antibiotic fermentations where thecontaminant would have to be resistant to thenormal inhibitory effects of the antibiotic anddegradation of the antibiotic is a common resistancemechanism, e.g. the degradation of *f3*lactam antibiotics by f3-lactamase-producingbacteria.

(vi) Contamination of a bacterial fermentation withphage 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 thefermentation during the process.

(v) Maintaining aseptic conditions during the fermentation.

The extent to which these procedures are adopted isdetermined by the likely probability of contaminationand the nature of its consequences. Some fermentationsare described as 'protected' - that is, the mediummay be utilized by only a very limited range of microorganisms,or the growth of the process organism mayresult in the development of selective growth conditions,such as a reduction in pH. The brewing of beerfalls into this category; hop resins tend to inhibit thegrowth of many micro-organisms and the growth ofbrewing yeasts tends to decrease the pH of the medium.Thus, brewing worts are boiled, but not necessarilysterilized, and the fermenters are thoroughly cleanedwith disinfectant solution but are not necessarily sterile.Also, the precautions used in the development ofinoculum for brewing are far less stringent than, forexample, in an antibiotic fermentation. However, thevast majority of fermentations are not 'protected' and,if contaminated, would suffer some of the consequencespreviously listed.

MEDIUM STERILIZATION

Media may besterilized by filtration, radiation, ultrasonic treatment,chemical treatment or heat. However, for practicalreasons, steam is used almost universally for the sterilizationof fermentation media. The major exception isthe use of filtration for the sterilization of media foranimal-cell culture - such media are completely solubleand contain heat labile components making filtrationthe method of choice. Filtration techniques will beconsidered later in this

chapter. Before the techniques _f\J,_which are used for the steam sterilization of culture Nomedia are discussed it is necessary to discuss the kineticsof sterilization. The destruction of micro-organismsby steam (moist heat) may be described as a first-orderchemical reaction and, thus, may be represented by thefollowing 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:

$$N_t/N_0 = e^{-kt}$$

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

Nt is the number of viable organisms presentafter a treatment period, *t.*

On taking natural logarithms, equation is reducedto:

$$\ln (N_t/N_0) = -kt$$

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 exponentiallyover the treatment period. A plot of the naturallogarithm of *N,.INo* against time yields a straight line,the slope of which equals - *k.* This kinetic descriptionmakes 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 oneviable cell present.

Thus, in this context, a value of *Nt* of less than one isconsidered in terms of the probability of an organismsurviving the treatment. For example, if it were pre-dieted that a particular treatment period reduced thepopulation to 0.1 of a viable organism, this implies thatthe probability of one organism surviving the treatmentis one in ten. This may be better expressed in practicalterms as a risk of one batch in ten becoming contaminated.This aspect of contamination will be onsideredlater.The relationship displayed in Fig. 5.1 would beobserved only with the sterilization of a pure culture inone physiological form, under ideal sterilization conditions.

The value of *k* is not only species dependent, butdependent on the physiological form of the cell; forexample, the endospores of the genus *Bacillus* are farmore heat resistant than the vegetative cells. Richards(1968) produced a series of graphs illustrating the deviationfrom theory which may be experienced in practice.Figures 5.2a, 5.2b and 5.2c illustrate the effect ofthe time of eat treatment on the survival of a populationof bacterial endospores. The deviation from animmediate exponential decline in viable spore numberis due to the heat activation of the spores, hat is theinduction of spore germination by the heat and moistureof the initial period of the sterilization process. InFig. 5.2a the activation of spores is significantly morethan their destruction during the early stages of theprocess and, therefore, viable numbers increase beforethe observation of exponential decline. In Fig. 5.2bactivation is balanced by spore death and in Fig. 5.2cactivation is less than spore death.

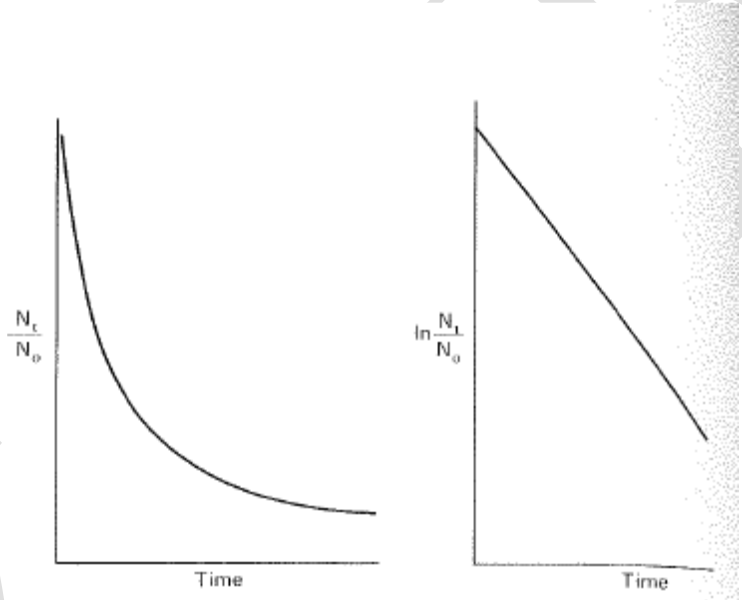


FIG. 5.1. Plots of the proportion of survivors and the natural logarithm of the proportion of survivors in a population of micro-organisms subjected to a lethal temperature over a time period.

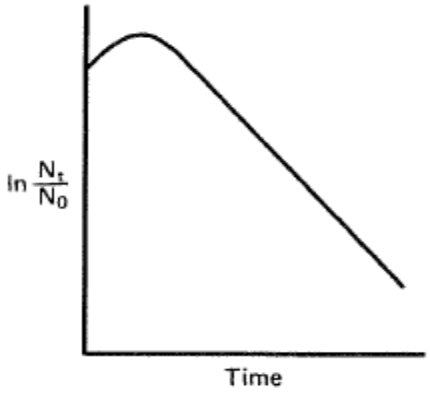


FIG. 5.2a. Initial population increase resulting from the heat activation of spores in the early stages of a sterilization process (Richards, 1968).

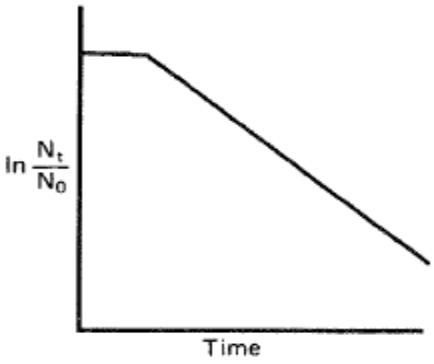


FIG. 5.2b. An initial stationary period observed during a sterilization treatment due to the death of spores being completly compensated by the heat activation of spores (Richards, 1968).

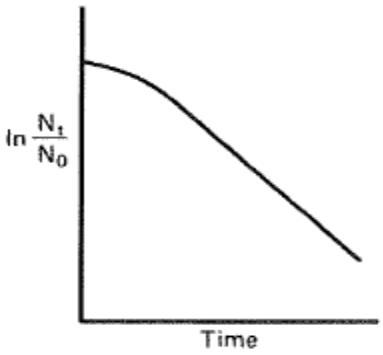


FIG. 5.2c. Initial population decline at a sub-maximum rate during a sterilization treatment due to the death of spores being compensated by the heat activation of spores (Richards, 1968).

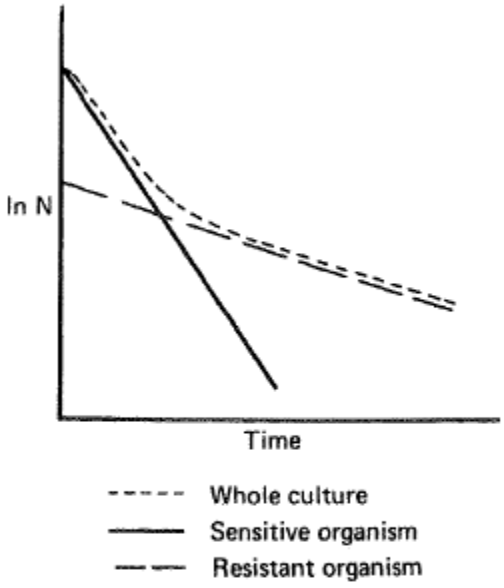


FIG. 5.3a. The effect of a sterilization treatment on a mixed culture consisting of a high proportion of a very sensitive organism (Richards, 1968).

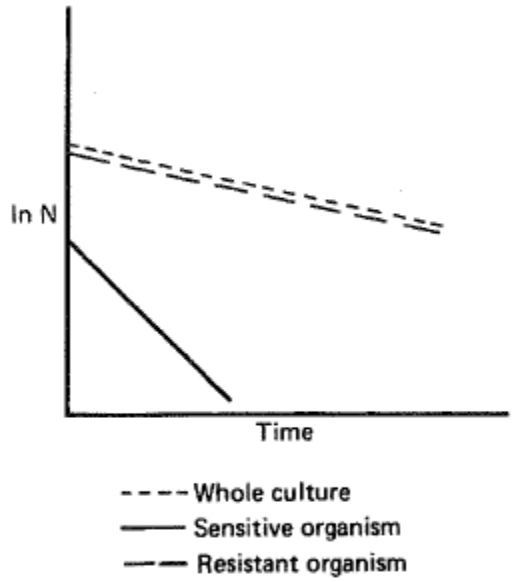


FIG. 5.3b. The effect of a sterilization treatment on a mixed culture consisting of a high proportion of a relatively resistant organism (Richards, 1968).

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?

DEPARTMENT OF MICROBIOLOGY, KARPAGAM ACADEMY OF HIGHER EDUCATION
BIOPROCESS ENGINEERING- 17MBP205C
UNIT I
UNIT II

-------method is the most preferred method compared to other agents for mass sterilization.
----------is the least expensive and reliable method for fermentor design and operation
Sterilization is necessary in fermentation as it may
The methods for sterilization involves both ----------and----------
Sterilization also help in ----------and----------of complex substrates used in fermentation.
Sterilization has disadvantage of destruction of heat ---------compounds in medium.
There is always a ----------relationship between time and temperature in sterilization of production mediu
During sterilization of fermentor tanks, it is very important to sterilize the----------attached to it
Ancillary equipments in fermentors means the ----------
The sterilization temperature of the fermentation equirement is
After sterilization, all parts of fermentor are kept sterile by maintaining at ----------pressure
There should be no permanent direct connection below ----------and----------parts of the fementor syster
High quality valves such as----------should be used where joints are needed connecting to fermentor
Sugar containing medium can't be sterilized by prolonged heating because sugars undergo
-----media gives lower yields and poor growth of microorganisms in fermentation
In continous sterilization, the fermentation medium is passed through ----------aiding in sterilizati
----------are simplest method employed for sterilizing production medium.
Jackets are used around fermentors to aid in ----------and-------of production media.
In ----------, the medium to be sterilized to maintained at particular temperature and time.
By steam injection method the high temperature steam is passed into the production medium where holdin
Continuous sterilization is highly advantageous
----------media require very long sterilization time period.
The technique of air filtration in fermentation industries was developed by----------
The technique developed by Bourdillon et al was studied by ----------and----------
In air filtration technique slab of slag wool used was of --------inches thick,--------lb/ft3 thick and less than
Cobalt increases the growth of _____.
Temperature of _____ in the production tank is satisfactory during fermentation.
Stabilization of mask is practiced by reducing the pH and adding reducing agents _____
Sterilization of air is done by passing it through columns filled with _____
_____ are the antifoam agents used to suppress the foam formation.
Mass transfer occurs in mixtures containing ------
Mass is transferred from one location to another under the influence of a ---------- in the system.
Concentration of oxygen at the surface of air bubbles is----- compared with the rest of the fluid,
(---------- is the movement of component molecules in a mixture under the influence of a concent
(----------occurs in the direction required to destroy the concentration difference.
According to ---------- molecules A will diffuse away from the region of high concentration until eve
According to ----------, mass flux is proportional to the concentration gradient.
The only mechanism for intra particle mass transfer is ----------
The ----------is a useful model for mass transfer between phase.
The -------- of solute from one phase to another involves transport from bulk of one phase to the interface,

The majority of fermentation processes are --------

The solubility of oxygen in pure water is -------at $4^{\circ}C$

The solubility of sucrose in pure water is -------at $4^{\circ}C$

The---------- of an industrial fermentation process is normally satisfied by aerating and agitating the ferm

The productivity of many fermentations is limited by ----------.

which law describes the solubility of O_2 in nutrient solution in relation to the O_2 partial pressure in the gas

The effect of dissolved oxygen concentration on the specific oxygen uptake rate follows the --------- type

The specific oxygen uptake rate increases with increase in the dissolved oxygen concentration up to a certa

Critical dissolved oxygen concentrations for *E.coli* is ------mMoles/dm3

Critical dissolved oxygen concentrations for *Saccharomyces* sp is ------mMoles/dm3

Critical dissolved oxygen concentrations for *Pencillium chrysogenum* is ------mMoles/dm3

At ---------- cultures may be aerated by means of the *shake-flask technique* where the culture is grown in

In ---------- fermentations broth or culture is aerated by *stirrers or agitators.*

In ---------- bioreactor are so designed that adequate supply of oxygen is obtained without agitation

In ---------- bioreactor are so designed that adequate supply of oxygen is obtained without agitation

In 1950, ---------- represented the transfer of oxygen from air to the cell, during a fermentation, as occurri

The transfer of oxygen from an air bubble into solution is the -----step in the oxygen transfer process

The transfer of the dissolved oxygen through the fermentation medium to the microbial cell is the -----step

The uptake of the dissolved oxygen by the cell is the -----step in the oxygen transfer process.

