BIO204 FINAL TERM SUBJECTIVE MEGA FILE

ALL DATA FROM PAST PAPERS (2018-2022)

GROUP: BIOTECH BRAINY BUNCH

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SHORT QUESTIONS

1. Differentiate between quasi steady state and steady state of chemo stat. Also write theeffect on the value of Y, U, max, Ks?

The major difference between steady state of chemo stat and quasi steady state of fed-batch culture is that μ is constant in the chemostate but decrease in fed batch Fed batch quasi steady state change over the time of the fermentation. Product concentration in a fed batch system over the time of fermentation will be dependent on the relationship between qp and μ (Hence D).

Quasi Steady State:

Thus, (ds/dt)≃0. Although the total biomass in the culture (X) increases with time, cell concentration (x) remains virtually constant, that is (dx/dt) ≃0. And therefore $\mu \simeq$ D. This situation is termed as quasi-steady state.

Steady State:

However, if an overflow device were fitted to the fermenter such as that the added medium displaced an equal volume of culture from the vessel then continuous production of cells could be achieved. If medium id fed continuously to such a culture at a suitable rate, steady state is achieved eventually, that is formation new biomass by the culture is balanced by the loss cells from the vessels.

Effects on Values of Y, U, max, Ks:

The characteristics of an organisms and (therefore its behavior in a chemo stat) are described by the numerical values of the constant Y, μmax and Ks. The value of Y affects the steady state biomass concentration, the value of $\mu max\ affects$ the maximum dilution rate that may be employed and the values affects the residual substrate concentration (and hence, the biomass concentration) and also the maximum dilution rate that may be used.

2. Differentiate between axial and radial flow / basic classification?

Axial Flow:

Axial flow impellers have low shear properties. The angle pitch of the agitators coupled with the thin trailing edges of the impeller blades reduce formation of the eddies in the wake of the moving blades. Axial flow impellers are pitched in an angle and thus direct the liquid flow towards the base of the bank. Example of axial flow mixing is considerably more energy efficient than radial flow mixing. They are more effective at lifting solids from the base of the tank.

Radial Flow:

As the high shear region exists only at the tip, the overall shear condition in the reactor is lower than would be generated by the radial flow impeller such as Rushton Turbine. Intermig impellers are used widely for agitation and aeration in fungal fermentation. High shear is effective at breaking up bubbles. For this reason, radial flow impellers are used for the culture of the aerobic bacteria.

3. Write down four accessories of fermentation process?

Accessories for a Fermenter

1. Baffle
2. Sterile compressed air (at 1,5 to 3.0 atmospheres)
3. Chilled water (12 to 15°C)
4. Cold water (4°C)
5. Hot water
6. Steam (High Pressure)
7. Steam Condensate
8. Electricity

9. Stand- by generator
10. Drainage of effluents
11. Motors
12. Storage facilities for media component

4. Convert Monod equation into linear equation?

The Monod equation is

$$\mu = \mu\, max\, \frac{S}{K_s+S}$$

This tells us that specific growth rate is generally found to be function of the three parameters

1. The concentration of growth limiting substrate (S)
2. The maximum specific growth rate (μmax)
3. A substrate-specific constant Ks

$$\mu = \mu\, max\, \frac{S}{K_s+S}$$

Taking the reciprocal values in the Monod equation and rearranging it

$$\mu = \frac{\mu\, max\, S}{K_s+S}$$

$$\frac{1}{\mu} = \frac{K_s + S}{\mu\, MAX\, S}$$

$$\frac{1}{\mu} = \frac{K_s}{\mu\, max\, S} + \frac{S}{\mu\, max\, S}$$

$$\frac{1}{\mu} = \frac{K_s}{\mu\, max} + \frac{1}{\mu\, max\, S}$$

$$\frac{1}{\mu} = \frac{1}{\mu\, max} + \frac{K_s}{\mu\, max\ S}$$

This equation is fit in linear form as Y = a + bx

Now by considering a = $\frac{1}{\mu\, max}$, b = $\frac{Ks}{\mu\, max\ S}$ and $\frac{1}{\mu}$ is the yield which is represented as Y.