Maximum ----------production may be achieved by satisfying the organism's maximum specific oxygen

Critical dissolved oxygen concentrations for *Azotobacter sp* is ------mMoles/dm3

Option A
Option A
steam
chemicals
contaminants are not affected
destruction and encourages microbes
breakdown and solubility
sensitive
linear
raw material
seed tank
120°C – 15 min
positive
medium and air
plastic
reaction with contaminants
cooled
heat exchange
continous fermentors
batch and continuous sterilization
heat exchange
1-3 minutes
saves production time and plant space
synthetic
Bourbon et al
and Cherry
3,15,4
Streptomyes olivaceus
75° F
Sodium citrate
Activated charcoal
Soyabean oil
local concentration variation
concentration gradient
high
Molecular diffusion
Molecular diffusion
Diffusion theory
Diffusion theory
passive diffusion
single film theory
Mass transfer

aerobic
2 mg/L
200 g/L
Mass transfer
Mass transfer
Ford's law
Diffusion theory
specific oxygen concentration
0.004
0.004
0.004
pilot scale
Pilot- and industrial-scale
bubble columns
AIR LIFT
Bartholomew et al
first
first
first
waste
0.004

Option B
Option B
UV light
passing of fire flames
affect the yield of fermentation products
destruction and removal of microbes
buildup and toxicity
resistant
inverse
vitamins
fermentation medium
120°C – 20 min
negative
sterile and non-sterile
β-hydroxybutyrate
charring
overcooled
holding coil
batch cookers
maintaining the pH and foam
holding coil
2-4 minutes
poor quality of medium
complex
Bourdillon et al
Terjesen and Cherry
3,16,6
S. griseus
80° F
Sodium sulphite
Dry charcoal
Cord-linee oil
same concentration
concentration defecient
low
passive diffusion
passive diffusion
passive diffusion
Molecular diffusion
molecular diffusion
two film theory
liquid transfer

anaerobic
8 mg/L
400 g/L
oxygen demand
nitrogen availability
Henry's Law
Henry's Law
critical oxygen concentration
0.008
0.008
0.008
laboratory-scale
pilot scale
CSTR
CSTR
Belquiren et al
second
second
second
biomass
0.008

Option C
Option C
ethidium bromide
moist heat
not remove pathogens
alters pH and growth inactivation
precipitation and agglomeration
soluble
non-linear
ancillary equipments
extra connection
115°C – 15min
no
mixing and air
silica gel
caramelization
undercooled
cooler
filtration
heating and cooling
cooler
1-5 minutes
high steam costs
cruder
Billy et al
Stanbury and Cherry
3,17,6
S. oryzae
85° F
Ammonium sulphate
Liquid paraffin
Palm oil
different solute
concentration reference
very low
active diffusion
active diffusion
active diffusion
Fick’s Law of diffusion
microbial diffusion
no film theory
oxygen transfer

facultative anaerobic
4mg/L
600 g/L
nutrient availibility
oxygen availability
Raman's law
Michaelis-Menten
microbial oxygen concentration
0.022
0.022
0.022
industrial scale
industrial scale
PACKED BED
PACKED BED
Barbitol et al
third
third
third
toxic
0.022

Option D
Option D
chlorine
dry heat
value the fermentation
removes and microbes growth
charring and precipitation
insoluble
reverse
valves
antifoamers
115°C – 20min
zero
probes and medium
rubber
reaction with phosphates
contaminant
heat exchange, holding coil and cooler
radiation
circulation of air and steering
heat exchange, holding coil and cooler
2-5 minutes
high sterilizing temperature and longer holding time
semi-synthetic
Cherey et al
Wahsman and Cherry
3,17,7
P. notatum
63° F
Sodium thiosulphate
Melted charcoal
Cedar-wood oil
different solvent
concentration base
medium
microbial diffusion
microbial diffusion
Molecular diffusion
Molecular diffusion
active diffusion
multiple film theory
ion transfer

microaerophilic
1 mg/L
800 g/L
nitrogen availability
oxygen demand
Libert's law
film theory
integrated oxygen concentration
0.018
0.018
0.018
semi-industrial scale
laboratory-scale
FLUIDISED BED
FLUIDISED BED
Batingulo et al
fourth
fourth
fourth
fourth
0.018

Answer Key
Answer Key
steam
moist heat
affect the yield of fermentation products
destruction and removal of microbes
breakdown and solubility
sensitive
linear
ancillary equipments
extra connection
120°C – 20 min
positive
sterile and non-sterile
rubber
caramelization
overcooled
heat exchange, holding coil and cooler
batch cookers
heating and cooling
holding coil
1-5 minutes
saves production time and plant space
cruder
Bourdillon et al
Terjesen and Cherry
3,17,6
Streptomyes olivaceus
80° F
Sodium sulphite
Activated charcoal
Soyabean oil
local concentration variation
concentration gradient
high
Molecular diffusion
Molecular diffusion
Diffusion theory
Fick's Law of diffusion
molecular diffusion
two film theory
Mass transfer

aerobic
8 mg/L
600 g/L
oxygen demand
oxygen availability
Henry's Law
Michaelis-Menten
critical oxygen concentration
0.008
0.004
0.022
laboratory-scale
Pilot- and industrial-scale
bubble columns
AIR LIFT
Bartholomew et al
first
second
third
biomass
0.018

Unit III

MEDIA FOR INDUSTRIAL FERMENTATIONS

All micro-organisms require water,sources of energy, carbon, nitrogen, mineral elementsand possibly vitamins plus oxygen if aerobic. On a smallscale it is relatively simple to devise a medium containingpure compounds, but the resulting medium, althoughsupporting satisfactory growth, may be unsuitablefor use in a large scale process.On a large scale one must normally use sources ofnutrients to create a medium which will meet as manyas 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 undesiredproducts.

5. It will be of a consistent quality and be readilyavailable throughout the year.

6. It will cause minimal problems during mediamaking and sterilization.

7. It will cause minimal problems in other aspectsof the production process particularly aerationand agitation, extraction, purification and wastetreatment.

The use of cane molasses, beet molasses, cerealgrains, starch, glucose, sucrose and lactose as carbonsources, and ammonium salts, urea, nitrates, com steepliquor, soya bean meal, slaughter-house waste and fermentationresidues as nitrogen sources, have tended tomeet most of the above criteria for production mediabecause they are cheap substrates. However, othermore expensive pure substrates may be chosen if theoverall cost of the complete process can be reducedbecause it is possible to use simpler procedures.

It must be remembered that the medium selectedwill affect the design of fermenter to be used. Forexample, the decision to use methanol and ammonia inthe single cell protein process developed by ICI picnecessitated the design of a novel fermenter design. The microbialoxidation of hydrocarbons is a highly aerobic and exothermicprocess. Thus, the production fermenter hadto have a very high oxygen transfer capacity coupledwith excellent cooling facilities. ICI pic solved theseproblems by developing an air lift fermenter. Equally, if a fermenter is already available thiswill obviously influence the composition of the medium. The optimum concentrationsof available nitrogen for griseofulvin productionshowed some variation with the type of fermenterused.

The problem of developing a process from thelaboratory to the pilot scale, and subsequently to theindustrial scale, must also be considered. A laboratorymedium may not be ideal in a large fermenter with alow gas-transfer pattern. A medium with a high viscositywill also need a higher power input for effectivestirring. Besides meeting requirements for growth andproduct formation, the medium may also influence pHvariation, foam formation, the oxidation-reduction potential,and the morphological form of the organism. Itmay also be necessary to provide precursors ormetabolic inhibitors. The medium will also affectproduct recovery and effluent treatment.Historically, undefined complex natural materialshave been used in fermentation processes because theyare much cheaper than pure substrates. However, thereis often considerable batch variation because of variableconcentrations of the component parts and impuritiesin natural materials which cause unpredictablebiomass and/or product yields. As a consequence ofthese variations in composition small yield improvementsare difficult to detect. Undefined media oftenmake product recovery and effluent treatment moreproblematical because not all the components of acomplex nutrient source will be consumed by the organism.

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^{-3}
$FeSO_4 \cdot 7H_2O$	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

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

Somenutrients are frequently added in substantial excess ofthat required, e.g. P, K; however, others are often nearlimiting values, e.g. Zn, Cu. The concentration of P isdeliberately raised in many media to increase the bufferingcapacity. These points emphasize the need forconsiderable attention to be given to medium design.Some micro-organisms cannot synthesize specificnutrients, e.g. amino acids, vitamins or nucleotides.Once a specific growth factor has been identified it canbe incorporated into a medium in adequate amounts asa pure compound or as a component of a complexmixture.

The carbon substrate has a dual role in biosynthesis and energy generation. The carbon requirement forbiomass production under aerobic conditions may beestimated from the cellular yield coefficient *(Y)* whichis defined as:

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

An adequate supply of the carbon source isessential for a product-forming fermentation process.In a critical study, analyses are made to determinethe observed conversion of the carbon source toproduct compares with the theoretical maximum yield.This may be difficult because of limited knowledge ofthe biosynthetic pathways. Theoretical yields for penicillin G biosynthesis onthe basis of material and energy balances using abiosynthetic pathway based on reaction stoichiometry.The other major nutrient which will be required isoxygen which is provided by aerating the culture. Thedesign of a medium will influence the oxygen demandof a culture in that the more reduced carbon sourceswill result in a higher oxygen demand. The amount ofoxygen required may be determined stoichiometrically.

WATER

Water is the major component of all fermentationmedia, and is needed in many of the ancillary servicessuch as heating, cooling, cleaning and rinsing. Cleanwater of consistent composition is therefore required inlarge quantities from reliable permanent sources. Whenassessing the suitability of a water supply it is importantto consider pH, dissolved salts and effluent contamination.The mineral content of the water is very importantin brewing, and most critical in the mashing process,and historically influenced the siting of breweries andthe types of beer produced. Hard waters containinghigh CaS04 concentrations are better for the EnglishBurton bitter beers and Pilsen type lagers, while waterswith a high carbonate content are better for the darkerbeers such as stouts. Nowadays, the water may betreated by deionization or other techniques and saltsadded, or the pH adjusted, to favour different beers sothat breweries are not so dependent on the local watersource.

ENERGY SOURCES

Energy for growth comes from either the oxidationof medium components or from light. Most industrialmicro-organisms are chemo-organotrophs, thereforethe commonest source of energy will be the carbonsource such as carbohydrates, lipids and proteins. Somemicro-organisms can also use hydrocarbons or methanolas carbon and energy sources.

CARBON SOURCES

Factors influencing the choice of carbon sourceIt is now recognized that the rate at which thecarbon source is metabolized can often influence theformation of biomass or production of primary or secondarymetabolites. Fast growth due to high concentrationsof 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 regulationmight be overcome by genetic modification of the producerorganism

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 alboniger*	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	Basek and Majumdar (1973)
Novobiocin	*S. niveus*	Citrate	Kominek (1972)
Siomycin	*S. sioyaensis*	Glucose	Kimura (1967)

The main product of a fermentation process willoften determine the choice of carbon source, particularlyif the product results from the direct dissimilationof it. In fermentations such as ethanol or single-cellprotein production where raw materials are 60 to 77%of the production cost, the selling price of the productwill be determined largely by the cost of the carbon source. It is oftenpart of a company development programme to test arange of alternative carbon sources to determine theyield of product and its influence on the process andthe cost of producing biomass and/or metabolite. Thisenables a company to use alternative substrates, dependingon price and availability in different locations,and remain competitive.The purity of the carbon source may also affect thechoice of substrate. For example, metallic ions must beremoved from carbohydrate sources used in some citricacid processes.

The method of media preparation, particularly sterilization,may affect the suitability of carbohydrates forindividual fermentation processes. It is often best tosterilize sugars separately because they may react withammonium ions and amino acids to form black nitrogen containing compounds which will partially inhibitthe growth of many micro-organisms. Starch suffersfrom the handicap that when heated in the sterilizationprocess it gelatinizes, giving rise to very viscous liquids,so that only concentrations of up to 2% can be usedwithout modification.

The choice of substrate may also be influenced bygovernment legislation. Within the European EconomicCommunity (EEC), the use of beet sugar and molassesis encouraged, and the minimum price controlled. Thequantity of imported cane sugar and molasses is carefullymonitored and their imported prices set so thatthey will not be competitive with beet sugar. If theworld market sugar price is very low then the EECfermentation industry will be at a disadvantage unlessit receives realistic subsidies.

EXAMPLES OF COMMONLY USED CARBON SOURCES

CARBOHYDRATES

It is common practice to use carbohydrates as thecarbon source in microbial fermentation processes. Themost widely available carbohydrate is starch obtainedfrom maize grain. It is also obtained from other cereals,potatoes and cassava. Maize and other cereals may also be useddirectly in a partially ground state, e.g. maize chips.Starch may also be readily hydrolysed by dilute acidsand enzymes to give a variety of glucose preparations(solids and syrups). Hydrolysed cassava starch is usedas a major carbon source for glutamic acid production in Japan. Syrups produced by acidhydrolysis may also contain toxic products which maymake them unsuitable for particular processes.Barley grains may be partially germinated and heattreated to give the material known as malt, whichcontains 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 veryimpure form as beet or cane molasses which are the residues left after crystallization of sugarsolutions in sugar refining. Molasses is used in theproduction of high-volumeflow-value products such asethanol, SCP, organic and amino acids and some microbialgums.The use of lactose and crude lactose (milk wheypowder) in media formulations is now extremely limitedsince the introduction of continuous-feeding processes utilizing glucose.

Corn steep liquor is a by-product afterstarch extraction from maize. Although primarily usedas a nitrogen source, it does contain lactic acid, smallamounts of reducing sugars and complex polysaccharides.Certain other materials of plant origin, usuallyincluded as nitrogen sources, such as soyabean mealand Pharmamedia, contain small but significantamounts 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.) mayalso be used as carbon substrates, particularly for theircontent of the fatty acids, oleic, linoleic and linolenicacid, 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 chosensubstrate in the intervening period.A typical oil containsapproximately 2.4 times the energy of glucose ona per weight basis. Oils also have a volume advantageas 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 beingadded as 50% w/w solutions. Ideally, in any fermentationprocess, the maximum working capacity of a vesselshould be used. Oil based fed-batch fermentationspermit this procedure to operate more successfullythan those using carbohydrate feeds where a largerspare capacity must be catered for to allow for responsesto a

sudden reduction in the residual nutrient level. Oils also have antifoam propertieswhich may make downstream processing simpler, butnormally 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-alkanesfor production of organic acids, amino acids,vitamins and co-factors, nucleic acids, antibiotics, enzymesand proteins.Methane,methanol and n-alkanes have all been used as substratesfor biomass production.On a weight basis n-alkanes have approximatelytwice the carbon and three times the energycontent of the same weight of sugar. Althoughpetroleum-type products are initially impure they canbe refined to obtain very pure products in bulk quantitieswhich would reduce the amount of effluenttreatment and downstream processing. At this time theview was also held that hydrocarbons would not besubject to the same fluctuations in cost as agriculturallyderived feedstocks because it would be a stable pricedcommodity and might be used to provide a substrate.

NITROGEN SOURCES

Most industrially used micro-organisms can utilizeinorganic or organic sources of nitrogen. Inorganicnitrogen may be supplied as ammonia gas, ammonium salts or nitrates. Ammonia has beenused for pH control and as the major nitrogen sourcein a defined medium for the commercial production ofhuman serum albumin by *Saccharomyces ceriuisiae*. Ammonium salts such as ammoniumsulphate will usually produce acid conditions as theammonium ion is utilized and the free acid will beliberated. On the other hand nitrates will normallycause an alkaline drift as they are metabolized. Ammoniumnitrate will first cause an acid drift as theammonium ion is utilized, and nitrate assimilation isrepressed.Organic nitrogen may be supplied as amino acid,protein or urea. In many instances growth will be fasterwith a supply of organic nitrogen, and a few microorganismshave an absolute requirement for aminoacids. It might be thought that the main industrial needfor pure amino acids would be in the deliberate additionto amino acid requiring mutants used in aminoacid production. However, amino acids are more commonlyadded as complex organic nitrogen sources whichare non-homogeneous, cheaper and readily available.In lysine production, methionine and threonine areobtained from soybean hydrolysate since it would betoo 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 toammonium ion, is repressed in the presence of ammonia. For this reason ammonia orammonium ion is the preferred nitrogen source. Infungi that have been investigated, ammonium ion repressesuptake of amino acids by general and specificamino acid permeases. In *Aspergillusnidulans,* ammonia also regulates the production ofalkaline 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 essentialcomponents, 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 usuallypresent as impurities in other major ingredients. Thereis obviously a need for batch analysis of media componentsto ensure that this assumption can be justified,otherwise there may be deficiencies or excesses indifferent batches of media.As a consequence of product compositionanalysis, as outlined earlier in this chapter, it ispossible to estimate the amount of a specific mineralfor medium design, e.g. sulphur in penicillins andcephalosporins, 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 metalformed, containing all the iron and most of the calcium, manganese and zinc present in the medium.The problem of insoluble metal phosphate(s) mayeliminated by incorporating low concentrationschelating agents such as ethylene diaminetetraaceticacid (EDTA), citric acid,

polyphosphates, etc., intomedium. These chelating agents preferentiallycomplexes with the metal ions in a medium. Theions then may be gradually utilized by the organism. The precipitate was eliminatedfrom Mandel and Weber's medium by the additionEDTA at 25 mg dm-3. It is important to check that achelating agent does not cause inhibition of growththe micro-organism which is being cultured.In many media, particularly those commonly used inlarge scale processes, there may not be a need to add achelating agent as complex ingredients such as yeastextracts or proteose peptones will complex with metalions and ensure gradual release of them during growth.

GROWTH FACTORS

Some micro-organisms cannot synthesize a full complementof cell components and therefore require preformedcompounds called growth factors. The growthfactors most commonly required are vitamins, but theremay also be a need for specific amino acids, fatty acidsor sterols. Many of the natural carbon and nitrogensources used in media formulations contain all or someof 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 occasionallymore economical to add the pure vitamin, instead ofusing a larger bulk of a cheaper multiple vitamin source. Calcium pantothenate has been used in one mediumformulation for vinegar production. Inprocesses 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.

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BIOPROCESS ENGINEERING- 17MBP205C
UNIT III

In Pencillin fementation the precursor added is ----------
Buffering agents used in media formulation is
Foaming during fermentation process creates
The antifoaming agent used in pencillin fermentation is
The citric acid fermentation *Aspergillus niger* culture is grown at ----------pH values prevent contamina
.----------media is mainly used in fermentation process.
Which is the common raw material source used in fermentation process
----------is rich in biotin, panthothenic acid , thiamine, phorphorus and sulphur.
In Beet molasses ----------is limiting compared to cane molasses
----------require biotin for growth in production.
In India there is very large utilization of cane blackstrap molasses in ----------industry
The ----------contain 17% sugar, 1%acid and 0.3%ash
In grapes the nitrogen content should be ----------as it may result in underisable fermentation.
----------is 6.6-7.1% in cheddar whey
Cheese whey is an important raw material in ---------production
.----------and----------are the main sources of starch
Wheat, maize and rice are example of ----------
Cellulose are carbohydrates made of repeating units of --------
In ----------industry the digestion process of wood with calcium bisulfate under heat and pressure to give
Sulfite waste liquor contains ----------sugars
Wood molasses syrup contain ------of fermentable sugars
The untreated cellulosic wastes have been used in production of
During manufacture of starch, gluten from corn---------is formed by steeping of corn.
The clean, yellow, fine powder prepared from embryo of cotton seed is called as ----------
Pharmamedia is used as production media for ---------production.
Initially fermentation industries used corn steep liquor for -------production.
Soya bean meal is used as production medium for ----------
Vegetable oils are used as
ATCC is ---------- founded in 1925
The specific gravity of oil used in presence of cultures is
.----------is the simplest and common method of maintaining microbial cultures.
The Drying-up of medium encourages good ---------of *Streptomyces sp*
.----------and----------are usually maintained in liquid medium
The mineral oil overlay method was firstused by Bwell and Weston in ----------
Temperature of liquid nitrogen is
The first commercial production of lactic acid in the US was in _____
Lyophilization is the most satisfactory method of long term preservation of microorganisms
---------- is perhaps the most popular form of suspended metabolism.
Hwang in ---------- year recommends precooling to 7 degree Celsius.
---------- stressed the importance of the elimination of air and moisture from lyophilized cultures prior t
The catabolism of sugars is an --------- process

The largest mechanical stirred fermentation vessels developed during stage 3 were in the range of _____

----------culture where a portion of the culture is harvested at regular intervals and replaced by an equal vo

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

-------- is a culture system which contain a initial limited amount of nutrient

_____ properties are temperature, concentration, pressure and specific heat.

_____ properties are mass, volume, entropy and energy.

----------is performed in order to mix the three phases with in the fermenter

----------phase dissolved nutrients and metabolism are present

In --------- phase o2 and co2 are present

In --------- phase cells and solid substrates are present

Transfer in to liquid from the gaseous phase is enhanced by --------

----------culture is a open system where fresh medium is continuously added

Aim of ATCC is to_____

Oil should be autoclaved at _____

Lyophilization refers to _____

Dessicant used in lyophilizer are

_____ is used in storage at very low temp

The soil culture tubes are kept in refrigerator at above _____ temperature

De Becze and Liebmann in the year _____ used the first large scale fermentor for the

phenyl acetic acid
mono and dihydropotassium phosphates
oxidation
lard oil
low
synthetic
food waste
cane molasses
biotin
Bacteria
textile
rust
high
total solids
lactic acid
molasses and cereals
molasses
α-glucose
cheese, cheese whey
1%
60-80%
ethanol
sulfite waste liquor
corn-steep liquor
penicillin
mushroom
penicillin
animal feed
American type culture collection
0.821-0.860
serial subculture
growth
Bacteriophages and *Actinomycetes*
1945
-130˚C
1881
long term
overlaying cultures with mineral oil
1966
Meryman
Oxidative

75000-80000dm3
Fed batch
Batch
Fed batch
Intensive
Intensive
Aeration
Gaseous
Gaseous
Gaseous
Agitation
Batch
Prevent contamination
15 lb/in^2 for 2 hr
Short term preservation of microorganisms
Phosphorus
glycerol
5-8^0 C
1941

benzyl alcohol
triiodosodium phosphates
reduction
lard oil with ocetadecanol
high
semi-synthetic
agricultural waste
beet molasses
pyridoxine
yeasts
animal fodder
just
medium
protein
aspartic acid
cheese whey and tubers
cereals
β-glucose
starch, starch liquor
2%
65-85%
single cell protein
corn steep liquor
soya bean meal
streptomycin
penicillin
streptomycin
antifoams
African type culture collection
)0.865-0.890
lyophilisation
sporulation
Bacteriophages and *clostridium*
1947
-150˚C
1882
short term
lyophilization
1969
Dewald
Non oxidative

80,000-150,000dm3
Batch
Fed batch
Batch
Extensive
Extensive
Agitation
Liquid
Liquid
Solid
Aeration
Continuous
To maintain antibiotics
30 lb/in^2 for 2 hr
long term preservation of Microorganisms
silica gel
mineral oil
4-6^0 C
1944

Benzene
Tetraiodiosodium phosphates
contamination
decanol
medium
non-synthetic
industrial toxic waste
fruit molasses
thiamine
viruses
alcohol
must
low
lactose
glutamic acid
cereals, roots and tubers
roots
α-galactose
paper pulp, sulfite waste liquor
3%
70-90%
fuel
wood molasses syrup
Pharmamedia
tetracycline
vitamin
tetracycline
mushroom production media
Auxenic type culture collection
0.752-0.812
cryopreservation
storage
Viruses and *Acetobacter*
1949
-176˚C
1880
both a and b
nitrogen storage
1974
leogetring
Reoxidised

150,000-175,000dm3
Semi continuous
Continuous
Semi continuous
Physical
Physical
Reaction
Solid
Aeration
Liquid
Fermentation
Fed batch
Preserve cultures
45 lb/in^2 for 2 hr
killing of Microorganisms
Magnesium
Paraffin wax
$2\text{-}4^0$ C
1948

pyridine
monophosphates
production
mustard oil with decanol
very high
differential
Biofuel waste
cheese molasses
pantothenic acid
phages
dyes
bust
no
fat
citric acid
cereals and cornsteep liquor
tubers
β-galactose
wood, molasses
4%
75-95%
vitamins
distillers soluble
distiller’s soluble
griseofulvin
organic acid
griseofulvin
pH adjustment
Australian type culture collection
0.718-0.835
dessication
collection
Bacteriophages and *Streptomyces*
1950
-196˚C
1883
none of the above
none of the above
1980
Louis Pasteur.
Deoxidation

10,000-50,000dm3
Continuous
Semi continuous
Continuous
Chemical
Chemical
Fermentation
Semisolid
Agitation
Semisolid
Precipitation
Airlift
None of the above
60 lb/in^2 for 2 hr
None of the above
Copper
All the above
6-8^0 C
1940

phenyl acetic acid
mono and dihydropotassium phosphates
contamination
lard oil with ocetadecanol
low
non-synthetic
agricultural waste
cane molasses
biotin
yeasts
alcohol
must
low
total solids
lactic acid
cereals, roots and tubers
cereals
β-glucose
paper pulp, sulfite waste liquor
2%
65-85%
single cell protein
corn steep liquor
Pharmamedia
tetracycline
penicillin
streptomycin
antifoams
American type culture collection
0.865-0.890
serial subculture
sporulation
Bacteriophages and *clostridium*
1947
-196˚C
1881
long term
lyophilization
1966
Dewald
Oxidative

80,000-150,000dm3
Semi continuous
Fed batch
Batch
Intensive
Extensive
Agitation
Liquid
Gaseous
Solid
Agitation
Continuous
Preserve cultures
15 lb/in^2 for 2 hr
long term preservation of Microorganisms
silica gel
glycerol
5-8^0 C
1944

Unit IV

DOWNSTREAMING PROCESS

The choice of recovery process is based on thefollowing criteria:

1. The intracellular or extracellular location of theproduct.

2. The concentration of the product in the fermentationbroth.

3. The physical and chemical properties of the desiredproduct (as an aid to selecting separationprocedures).

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 recoveryof an extracellular product is the removal of large solidparticles and microbial cells usually by centrifugationor filtration. In the next stage, the broth isfractionated or extracted into major fractions usingultrafiltration, reverse osmosis, adsorption/ion-exchange/gel filtration or affinity chromatography, liquid-liquid extraction, two phase aqueous extraction orprecipitation. Afterwards, the product-containing fractionis purified by fractional precipitation, further moreprecise chromatographic techniques and crystallizationto obtain a product which is highly concentrated andessentially free from impurities. Other products areisolated using modifications of this flow-stream.Attempts to simplify this outline extraction procedurefor antibiotic recovery using 'whole broth' processinghave met with limited success. The technique of'whole broth' processing involves initial removal oflarge particles, which is then followed by passage of thebroth (including cells) through, for example, well mixedion-exchange columns or counter-current liquid-liquidextraction units to extract the product directly.

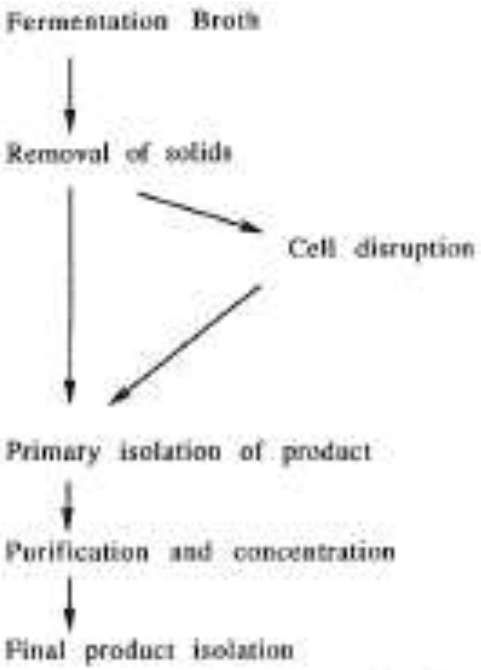

FIG. 10.1. Stages in the recovery of product from a harvested fermentation broth.

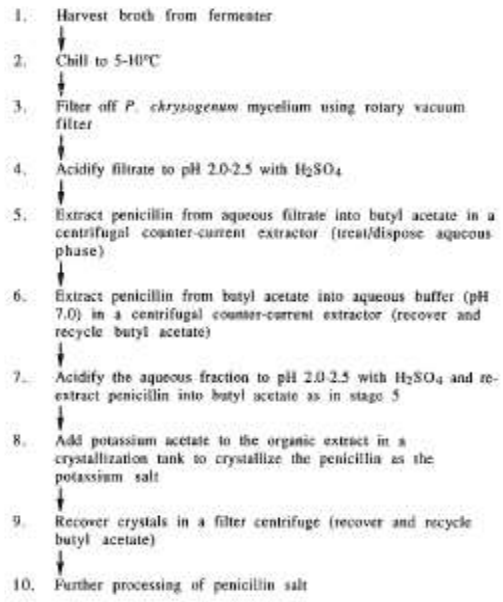

FIG. 10.2. Recovery and partial purification of penicillin G.