Hence, the linear equation is

$$\frac{1}{\mu} = \frac{1}{\mu\, max} + \frac{K_s}{\mu\, max\ S}$$

5. What is the equation of the quasi-steady state?

Thus, (ds/dt)≃0. Although the total biomass in the culture (X) increases with time, cell concentration (x) remains virtually constant, that is (dx/dt) ≃0. And therefore $\mu \simeq$ D. This situation is termed as quasi-steady state.

6. What is the range / scale of fermenter in daily life?

Lab Scale Fermenters:
Ex-situ sterilization but seed fermenter is not required for inoculums development, slants and flask cultures can be utilized.

Pilot Scale Fermenters:

In-situ sterilization but seed fermenter is not required for inoculums developments, slants and flasks cultures can be utilized.

Industrial Scale Fermenters:

In-situ sterilization and seed fermenters are required for inoculums development, slants and flask cultures can be utilized.

7. What is agitators radial flow and axial flow characteristics?
Agitators are classified as having radial flow and axial flow characteristics.

i. With radial flow mixing, the liquids flow from the impeller is initially directed towards the walls of the reactor such as along the radius of the tank.

ii. Radial flow impellers are primarily used for gas-liquid contacting such as in the mixing of sparged bioreactors and blending processes.

iii. Radial flow impellers contain two or more impeller blades which are set at a verticalpitch.

iv. Radial flow mixing is not as efficient as axial flow mixing. For radial flow impellers, a much high energy is required to generate a given level of flow.

v. Radial flow impellers do and are designed to, generate high shear conditions. This isachieved by the formation of the vortices in the wake of the impellers.

vi. With axial flow mixing, the liquid from the impeller is directed downwards toward thebase of the reactor such as in the direction of the axis of the tank.

vii. Axial flow impellers provide more gentle but efficient mixing and are used for reactions involving shear sensitive cells and particles.

8. How bottom entry agitators and top entry agitators?

Top Entry Agitation:
The impellers shaft can enter from the bottom of the tank or from the top. A top entry impeller ("Overhung Shaft") is more expensive to install as the motor and shaft will need to be structurally supported.

Bottom Entry Agitation:

Bottom entry agitation tends to require more maintenance than top entry impeller due to the formation of crystals and other solids in the seals.

9. What is Fed-Batch culture?

Fed-Batch Culture:

Yoshida et al (1973), introduced this term to describe batch cultures which are fed continuously, or sequentially, with medium and without removal of the culture fluid. It is established initially in batch mode and then it is fed according to one of the following feed strategies.

i) The same medium used to establish the batch culture in added, resulting in an increase involume.

ii) A solution of the limiting substrate at the same concentration as that in the initial mediumis added, increase in volume.

iii) A concentrated solution of the limited substrate is added at the rate less than in step i andii which results in increase in volume.

iv) A very concentrated solution of the limited substrate is added at the rate less than in step i,ii, and iii, resulting in an insignificant increase in volume.

10. Why glass is used in fermenter?

In fermentation with strict aseptic requirements, it is important t select materials that can withstand repeated steam sterilization cycles. On a small (1 to 30 L) it is possible to use glass/ stainless steel. Glass is useful because it gives smooth surface, is nontoxic, corrosion proof and it is usually easy to examine the interior of the vessel. So, it is used.

11. **What is appropriate containment requirements can be applied for hazardous group?** When the organisms are allocated to a hazardous group, the appropriate containment requirements can be applied P, prevent release number. No detectable containment during work should be found in the air, working surface and personnel, unless required for productquality. The negative sign shows not required and positive sign means required.

12. What is chemo stat?

The growth of the cells in a continuous culture of this type is controlled by the availability of the growth limiting chemical components of the medium and, thus, this system is described as a chemo stat.

13. Give biomass relation U and max?

The final biomass concentration is produced when s=0 may be described as x max and, provided ≃ that xo is small as compared with x max: x max Y.SR. If at the time when x=x' a medium feed is started such as that dilution rate is less than μmax, virtually all the substrate will be consumed as fast as it enters the culture.

14. What are important things to consider for the fermentation of the fermenter?

In fermentation with strict aseptic requirements, it is important to select materials that can withstand repeated steam sterilization cycles. On a small (1 to 30 L) it is possible to use glass/ stainless steel. Glass is useful because it gives smooth surface, is nontoxic, corrosion proof and it is usually easy to examine the interior of the vessel. So, it is used.