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

1. Selection of a micro-organism whichproduce pigments or undesirable metatlolites.

2. Modification of the fermentationreduce the production ofmetabolites.

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 fermentationproduct recovery are integral parts of an overallBecause 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, ionicstrength and culture medium constituents. Largevolumes of supernatants containing extracellular enzymesneed immediate processing while harvestingtimes and enzyme yields might not be predictable. Thiscan make recovery programmes difficult to plan. Pigmentproduction might make some recovery proceduresdifficult, when the pigment binds to the sameresin as the enzyme. Changes in fermentation conditionsmay reduce pigment

formation. Certain antifoamsremain 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 productionmedium may be too high, resulting in the harvestedsupernatant needing dilution with demineralized waterbefore it can be processed. Such a negative procedureshould be avoided if possible by unified research anddevelopment programmes. Media formulation is dominatedby production requirements, but the protein contentof complex media should be critically examined inview of subsequent enzyme recovery. When consideringwater recycle in biomass production. Theystated that the interaction between the different unitoperations in a recycle process made it imperative that commercial plant design and operation should beviewed 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 oftechniques 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 proteinsand vitamin. Other reviews on separation and purificationare available for penicillin,amino acids, enzymes, single-cell protein and polysaccharides. In the selection of processes forthe recovery of biological products it should always beunderstood that recovery and production are interlinked,and that good recovery starts in the fermentationby the selection of, amongst other factors, thecorrect media and time of harvesting.

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

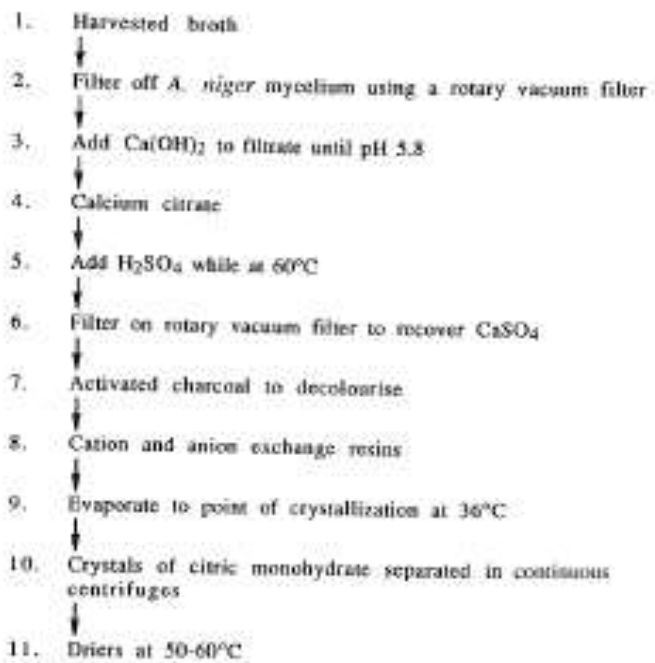

FIG. 10.4. Recovery and purification of citric acid (Sodesk *et al.*, 1981).

The recovery and purification of many compoundsmay be achieved by a number of alternative routes.The decision to follow a particular route involves comparingthe following factors to determine the mostappropriate 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 recoveryis the large-scale purification of biologically activemolecules. For a process to be economically viablelarge-scale production is required, and therefore largescaleseparation, recovery, and purification. This thenrequires the transfer of small-scale preparative/analyticaltechnologies (e.g. chromatographic techniques)to the production scale whilst maintaining efficiency ofthe process, bio-activity of the product and purity ofthe product so that it conforms with safety legislationand regulatory requirements.

REMOVAL OF MICROBIAL CELLS AND OTHER SOLID MATTER

Microbial cells and other insoluble materials arenormally separated from the harvested broth by filtrationor centrifugation. Because of the small size ofmany microbial cells it will be necessary to consider theuse of filter aids to improve filtration rates, while heatand flocculation treatments are employed as techniquesfor increasing sedimentation rates in centrifugation.The methods of cell and cell debris separationdescribed in the following sections have been practiced for many years. Somepotential developments in cell recovery. These includethe use of electrophoresis and di-electrophoresis toexploit the charged properties of microbial cells, ultrasonictreatment to improve flocculation characteristicsand magnetic separations. All these techniques sufferfrom high cost and scale-up difficulties and currentlyare not appropriate technologies. Of more current interestis the use of two-phase liquid extraction. Thoughstill most appropriately used for separation of selectedsoluble components, it is easy to scale up and usesconditions which are gentle on the product.

FOAM SEPARATION

Foam separation depends on using methods whichexploit differences in surface activity of materials. Thematerial may be whole cells or molecular such as aprotein or colloidal, and is selectively adsorbed orattached to the surface of gas bubbles rising through aliquid, to be concentrated or separated and finallyremoved by skimming. It may be possible tomake some materials surface active by the applicationof surfactants such as long-chain fatty acids,and quaternary ammonium compounds. Materialssurface active and collected are termedwhereas the surfactants are termed collectors when developing this method of separation, thevariables 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*Chiarella*sp. and *Chlamydomonas*sp. In otherwith *E. coli*, Grieves and Wang (1966) were able to achieve cell enrichment ratios of between 10 and 1 X10 6 using ethyl-hexadecyl-dimethyl ammoniumbromide.

PRECIPITATION

Precipitation may be conducted at various stages ofthe product recovery process. It is a particularly usefulprocess in that it allows enrichment and concentrationin one step, thereby reducing the volume of materialfor further processing.It is possible to obtain some products (or to removecertain impurities) directly from the broth by precipitation,or to use the technique after a crude cell lysatehas 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 solutionuntil the isoelectric point of the compound isreached and pH equals pI, when there is thenno overall charge on the molecule and its solubilityis decreased.

(b) Salts such as ammonium and sodium sulphateare used for the recovery and fractionation ofproteins. The salt removes water from the surfaceof the protein revealing hydrophobicpatches which come together causing the proteinto precipitate. The most hydrophobic proteinswill precipitate first, thus allowing fractionationto take place.

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

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

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

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

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

FILTRATION

Filtration is one of the most common processes usedat all scales of operation to separate suspended particlesfrom a liquid or gas, using a porous medium whichretains the particles but allows the liquid or gas to passthrough. Gas filtration has been discussed in detail elsewhere. It is possible to carry outfiltration under a variety of conditions, but a number offactors will obviously influence the choice of the mostsuitable type of equipment to meet the specified requirementsat 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 andpacking 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 whichthe simplest form consists of plates and frames arrangedalternately. The plates are covered with filterclothsor filter pads. The plates and framesare assembled on a horizontal framework and heldtogether by means of a hand screw or hydraulic ram sothat there is no leakage between the plates and frameswhich form a series of liquid-tight compartments. Theslurry is fed to the filter frame through the continuouschannel formed by the holes in the corners of theplates and frames. The filtrate passes through the filtercloth or pad, runs down grooves in the filter plates andis then ischarged through outlet taps to a channel.Sometimes, if aseptic conditions are required, the outletsmay lead directly into a

pipe. The solids are retainedwithin the frame and filtration is stopped whenthe frames are completely filled or hen the flow of filtrate becomes uneconomically low.

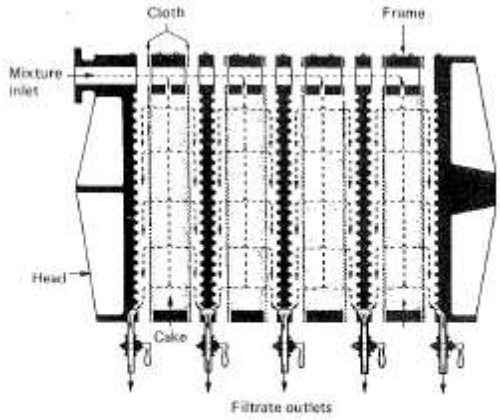

FIG. 10.8. Flush plate and frame filter assembly. The cloth is shown away from the plates to indicate flow of filtrate in the grooves between pyramids (Purchas, 1971).

PRESSURE LEAF FILTERS

There are a number of intermittent batch filtersusually called by their trade names. These filters incorporatea number of leaves, each consisting of a metal framework of grooved plates which is covered with afine wire mesh, or occasionally a filter cloth and oftenprecoated with a layer of cellulose fibres. The processslurry is fed into the filter which is operated underpressure or by suction with a vacuum pump. Becausethe filters are totally enclosed it is possible to sterilizethem with steam. This type of filter is particularlysuitable for 'polishing' large volumes of liquids withlow solids content or small batch filtrations of valuablesolids.

(i) *Vertical metal-leaffilter*

This filter consists of a number of vertical porousmetal leaves mounted on a hollow shaft in a cylindricalpressure vessel. The solids from the slurry graduallybuild up on the surface of the leaves and the filtrate isremoved from the plates via the horizontal hollowshaft. In some designs the hollow shaft can be slowlyrotated during filtration. Solids are normally removedat the end of a cycle by blowing air through the shaft and into the filter leaves.

(ii) *Horizontal metal-leaffilter*

In this filter the metal leaves are mounted on avertical hollow shaft within a pressure vessel. Often,only the upper surfaces of the leaves are porous. Filtrationis continued until the cake fills the space betweenthe disc-shaped leaves or when the operational pressurehas become

excessive. At the end of a processcycle, the solid cake can be discharged by releasing thepressure and spinning the shaft with a drive motor.

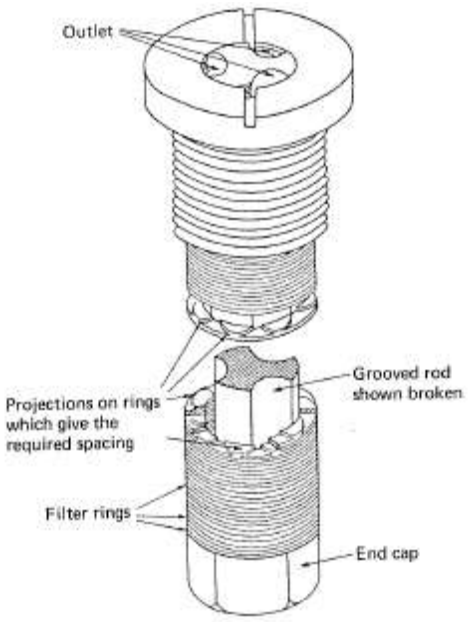

FIG. 10.9a. Metafilter pack (Coulson and Richardson, 1991).

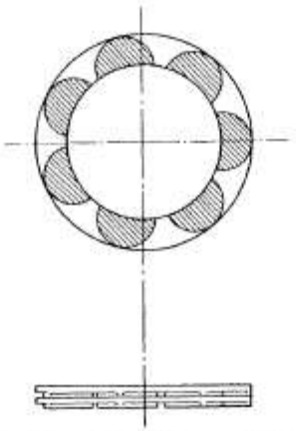

FIG. 10.9b. Rings for metafilter (Coulson and Richardson, 1991).

ROTARY VACUUM FILTERS

Large rotary vacuum filters are commonly usedindustries which produce large volumes of liquidneed continuous processing. The filter consistsrotating, hollow, segmented drum covered with aor metal filter which is partially immersed in acontaining the broth to be filtered.slurry is fed on to the outside of the revolving drumand vacuum pressure is applied internally so that thefiltrate is drawn through the filter, into the drum andfinally to a collecting vessel. The interior of the drum isdivided into a series of compartments, to which thevacuum

pressure is normally applied for most of eachrevolution as the drum slowly revolves (~ 1 rpm). However,just before discharge of the filter cake, air pressuremay be applied internally to help ease the filtercake off the drum. A number of spray jets may becarefully positioned so that water can be applied torinse the cake. This washing is carefully controlled sothat 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 oneatmosphere (l00 kN m-Z) and in practice it is significantlyless than this. In contrast, pressure filters can beoperated at many atmospheres pressure. A number perforatoryvacuum drum filters are manufactured, whichdiffer in the mechanism of cake discharge from thedrum:

(i) String discharge.

(ii) Scraper discharge.

(iii) Scraper discharge with precoating of the drum.

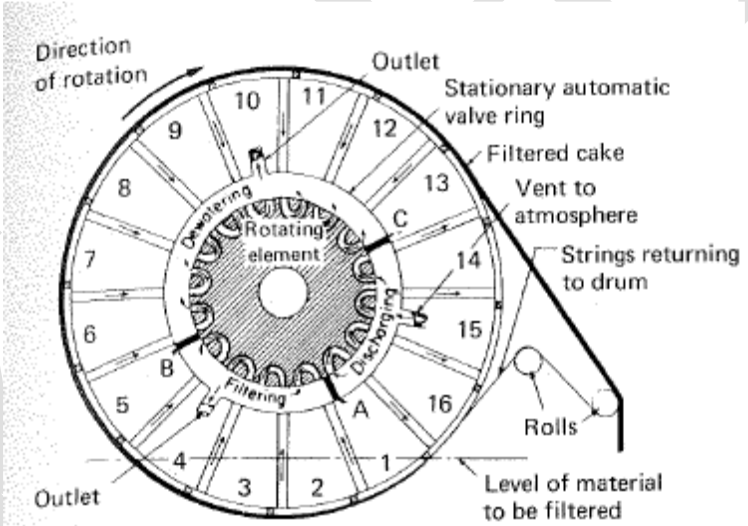

FIG. 10.10. Diagram of string-discharge filter operation. Sections 1 to 4 are filtering; sections 5 to 12 are dewatering; and section 13 is discharging the cake with the string discharge. Sections 14, 15 and 16 are ready to start a new cycle. A, B and C represent dividing members in the annular ring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

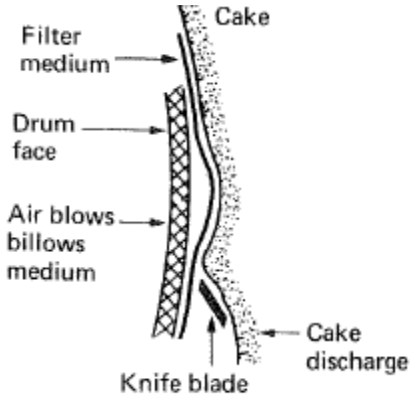

FIG. 10.12. Cake discharge on a drum using a scraper (Talcott *et al.*, 1980).

CENTRIFUGATION

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

1. Filtration is slow and difficult.

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

3. Continuous separation to a high standard ofhygiene is required.Non-continuous centrifuges are of extremely limitedcapacity and therefore not suitable for large-scale separation.The centrifuges used in harvesting fermentationbroths are all operated on a continuous or semi-continuousbasis. Some centrifuges can be used for separatingtwo immiscible liquids yielding a heavy phaseand light phase liquid, as well as a solids fraction. Theymay also be used for the breaking of emulsions.According to Stoke's law, the rate of sedimentationof spherical particles suspended in a fluid of Newtonianviscosity characteristics is proportional to the square ofthe diameter of the particles, thus the rate of sedimentationof 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 centrifugecompared with gravity settling. It is often referredto as the relative centrifugal force and given thesymbol Z.It is evident from this formula that factors influencingthe rate of sedimentation over which one has littleor no control are the difference in density between thecells and the liquid (increased temperature would lowermedia density but is of little practical use with fermentationbroths), the diameter of the cells (could beincreased by coagulation/flocculation) and the viscosityof the liquid. Ideally, the cells should have alarge diameter, there should be a large density differencebetween cell and liquid and the liquid should havea low viscosity. In practice, the cells are usually verysmall, of low density and are often suspended in viscousmedia. Thus it can be seen that the angularvelocity and diameter of the centrifuge are the majorfactors to be considered when attempting to maximizethe rate of sedimentation (and therefore throughput)of fermentation broths.

TYPES OF CENTRIFUGES

A number of centrifuges will be describedvary 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 itimportant to ensure that the centrifuge will be ableperform the separation at the planned productionand operate reliably with minimum manpower. Largescaletests may therefore be necessary with fermenta"tion broths or other materials to check that the correctcentrifuge is chosen.

THE BASKET CENTRIFUGE (PERFORATED-BOWL BASKET CENTRIFUGE)

Basket centrifuges are useful for separating mouldmycelia or crystalline compounds. The centrifuge ismost commonly used with a perforated bowl lined witha filter bag of nylon, cotton, etc.. A continuousfeed is used, and when the basket is filled with thefilter cake it is possible to wash the cake before removingit. The bowl may suffer from blinding with softbiological materials so that high centrifugal forces cannotbe used. These centrifuges are normally operatedat speeds of up to 4000 rpm for feed rates of 50 to 300dm3 min -1 and have a solids

holding capacity of 30 to500 dm3. The basket centrifuge may be considered tobe a centrifugal filter.

THE TUBULAR-BOWL CENTRIFUGE

This is a centrifuge to consider using for particle sizeranges of 0.1 to 200 p.m and up to 10% solids in thein-going slurry. Figure 1O.16a shows an arrangementused in a Sharples Super-Centrifuge. The main componentof 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 thebottom bearing (D). The feed which may consist ofsolids and light and heavy liquid phases is introducedby the nozzle (E). During operation solids sediment onthe bowl wall while the liquids separate into the heavyphase in zone (0) and the light phase in the centralzone (H). The two liquid phases are kept separate intheir exit from the bowl by an adjustable ring, with theheavy phase flowing over the lip of the ring. Rings ofvarious sizes may be fitted for the separation of liquidsof 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 phaseseparation.

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

THE SOLID-BOWL SCROLL CENTRIFUGE (DECANTER CENTRIFUGE)

This type of centrifuge is used for continuous handlingof fermentation broths, cell lysates and coarsematerials such as sewage sludge. The slurry is fed through the spindle of an archimedean screwwithin the horizontal rotating solids bowl. Typically thespeed differential between the bowl and the screw is inthe range 0.5 to 100 rpm. The solids settling on the walls of the bowl arescraped to the conical end of the bowl. The slope of thecone helps to remove excess liquid from the solids before discharge. The liquid phase is discharged fromthe opposite end of the bowl. The speed of this type ofcentrifuge 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 thediameter. Feed rates range from around 200 dm3 h- 1to 200 m3 h -1 depending on scale of operation andmaterial being processed. A number of variants on theasic 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 mechanicalseals, control of aerosols, containment casingsand the use of high pressure sterile gas in sealsto prevent the release of micro-organisms

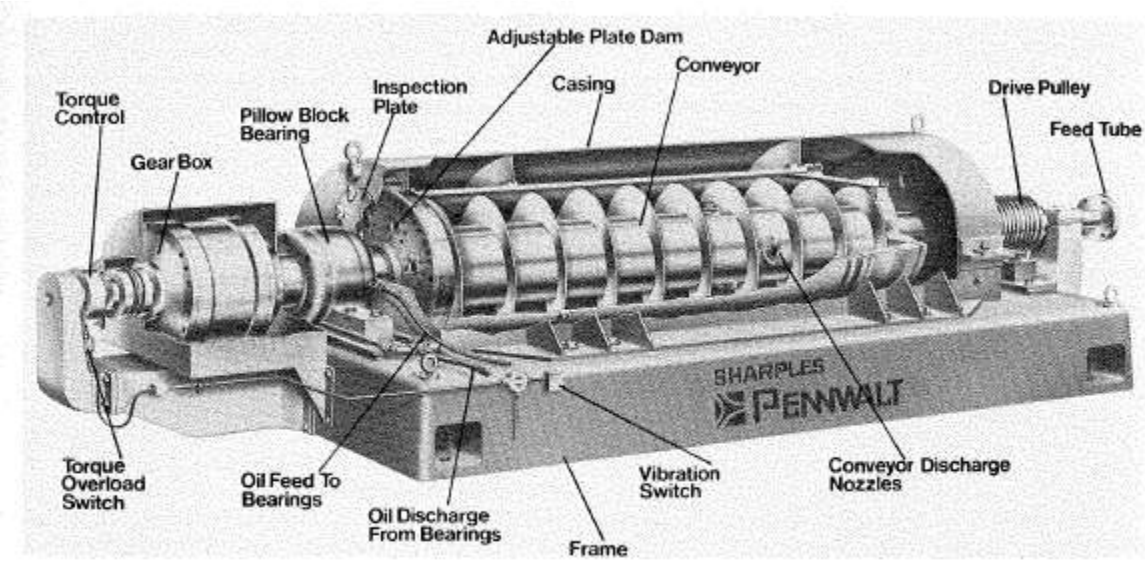

Fig. 10.17b. Cutaway view of a Sharples Super-D-Canter continuous solid-bowl centrifuge, Model P-5400 (Alfa Laval Sharples Ltd, Camberley, U.K.).

FREEZING-THAWING

Freezing and thawing of a microbial cell paste willinevitably cause ice crystals to form and their expansionfollowed by thawing will lead to some subsequentdisruption of cells. It is slow, with limited release ofcellular materials, and has not often been used as atechnique on its own, although it is often used incombination with other techniques. f3-Glucosidase hasbeen obtained from S. *cerevisiae*by this method. A sample of 360 g of frozen yeastpaste was thawed at 50 for 10 hours. This cycle wasrepeated twice before further processing.

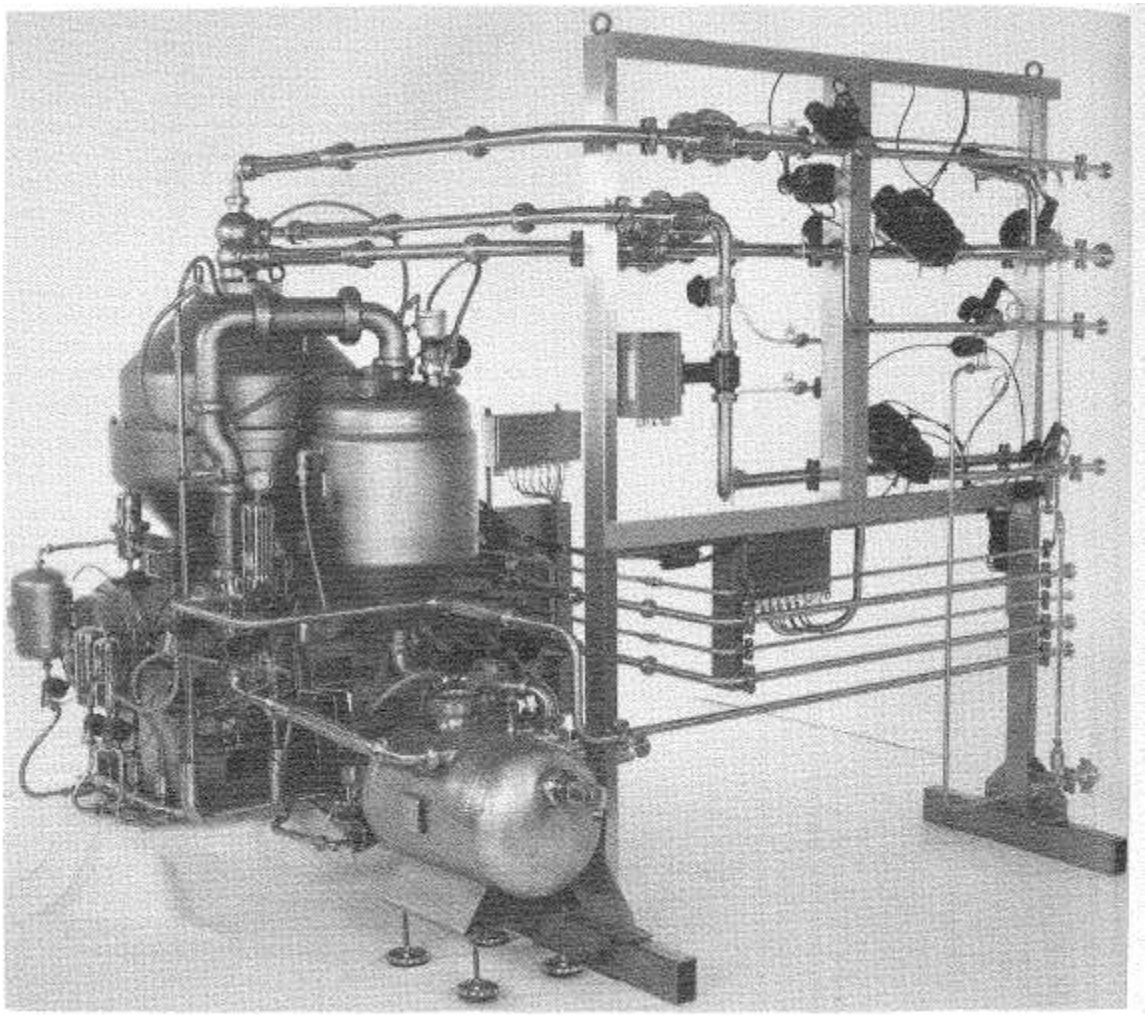

FIG. 10.20. Alfa Laval BTUX 510 disc stack centrifuge (Alfa Laval Sharples Ltd. Camberley, U.K.).

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?

DEPARTMENT OF MICROBIOLOGY, KARPAGAM ACADEMY OF HIGHER EDUCATION
BIOPROCESS ENGINEERING- 17MBP205C
UNIT IV

Fungus Mycelium should be suspended in _____ medium to maintain its structure.
The volume of inoculum used to cultivate bacteria are _____.
Organic acids are produced in _____
Antibiotics from soil are easily isolated from_____
Optimum Prodution of lysine takes place in medium containing _____ mg of biotin
Optimum Production of Arnithine occurs in a medium containing _____ mg of Arginine.
-------- developed a fermentor for the production of acetone.
In -------- year Straunch patented a system in which the aeration tubes were introduced with water and
Carbon source used for production of tctracycline is _____
Mycellium undergoes autolysis with raise in _____
Example for non ionic detergents
Example for coagulating agent
_____ is an established and final purification of a diverse range of compounds.
---------- filters are used to filter.
_____ is the organism used in the first truly large scale aseptic fermentation vessels.
_____ is to provide microorganisms in submerged culture with sufficient oxygen for metabolic requireme
_____ ensures uniform suspension on microbial cells.
---------device is used to introduce air in fermenter
Aeration and agitation of a liquid medium may lead to the formation of
---------- are surface active agents reducing the Surface tension in the foam t.
-------- fermenter is called as elongated non-mechanically stirred fermenter
_____ fermentor is a gas tight baffled rise tube connected to a down comer tube.
Multiple air lift fermenter Is designed by
Silicon compound are example are of inert ------- agent
---------- is the main compound in corn steep liquor
Impeller are used in the fermentor helps in
_____ or _____ is added to adjust pH if too acidic.
_____ chromatography separates according to the affinity of the protein, for the surface of the solid m
-------- chromatography is a powerful and highly selective purification technique.
Microbial cells and other insoluble materials are normally separated from the harvested broth by_____ c
Salts such as ammonium and ----------are used for the discover of protein
Dextrans can be precipitated out of a broth by the addition of _____.
---------- method is used for large scale enzyme purification
Ultrasonication has frequency of ----------khz
---------- damage the cell membrane and lead to the release of intracellular components
_____ caused by a sudden change in salt concentration will cause disruption of a number of cell types.
_____ is the separation process where the solvent molecules are passed to flow through Semipermi
_____ of any product is often the last stage of a manufacturing process.
--------- drier is mostly used for drying of biological materials
_____ device are the most economical available for handling large volumes.
------- drying is an important operation in the production of biological and pharmaceuticals

_____ is an established and final purification of a diverse range of compounds.
_____ are metal strips roughly one- tenth of the vessel diameter and attached radically to the wall
A combined sparger and agitator may be used in ---------fermenter
---------were first used as careeier for antifoam in antibiotic processes
Which is the by-product after starch extraction from maize.
Chemically defined amino acid media devoid of protein are used in production of _____
_____does not appear to play a nutritional role in the metabolism of fungi.
_____ is used to carry out microbiological process on batch basis.
Small lab fermentor is in the size range of
Larger fermentor range from _____ gallous.
pH control is achieved by _____ device.
_____ consists of circular discs to which blades are fitted with bolts.
Size of the holes in the sparger ranges from_____
Steady state condition can be achieved by operation on _____ principles.
CSTF is expanded as
The temperature of _____ in the production tanks is satisfactory during fermentation.
Industrial alcohol production can be carried out in very large fermentor upto _____ Gallous.
_____ chromatography separates according to the affinity of the protein, for the surface of the solid
-------- chromatography is a powerful and highly selective purification technique.
Microbial cells and other insoluble materials are normally separated from the harvested broth by_____
---------- stressed the importance of the elimination of air and moisture from lyophilized cultures prior

Option A

Dimethyl sulphoxide
0.1-2%
Crowded plate technique
giant colony technique
5 mg/litre
100 mg
Liebmann
1930
Molasses
pH value
Tween 80
Calcium phosphate
Drying
Polytetra flouro ethylene
Clostridium acetobutylicum
Aeration
Aeration
Spargers
Acid
Antifoam
Tower
Tower
Bakker etal
Antibacterial
Lactic acid
Aeration
Ammonia
Adsorption
Adsorption
Filtration
NH3SO4
Methanol
Liquid shear
200
Osmotic shock
Osmotic shock
Ultra filtration
Filtration
Freeze
Freeze
Freeze

Drying
Sparger
Laboratary
Oils
Corn steep liquor
Acids
Chlorine
Batch fermentor
1-21+
5000-10000
Anti titrator
Impellers
1/64-1/32
Chemostatic
Continuous stirred tank fermentor
80°F
12500
Adsorption
Adsorption
Filtration
Meryman

Option B

Rose Bengal medium
0.5-5%
giant colony technique
Crowded plate technique
20 mg/litre
200 mg
Robert Koch
1934
Corn steep liquor
temp
Tween 20
Calcium carbonate
Crystallization
Poly vinyl chloride
C. perfringens
Agitation
Agitation
Impellers
Alkali
Buffles
Airlift
Air lift
Okabe etal
Antifoam
Amino acid
Antifoaming
Sodium hydroxide
Affinity
Affinity
Centrifugation
Na2SO4
Ethanol
Solid shear
2
Alkali treatment
Alkali
Reverse osmosis
Centrifugation
Spray
Spray
Drum

Crystallization
Baffler
Tower
Fats
Barley
Vitamin
Fluoride
Continuous fermentor
0.5-11+
100-1000
Aerator
Sparger
1/32- 1/64
Turbidostatic
Continuous solid tank fermentor
70°F
125
Affinity
Affinity
Centrifugation
Dewald

Option C

Sabouraud's dextrose agar medium
3-5%
Primary screening
Primary screening
15 mg/litre
300 mg
Weizmann
1940
Barley
Ionic conc
Tween 40
Caciumsulphate
Filtration
Glass and mineral fibres
S. cereviseae
Impeller
Sparger
Baffles
Foam
Yeast
Cylindraconical
Cylinder conical
Bacon etal
Anti fungal
Tartaric acid
Agitation
Both a or b
Ion exchange
Ion exchange
Filtration OR centrifugation
CaCl2
Butanol
Ultrasonication
20
Detergent
Protease
Liquid membranes
Drying
Drum
Drum
Spray

Filtration
Magnetic devices
Airlift
Carbohydrate
Molasses
Vaccines
Copper
Fed batch fermentor
1-101+
1000-5000
Baffler
Baffler
1/32- 1/32
Both chemostat and turbidostat
Cell suspended tank fermentor
90°F
25000
Ion exchange
Ion exchange
filtration or centrifugation
leogetring

Option D
None of the above
06-Apr
Pour plate technique
Pour plate technique
25 mg/litre
400 mg
chain weizmann
1944
None of the above
none
none of the above
All the above
HPLC
All the above
E.coli
Baffler
Baffler
Turbines
Air
Cell
Deep jet
Deep jet
Dawsa
Antiprotozal
Lactose
Absorption
Sulphuric acid
Column
Column
Sedimentation
Ca
Alcohol
Freeze thawing
2000
Enzyme
SDS
pumping
Packing
Tray
Tray
Tray

HPLC
Impellers
Batch
Acids
Soybean oil
Antibiotics
Cadmium
Semi continuous fermentor
5-101+
10000-20000
Impeller.
Aerator
1/64 -1/64
Photostat
Continuous solid type fermentor
100°F
100000
Column
Column
Sedimentation
Louis Pasteur.

Answer Key

Dimethyl sulphoxide
0.5-5%
Primary screening
giant colony technique
20 mg/litre
200 mg
chain weizmann
1934
Molasses
pH value
Tween 20
Calcium phosphate
Crystallization
All the above
Clostridium acetobutylicum
Aeration
Agitation
Spargers
Foam
Antifoam
Tower
Air lift
Bakker etal
Antifoam
Lactic acid
Agitation
Both a or b
Adsorption
Affinity
Filtration OR centrifugation
Na2SO4
Methanol
Liquid shear
20
Osmotic shock
Osmotic shock
Reverse osmosis
Drying
Spray
Spray
Freeze

Crystallization
Baffler
Laboratary
Oils
Corn steep liquor
Vaccines
Chlorine
Batch fermentor
1-21+
5000-10000
Anti titrator
Impellers
1/64-1/32
Both chemostat and turbidostat
Continuous stirred tank fermentor
80°F
12500
Adsorption
Affinity
filtration or centrifugation
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.

1.1 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.

1.2 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.

1.3 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.

1.4 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 commercialprocess may be a long and very expensive procedureand it is therefore essential that it retains thedesirable characteristics that led to its selection. Also,the culture used to initiate an industrial fermentationmust be viable and free from contamination. Thus,industrial cultures must be stored in such way as toeliminate genetic change, protect against contaminationand retain viability. An organism may be keptviable by repeated sub-culture into fresh medium, but,at each cell division, there is a small probability ofmutations occurring and because repeated sub-cultureinvolves very many such divisions, there is a highprobability that strain degeneration would occur. Also,repeated sub-culture carries with it the risk of contamination.Thus, preservation techniques have been developedto maintain cultures in a state of 'suspendedanimation' by storing either at reduced temperature orin 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 bereduced considerably by storage at the very low temperatures(-150° to -196°) which may be achievedusing a liquid nitrogen refrigerator. Snell (1991) claimedthat this aproach is the most universally applicable ofall preservation methods. Fungi, bacteriophage, viruses,algae, yeasts, animal and plant cells and tissue cultureshave all been successfully preserved. The techniqueinvolves growing a culture to the maximum stationaryphase, 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 freezingand thawing stages but there is virtually no loss duringthe storage period. Thus, viability may be predictableeven after a period of many years. Snell (1991) suggestedthat liquid nitrogen is the method of choice forthe preservation of valuable stock cultures and may bethe only suitable method for the long term preservationof cells that do not survive freeze-drying. Although theequipment is expensive the process is economical onlabour. However, the method has the major disadvantagethat liquid nitrogen evaporates and ustbe replenishedregularly. If this is not done, or the apparatusfails, then the consequences are the loss of the collection.

Storage in a dehydrated form

DRIED CULTURES

Dried soil cultures have been used widely for culturepreservation, particularly for sporulatingmycelial organisms.Moist, sterile soil may be inoculated with aculture and incubated for several days for some growthto occur and then allowed to dry at room temperaturefor 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 Pridham*et 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 detailedmethods are given for these simple, inexpensive techniquesin Malik's discussion.

LYOPHILIZATION

Lyophilization, or freeze-drying, involves the freezingof a culture followed by its drying under vacuum,technique involves growing the culture to the maximumstationary phase and resuspending the cells in a protectivemedium 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 highvacuum until sublimation is complete, after which theampoule is sealed. The ampoules may be stored in arefrigerator and the cells may remain viable for 10years or more (Perlman and Kikuchi, 1977).Lyophilization is very convenient for service culturecollections (Snell, 1991) because, once dried, the culturesneed no further attention and the storage equipment(a refrigerator) is cheap and reliable. Also, thefreeze dried ampoules may be dispatched as such, stillin a state of 'suspended animation' whereas liquidnitrogen stored cultures begin to deteriorate.

However,freeze-dried cultures are tedious to open and revitalizeand several sub-cultures may be needed before thecells regain their typical characteristics. Overall, thetechnique appears to be second only to liquid nitrogenstorage and even when liquid nitrogen is used makes an excellent insurance against the possibility of thebreakdown 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 appreciatedthat 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,Pontecorvo*et aZ.*(1953) demonstrated that nuclearfusion and gene segregation could take place outside,or in the absence of, the sexual organs. The processwas termed the parasexual cycle and has been demonstratedin 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 betweenunlike nuclei in the vegetative hyphae of the organism.Thus, recombination may be achieved only in an organismin which at least two different types of nucleicoexist, i.e. a heterokaryon. The heterozygous diploidnucleus resulting from the fusion of the two differenthaploid nuclei may give rise to a diploid clone and, inrare cases, a diploid nucleus in the clone may undergoan 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 theheterokaryon. Analysis of the recombinants normallydemonstrates them to be segregant for only one, or afew linked, markers and culture of the sergeantsresultsin the development of clones displaying morerecessive characters than the initial segregant. Theprocess of recombination during the growth of theheterozygous 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 ab:nol:m~ILmitosis. The normal mitosis of a heterozygouscell is shown in Fig. 3.26. During mitosis, each pairhomologous chromosomes replicate to producepairs of chromatids and a chromatid of one pair migrates to a pole of the cell with a chromatid ofother pair. Division of the cell at the equator resultsthe production of two cells, both of which are heterozvgousfor all the genes on the chromosome.crossing over involves the exchange of distal segments between chromatids of homologous chromosomesshown in Fig. 3.27. This process may result inproduction of daughter nuclei homozygous for ation of one pair of chromosomes and in the expressions of any recessive alleles contained in that portion.the clone arising from the

partial homozygote willrecombinant and further mitotic crossing over inrecombinant will result in the expression of more recessive alleles.

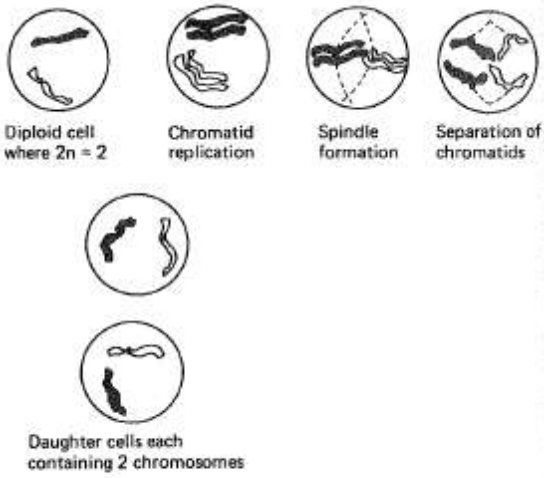

FIG. 3.26. Diagrammatic representation of the mitotic division of a eukaryotic cell containing two chromosomes. The nuclear membrane has not been portrayed in the figure.

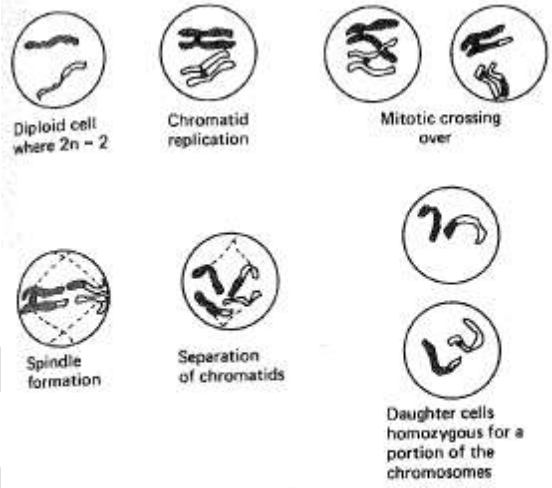

FIG. 3.27. Diagrammatic representation of mitosis including mitotic crossing over.

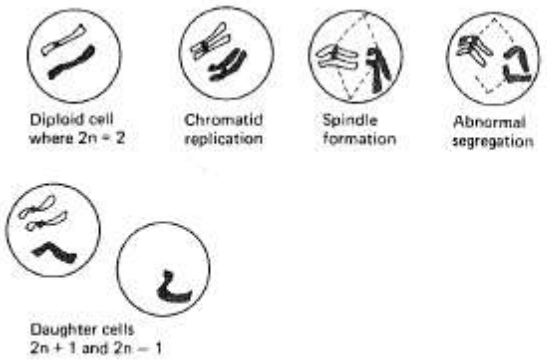

FIG. 3.28. Diagrammatic representation of mitosis involving haploidization.

THE APPLICATION OF PROTOPLAST FUSION TECHNIQUES

Protoplasts are cells devoid of their cell walls andmay be prepared by subjecting cells to the action ofwall degrading enzymes in isotonic solutions. Protoplastsmay regenerate their cell walls and are thencapable of growth as normal cells. Cell fusion, followedby nuclear fusion, may occur between protoplasts ofstrains which would otherwise not fuse and the resultingfused protoplast may regenerate a cell wall andgrow as a normal cell. Thus, protoplasts may be used to overcome some recombination barriers. Protoplast fusionhas been demonstrated in a large number ofindustrially important organisms including *Streptomyces*spp. (Hopwood *et al.*, 1977), *Bacillus* spp. (Fodor andAlfoldi, 1976), corynebacteria (Karasawa*et al., 1986),*filamentous fungi (Ferenczy*et al.*, 1974) and yeasts(Sipiczki and Ferenczy, 1977).Fusion of fungal protoplasts appears to be an excellenttechnique to obtain heterokaryons between strainswhere 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 ofPeberdy*et at.* (1977) who succeeded in obtaining heterokaryonsbetween *P. chrysogenum*and *P. cyaneofuluum*and demonstrated the formation of diploids whichgave rise to recombinants after treatment with *p-ftuo*rophenylalanineor benomy!. Although it has beenclaimed that *P. chrysogenum*and *P. cyaneofulvum*arenot different species of *Penicillium*(Samson *et al.*,1977), Peberdy*et al.* still demonstrated that protoplastfusion could be successful where conventional techniqueshad 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 protoplastfusion technique. The results from conventional techniquessuggested that nuclear fusion was difficult toachieve. Electron microscopic examination of fusedprotoplasts indicated that up to 1% underwnt immediatenuclear fusion. Recombinants were obtained inboth sister and divergent crosses. A cross between anasporulating, slow-growing strain with a sporulatingfast-growing strain which only produced one-third ofthe cephalosporin level of the first strain eventuallyresulted in the isolation of a recombinant which combinedthe desirable properties of both strains, i.e. astrain which demonstrated good sporulation, a highgrowth rate and produced 40% more antibiotic thanthe higher-yielding parent. Chang *et al.* (1982) utilizedprotoplast fusion to combine the desirable

qualities oftwo strains of *Penicilliumch'ysogenum.* Protoplasts fromtwo strains, differing in colony morphology and theabilities to produce penicillin V.

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

Protoplast fusion has also been applied to the improvementof amino acid producing strains. Karasawa*et al.* (1986) used the technique to improve the fermentationrates of lysine producers developed using repeatedmutation and directed selection. Such strainswere good lysine producers but showed low glucoseconsumption and growth rates, undesirable featureswhich had been inadvertently introduced during theselection programme. A protoplast fusion was performedbetween the lysine producer and a fast growingstrain and a fusant was isolated displaying the desirablecharacteristics of high lysine production and high glucoseconsumption rate resulting in a much faster fermentation.

TABLE 3.7. *The use of protoplast fusion for the improvement of a pencillin 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 ofbacteria has been achieved experimentally using both*in vivo* and *in vitro* techniques (Atherton *et al., 1979).*Thus, genetic material derived from one species may beincorporated into another where it may be expressed.*In vivo* techniques make use of phage particles whichwill pick up genetic information from the chromosomeof one bacterial species, infect another bacterial speciesand in so doing introduce the genetic information fromthe first host. The information from the first host maythen be expressed in the second host. Whereas, the *invivo*techniques depend on vectors collecting informationfrom one cell and incorporating it into another,the *in vitro* techniques involve the insertion of theinformation into the vector by *in vitro* manipulationfollowed by the insertion of the carrier and its associated'extra' DNA into the recipient cell. Because theDNA is incorporated into the vector by *in vitro* methodsthe source of the DNA is not limited to that of thehost organism of the

vector. Thus, DNA from humanor animal cells may be introduced into the recipientcell. Atherton *et al.* (1979) listed the basic requirementsfor 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 onesite where integration of foreign DNA will notdestroy 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 simplecharacteristics include drug resistance, immunity,plaque formation, or an inserted generecognizable by its ability to complement a known auxotroph.

(iv) A method of assaying for the 'foreign' geneproduct of choice from the population of recombinantscreated.

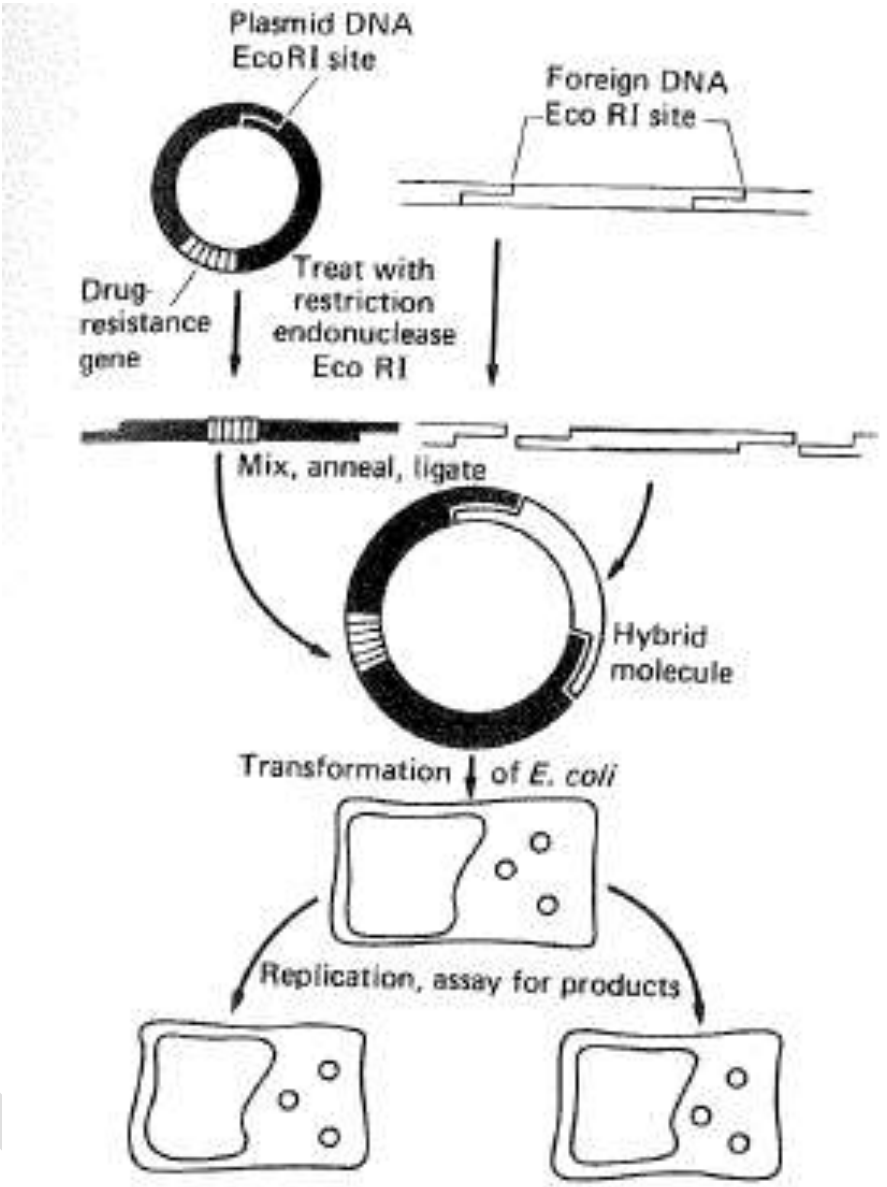

FIG. 3.29. A summary of the steps in *in vitro* genetic recombination. Both plasmid vector and foreign DNA are cut by the restriction endonuclease, EcoRI, producing linear double-stranded DNA fragments with single-stranded cohesive projections. EcoRI recognizes the oligonucleotide sequence $\frac{GAATTC}{CTTAAG}$ and will cut any double-stranded DNA molecule to yield fragments with the same cohesive ends $\frac{GAATT}{C}$ + $\frac{C}{TTAAG}$. 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 engineeredstrain is altered only in the number of copies of thegene and does not contain genes which were presentoriginally in a different organism. However, the techniquesemployed in the construction of these strainsare the same as those used in the construction ofchimeric strains, so it is logical to consider this aspecthere.The first application of gene amplification to industrialstrains was for the improvement of enzyme production.Indeed, some regulatory mutants isolated byconventional means owed their productivity to their containing multiple copies of the relevant gene as wellas 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.

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UNIT V

Industrial microbiology deals with areas of microbiology involving economic aspects, where valua
In history of industrial microbiology, the period of ignorance is
The period of discovery in history of industrial microbiology is from
The period of industrial development in history of industrial microbiology is
Bread was 1st baked around ----------B.C
Wine is produced from
The compound Microscope produced by Zaccharies Jensen had no provision for
Anton von Leewanhoek was able to obtain magnification upto ----------diameters.
In middle of last century fermentation was consider to be a ------process.
Fermentation was first described as chemical process by
In 1873,----------described that yeastsare involved in fermentation process for alcohol production
In fermentation, yeast converts ----------to----------and -----
who isolated the microbes associated fementation.
The optimum temperature condition for fermentation process was in range from-----°C
Pasteur identified the organisms involved in the transformation of sugar to ----------
Lactic acid organism is a ----------
who made an important discovery that fermentation takes place in absence of oxygen.
In 1861 pasteur did experiments on ---------and----------fermentation
)---------and----------requested Pasteur to study the problem of sowing wine that thereatened French wine i
The process of sterilization of wine introduced by Pasteur is called
which method is most satisfory method for long time preservation of microbes
The major antibiotics such as streptomycin and neomycin etc were isolated from----------
During 1910-1920,----------and---------were produced by Industrial fermentation
During 1920-1930,---------- was produced by industrial fermentation
During 1930-1940, first vitamin to be produced by industrial fermentation is------
The acetone-butanol fermentation is also called as ----------process
The important quality of production strain is
The screening techniques involves ----------and----------
Primary screening technology involves the isolation of new microbial species exhibiting the
Crowded plate technique is an example of ----------screening
The primary screening technique which is employed for a detect and isolating antibiotic producing strain is
Enrichment culture technology was designed by soil microbiologist----------
which technique is largely employed to identify the growth factor producing strain extracellularly
Neutral red, bromothymol blue dyes are added to partly buffered nutrient agar media to detect microorgani
Example of enrichment substrate used is
which screening helps in segregation microbe that have real potential in fermentation industry.
The suitable protective medium used at the ----------is 10% inositol in dissolved water
The fermentation product produced by the identified industrial strain should be ----------
The selected industrial strain is----------by secondary screening.
The process of lyophilization was first applied to microfungi on layers scale by ---------and ----------in 194
The important criteria in handling the industrially productive strain is to prevent

Mutation is done by----------and----------methods
Phosphorous pentoxide silica gel/freezing trap are examples of----------
In multivalent regulatory mechanism of a branched biosynthetic pathway, ----------end products inhibit t
The two categories of mutants are----------and----------
The wild strain of *Coryrebacterium glutamicus* secretes both -------and----------
The mutant strain of *Coryrebacterium glutamicus* produces upto ----------g of lysine litre in medium
The oil used in oil overlay method is
The lysine biosynthesis, the end products lysine and threonine inhibit the enzyme.
An analogue of threonine is ----------
The analogue is ----------to sensitive mutant cells in the population.
An example of analogue resistant mutant is ----------capable of excreating threomine upto 12.6g/l.
An example of revertant mutant is----------for the enzyme threonine deaminase.
.----------mutants are important in fermentation industry as produce high yields of particular enzymes
ATCC is ---------- founded in 1925
Secondary metabolites are produced during _____.
Extra chromosomal elements which carry information for synthesis of products is called _____.
_____ is the industrially used promoter.
.----------and----------are usually maintained in liquid medium
Lyophilization is the most satisfactory method of long term preservation of microorganisms
---------- is perhaps the most popular form of suspended metabolism.
Hwang in ---------- year recommends precooling to 7 degree Celsius.
---------- stressed the importance of the elimination of air and moisture from lyophilized cultures prior

Option A

from costly substrates
pre-1800
pre-1800
pre-1800
1000
malt
resolution power
150-300
biological
Pasteur
Pasteur
carbohydrate, alcohol, carbonic acid
Schwann
20-40
pyruvic acid
fungi
Schwann
butyric acid and acetic acid
Dumas and Napoleon 111
pasteurization
mineral oil overlay
Bacillus sp
ethanol and glycerol
lactic acid
riboflavin
Watsmann
should be a high yielding strain
primary and secondary
desined color
primary
crowded plate technique
Pasteur
crowded plate technique
vitamins
nutrient broth
primary
Commonwealth Mycological Institute
old
optimized
Raper and Alexander
stability

physical and chemical
crypreservation
single
autotropic mutants and mutants resistant to analogues
lysine and threonine
50
British Pharmacopoeia Medicinal Paraffin oil.
aspartate kinase
α-amino,β-hydroxyvaleric acid
ecofriendly
Brevibacterium flavum
Hydrophiles
auxotrophic
American type culture collection
Lag phase
Protoplast
CaMV
Bacteriophages and *Actinomycetes*
long term
overlaying cultures with mineral oil
1966
Meryman

Option B

from cheaper and disposable substrates
1800-1900
1800-1900
1800-1900
2000
molasses
focusing
160-270
physical
Robert Koch
Schwann
fatty acids, alcohol, carbonic acid
Bertholet
30-50
lactic acid
bacteria
Pasteur
acetone and butanol
Dumas and Flemming
ultrafiltration
lyophilisation
Staphylococcus sp
lactic acid and amylases
acetic acid
vitamin B12
Walksman
unstable biochemical characteristics
secondary and tertiary
desired shape
secondary
auxanography
Koch
auxanography
growth factors
cellulose powder
secondary
American type culture collection
novel
priotized
Thomas and Alexander
contamination

chemical and political
desiccant
double
phototropic mutants and mutants resistant to analogues
lysine and methionine
60
American Pharmacopoeia Medicinal Paraffin oil.
homoserine phosphatase
β-amino,β-hydroxyvaleric acid
toxic
Brevibacterium lactum
Hydromonas
mutants resistant to analogue
African type culture collection
Log phase
Chloroplast
TMV
Bacteriophages and *clostridium*
short term
lyophilization
1969
Dewald

Option C

from unavailable substrates
post-1900
post-1900
post-1900
3000
grapes
light facility
140-250
chemical
Liebig
Robert Koch
sugar, alcohol, carbonic acid
Pasteur
40-60
citric acid
virus
Koch
lactic acid and acetic acid
Napoleon111 and Schwann
low temperature,high holding time
cryopreservation
Streptococcus sp
acetone and n-butanol
citric acid
vitamin C
Websmann
produce underisable substances
primary and quartenary
desired quality
tertiary
enrichment culture technique
Ehrlich
enrichment culture technique
organic acids
peptone
tertiary
African type culture collection
gold
compared
Koch and Alexander
oxidation

physical and botanical
preservants
triple
auxenic mutants and mutants resistant to analogues
threonine and methionine
70
Australian Pharmacopoeia Medicinal Paraffin oil.
serine kinase
ч-amino,β-hydroxyvaleric acid
nutrient
Brevibacterium aseptum
Hydrogenomonas
constitutive
Auxenic type culture collection
Trophophase
Plasmid
BMV
Viruses and *Acetobacter*
both a and b
nitrogen storage
1974
leogetring

Option D

from foreign countries
post-2000
post-2000
post-2000
4000
sugarcane
specimen holding
150-200
electrical
Anton van Leewanhoek
Berzelius
starch, alcohol, carbonic acid
Koch
20-30
stearic acid
protozoa
Bertholet
acetic acid and citric acid
Duman and Leewanhoek
high temperature,low holding time
periodic transfer
Streptomyces sp
acetone and lactic acid
glutanic acid
vitamin D
Weizmann
not easily cultivate
secondary and quartenary
desired property
quartenary
use of indicator dye
Beijerinck
use of indicator dye
amines
minimal media
quartenary
Indian Mycological Institute
critical
deselected
Koch and Thomas
reduction

chemical and zoological
mineral oil overlay
all
auxotropic mutants and mutants sensitive to analogues
threonine and pectin
80
African Pharmacopoeia Medicinal Paraffin oil.
tryptophan synthase
)£-amino,β-hydroxyvaleric acid
non-toxic
Brevibacterium glutans
Hydromonothrobis
mutants sensitive to analogues.
Australian type culture collection
Idiophase
Spheroplast
HMV
Bacteriophages and *Streptomyces*
none of the above
none of the above
1980
Louis Pasteur.

Answer Key

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

physical and chemical
desiccant
all
autotropic mutants and mutants resistant to analogues
lysine and threonine
60
British Pharmacopoeia Medicinal Paraffin oil.
aspartate kinase
α-amino,β-hydroxyvaleric acid
toxic
Brevibacterium flavum
Hydrogenomonas
constitutive
American type culture collection
Trophophase
Plasmid
CaMV
Bacteriophages and *clostridium*
long term
lyophilization
1966
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DEPARTMENT OF MICROBIOLOGY

M. Sc., DEGREE FIRST CIA EXAMINATION (January – 2018)

BIOPROCESS ENGINEERING

TIME: 2Hours **Total: 50 Marks**

PART –A (Answer all the questions) 1 x 20 = 20 marks

1. _____tanks are used in production of all and lactic acid fermentation.
   (a)Stainless steel (b)Glass
   (c)Copper (d)Wooden
2. Soya bean meal is used as production medium for_____.
   (a)Penicillium (b)Streptomycin
   (c)Griseofulvin (d)VitaminB complex
3. _____type of bubbles facilitate high oxygen transfer than _____bubbles.
   (a)Larger, smaller (b)Smaller, larger
   (c)Medium, large (d) Small, medium
4. The ideal aspect ratio for a fermentor is _____
   (a)3:1 (b)4:1
   (c)5:1 (d)6:1
5. In Penicillin fermentation the precursor added is _____.
   (a)Phenyl acetic acid (b)Benzylalcohol
   (c)Benzene (d)Pyridine
6. The specific gravity of oil used in presence of cultures is_____.
   (a)0.821-0.860 (b)0.865-0.890
   (c)0.752-0.812 (d)0.718-0.835
7. Example of In-line sensors are_____.
   (a)Ion-specific sensors (b)Massspectrophotometer
   (c)Antifoam probe (d)Mediumaddition probe
8. In _____ fermentors aspect ratio is 6:1 and used for citric acid production.
   (a) Tower (b)Airlift
   (c)Cyclone (d)Bubblecolumn
9. The_____ are used to prevent shearing of microbes inside the fermentor
   (a)Baffle (b)Impeller
   (c)Sparger (d)Agitator
10. _____bioreactors are used in waste water engineering.
    (a)Packed bed (b)Airlift
    (c)Stirred tank (d)Cyclone
11. Dissolved oxygen in fermentation process medium measured by using _____.
    (a)Galvanic electrode (b)pH electrode
    (c)Thermometers (d)Thermistors
12. Oxygen diffuses from tubing into medium is measured by_____.
    (a)Galvanic electrode (b)Paramagnetic gas analyzer
    (c)Platinum electrode (d)Thermistors

13. The clean, yellow, fine powder prepared from embryo of cotton seed is called as _____.

(a)Corn-steep liquor (b)Soyabean meal

(c)Pharmamedia (d)Distiller's soluble

14. The interface converts the _____signal to _____.

(a)Analogue to digital (b)Digital to analogue

(c)Voltage to pulse (d)Pulse to voltage

15. _____is the least expensive and reliable method for fermentor design and operation

(a)Chemicals (b)Passing of fire flames

(c)Moist heat (d)Dryheat

16. Ancillary equipment in fermentors means the _____.

(a)Seed tank (b)Fermentation medium

(c)Extra connection (d)Antifoamers

17. In continuous sterilization, the fermentation medium is passed through _____aiding in sterilization.

(a)Heat exchange (b)Holding coil

(c)Cooler (d)Heat exchange, holding coil and cooler

18. The solubility of oxygen in pure water is _____mg/L at 4˚C.

(a)4 (b)6

(c)8 (d)10

19. Mass transfer occurs in mixtures containing_____.

(a)Equal concentration (b) Concentration gradient

(c)Isothermal concentration (d)Anisoisomers

20. The critical oxygen concentration of*E.coli* cells are_____.

(a)0.018 (b) 0.008

(c)0.004 (d)0.022

PART B (Answer all the questions) 3 x 2 = 6 marks

21. What is a bioreactor?

Instrument – fermentation- metabolite production-small and/or large scale.

22. What are impellers?

Stainless steel – fans – used for mixing the contents of the fermentation medium.

23. Define fermentation.

Metabolic process – acid + gas production – sugar utilization.

PART C (Answer all the questions) 3 x 8 = 24 marks

24. a. With clear figure explain the design of a basic fermentor and bioreactor configuration.

Definition of fermentor and bioreactor – criteria for constructing a fermentor – bioreactor configuration – stainless steel AWS – agitator – impeller – baffle – sparger – heating coil – cooling coil – media inlet – media out let – sample point – thermostat - probes – man hole – gas out let – dead space.

(Or)

b. Explain the computer control of fermentation process.

Automated and manual control – thermostat – chemostat – turbidostat – critical control point – time based automated program control – ultra accurate monitor - digital interface – analog to digital conversion of data – automated cleaning and sterilization system .

25. a. Discuss about the oxygen transfer from medium to cells during fermentation process.

Metabolism of substrate by microbes – energy production – role of oxygen in growth – phases of growth - different source of oxygen – size : particle ratio and microbial growth – microbial cell shearing effect – oxygen transfer and bubble phenomenon – sparger effect on oxygen supply – biomass production based on oxygen input.

(Or)

b. Explain the mass transfer taking place in fermentation process.

Growth of microbes – media formulation and biomass gain – different substrate utilization - acid and base utilization – growth phases and increase in cell biomass – steps to increase mass transfer rate - substrate : cell biomass volume – relation between different types of fermentor design and mass transfer.

26.a. Notes on the types of carbon sources from agricultural wastes employed in fermentation medium.

Role of carbon in microbial growth – cheap and cost effective carbon sources – carbon sequesterization in microbial cell – optimization of microbial growth based on carbon supplement – sugarcane molasses – corn steep liquor - bagasses.

(Or)

b. Describe the methods of preservation of microorganisms with its advantages and disadvantages.

Auxenic culture maintenance – mineral oil preservation – preservation using cryo preservation – deep freeze (– 80˚C) preservation – Liquid Nitrogen usage for preservation – criteria for long term preservation.

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

[16MBP205C]

KARPAGAM ACADEMY OF HIGHER EDUCATION

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

COIMBATORE – 641 021.

M.Sc. DEGREE EXAMINATION, APRIL 2017

MICROBIOLOGY

BIOPROCESS ENGINEERING

Time: 3 hours **Maximum: 60 marks**

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

(Multiple Choice Question No. 1 to 20 Online Exam)

1. 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
2. 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
3. The process of sterilization of wine introduced by Pasteur is called_____.
   (a)Pasteurization (b)Ultrafiltration
   (c)LTHT (d)HTLT
4. _____technique is largely employed to identify the strain producingextracellular growth factor
   (a)Crowded plate technique (b)Auxanography
   (c)Indicator dye (d)Enrichment technique
5. In lysine biosynthesis, the end products lysine and threonine inhibit _____ enzyme.
   (a)Aspartate kinase (b)Homoserine phosphatase
   (c) Serine kinase (d) Tryptophan synthase
6. 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
7. Buffering agents used in media formulation is_____
   (a)Mono and dihydropotassium phosphates (b)Tri-iodosodium phosphates
   (c)Tetraiodiosodium phosphates (d)Monophosphates
8. Which is the common raw material source used in fermentation process?
   (a)Food waste (b)Agricultural waste
   (c)Industrial toxic waste (d)Biofuel waste
9. _____culture where medium is fed to the culture resulting in an increases in volume
   (a) Batch (b)Fed batch
   (c)Continuous (d)Semi continuous
10. In _____ the solvent molecule are passed through a semi permeable membrane in opposite direction.
   (a) ultra filtration (b)reverse osmosis
   (c)liquid membranes (d)pumping
11. Steady state condition can be achieved by operation on _____ principles.
   (a)Chemostatic (b)Turbidostatic
   (c)Chemo-Turbidostat (d)Photostat
12. _____ of any product is often the last stage of a manufacturing process.
   (a)Filtration (b)Centrifugation
   (c)Drying (d)Packing

13. _____require biotin for growth in production medium.
    (a)Bacteria (b)Yeasts
    (c)Viruses (d)Phages
14. Griseofulvin is produced by _____.
    (a)*Penicillium patulum* (b)*Aspergillus*
    (c)*Pencilium chrysogenum* (d)*Mucor*
15. The production of amino acids through fermentation process was initiated by _____.
    (a)Kinoshita *et al* (b) Wells *et al*
    (c)Adamson *et al* (d)Dubey *et al*
16. Sulfite waste liquor contains _____sugars.
    (a)1% (b)2%
    (c)3% (d)4%
17. The solubility of oxygen in pure water is _____mg/l at $4^{\circ}C$.
    (a)2 (b)8
    (c)4 (d)1
18. Critical dissolved oxygen concentrations for *E. coli* is _____mM/dm^3
    (a)0.004 (b)0.008
    (c)0.022 (d)0.018
19. In _____ bioreactor are so designed that adequate supply of oxygen is obtained without agitation.
    (a)Air lift (b)CSTR
    (c)Packed bed (d)Fluidized bed
20. 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

PART-B (5x 6=30 marks)

(Answer all questions)

21. a. Elaborate on the basic design of a fermentor.

(Or)

b. With clear figure explain the principle and application of air lift reactors.

22. a. Explain the oxygen transfer mechanism from the medium to the cell.

(Or)

b. Discuss on importance of sterilization of fermentors, medium.

23. a. What is media formulation and ideal characteristics of fermentation medium?

(Or)

b. Write about the growth kinetics of batch fermentation.

24. a. What are the methods involved in of cell disintegration?

(Or)

b. Brief on methods of filters used and flocculation techniques.

25. a. Explain in detail about the primary screening methods.

(Or)

b. Write short notes on mutation methods used in strain improvement.

PART-C (1x 10=10 marks)

(Compulsory question)

26. Discuss on the preservation methods used for preserving microbial cultures with its advantages and disadvantages.

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

Set II

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

[5MBP205C]

KARPAGAM ACADEMY OF HIGHER EDUCATION

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

COIMBATORE – 641 021.

M.Sc. DEGREE EXAMINATION, APRIL 2015

MICROBIOLOGY

BIOPROCESS ENGINEERING

Time: 3 hours **Maximum: 60 marks**

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

(Multiple Choice Question No. 1 to 20 Online Exam)

1. Fermentation was first described as chemical process by_____.
   (a)Pasteur (b)Robert Koch
   (c)Liebig (d)Anton van Leuwenhoek
2. In 1873, _____described that yeasts are involved in fermentation process for alcohol production.
   (a)Pasteur (b)Schwann
   (c)Robert Koch (d)Berzelius
3. Enrichment culture technology was designed by soil microbiologist_____.
   (a)Pasteur (b)Koch
   (c)Ehrlich (d)Beijerinck
4. The suitable protective medium used at the _____is 10% inositol in dissolved water.
   (a)Commonwealth Mycological Institute (b)American type culture collection
   (c)African type culture collection (d)Indian Mycological Institute
5. The mutant strain of *Coryrebacterium glutamicus* produces upto _____g/L of lysine.
   (a)50 (b)60
   (c)70 (d)80
6. ATCC is the abbreviation of _____founded in 1925.
   (a)American type culture collection (b)African type culture collection
   (c)Auxenic type culture collection (d)Australian type culture collection
7. Membrane permeability is altered by changing the concentration of _____.
   (a)Fat (b)Carbohydrate
   (c)Phospholipids (d)Protein
8. Protoplast fusion is mainly used to improve_____industrial microbes
   (a)Algae (b)Fungi
   (c)Only yeast cells (d)Bacteria
9. _____is a culture system which contain a initial limited amount of nutrient
   (a)Fed batch (b)Batch
   (c)Semi continuous (d)Continuous
10. In Pencillin fementation the precursor added is _____.
   (a)phenyl acetic acid (b)benzyl alcohol
   (c)Benzene (d)pyridine
11. Cellulose are carbohydrates made of repeating units of _____.
   (a)α-glucose (b)β-glucose
   (c)α-galactose (d)β-galactose
12._____culture where medium is fed to the culture resulting in an increases in volume.
   (a)Fed batch (b)Batch
   (c)Semi continuous (d)Continuous

13. In _____bioreactor adequate supply of oxygen is obtained without agitation.

(a)Bubble columns (b)CSTR

(c)Packed bed (d)Fluidized bed

14. _____ industrial fermentation process is normally satisfied by aerating and agitating the fermentation broth.

(a)Mass transfer (b)Oxygen demand

(c)Carbon availability (d)Nitrogen availability

15. Critical dissolved oxygen concentrations for *Saccharomyces* sp is _____mMoles/dm^3

(a)0.004 (b) 0.008

(c)0.022 (d)0.01816. Mass transfer occurs in mixtures containing _____.

(a)Local concentration variation (b) Same concentration

(c)Different solute (d)Different solvent

17. An example of anionic surfactants is _____.

(a)Antiseptics (b)Shampoo

(c) Disinfectants (d) Emulsifiers

18. The ideal aspect ratio for a fermentor is _____.

(a)3:1 (b)4:1

(c)5:1 (d)6:1

19. Riboflavin fermentation employs media containing _____.

(a)Protein and lipid (b)Organic acid and inorganic acid

(c)Sugar and lipid (d)Protein

20. In _____ process the medium to be sterilized to maintained at particular temperature and time.

(a)Heat exchange (b)Holding coil

(c)Heat exchange, holding coil and cooler (d)Cooler

PART-B **(5x 6=30 marks)**

(Answer all questions)

21. a. How computers are used for control of fermentation process?

(Or)

b. With clear figure explain the principle and application of packed bed reactors?

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

(Or)

b. Explain the mass transfer mechanism.

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

(Or)

b. Write about the growth kinetics of batch fermentation.

24. a. Discuss on any one of the purification techniques used in down streaming process.

(Or)

b. Write a note on lyophilization with diagram.

25. a. Explain the secondary screening for strain isolation.

(Or)

b. Write notes on protoplast fusion technique used in strain improvement.

PART-C **(1x 10=10 marks)**

(Compulsory question)

26. Describe on the sterilization methods employed for fermentation medium, fermentor and air with clear figures.

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