15. G and Y stands for in the equation Dt/dt=GY?

G stands for the substrate feed rate (gdm-3 h-1) and Y stands for the yield factor.

16. On which factor, productivity of biological products depends upon?

Better productivity of biological products depends on:

1. Strain improvement

2. Media development
3. Process control and design

17. Derive the relationship equation for change in biomass and change in time?

Cell density (Xt) after time "t" will be
Xt = Xo2n = Xo2t/td
X/t
DX/dt
Change in biomass change in time

DX = $\mu.X.dt$
DX/dt= $\mu.X$
Where, X = concentration of microbial biomass
t= time (mostly in hours)
μ= is the specific growth rate per unit cell mass
On applying integration equation this equation (dX/dt=$\mu.X$ becomes:
Xt=xo eμt Xo= original biomass concentration
Xt= biomass concentration after time interval
To convert equation xt=x eμt into linear equation, take natural logarithm by which this equation becomes:
In Xt=InXo+μt (In e)

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Xt=xo eμt Xo= original biomass concentration
Xt= biomass concentration after time interval
To convert equation xt=x eμt into linear equation, take natural logarithm by which this equation becomes:
In Xt=InXo+μt (In e)
In Xt=InXo+μt because Ine=1
This equation fit to linear regression
Y= a + bx

19. **Can change in result find in chemo stat experiment and predicted/ foregoing theory?**

The results of the chemo stat experiments are differ from those predicted by the foregoing theory. The reasons for these deviations may be anomalies associated with equipment or the theory not predicting the behavior of organisms under certain circumstances. Practical anomalies include imperfect mixing and wall growth. Imperfect mixing would cause an increase in the degree of homogeneity in the fermenter when some organisms being subjected to nutrient excess while other are under severe limitations.
This method is particularly relevant to very low diluted rate system when the flow of medium is likely to be very intermittent. This problem could be overcome by use of feedback system. Wall growth is also another commonly encountered difficulty in which organisms adhere to inner surface of reactor that result in increase in homogeneity.

20. How we can calculate live and dead cell?

Cell growth is also measured by counting total cell number of the microbes present in that sample. Total cells (live and dead) of liquid sample are counted by using a Hemocytometer or Neubauer Chamber.
If there is dilute culture, direct cell counting can be done. However, the cell culture of high density can be diluted. Otherwise clumps of the cells would be formed which could create problem in exact counting of bacterial cells. The immobilized cells are not subjected to removal from the vessel but will consume substrate resulting in the suspended biomass concentration being lower than predicted. Wall growth may be limited by coating the inner surface of the vessel with Teflon.

21. Relationship between qp and u according to fed batch profile?

Pirt (1979) described the product balance in a fixed volume fed-batch system as:
dp / dt=qpx
butt substituting for x from equation gives:
dp/dt = qp(xa + GYt)
If qp is strictly growth-rate related then product concentration will rise linearly as for biomass. However, if qp is constant then the rate of increase in product concentration will rise as growth rate declines, i.e.as time progresses and x increases. If qp is related to μ in a complex manner then the product concentration will vary according to that relationship. As in the case of variable volume fed-batch the feed profile would be optimized according to the relationship between qp and μ.
For example, qP = YP/x.μ

22. Why the bottom entry agitators tend to require more maintenance than top entryimpellers?

A reactor with bottom entry impeller however will need higher maintenance due to damage of the seal by particulate in the medium and by medium components that crystallize in the seal when reactor is not in use.
Bottom entry agitators tend to require more maintenance than top entry impellers due to the formation of crystals and other solids in the seals.

23. Which is used as an alternative type in microbial growth?

An alternative type of continuous culture to the chemo stat is the turbidostat where the concentration of cells in the culture is kept constant by controlling certain, narrow limits. This may be achieved by monitoring the biomass with a photoelectric cell and feeding the single to a pump supplying medium to the culture such that the pump is switched on if the biomass exceeds the set point and is switched off if the biomass falls below the set point. Systems other than turbidity may be used to monitor the biomass concentration, such as CO_2 concentration or pH in which case it would be more correct to term the culture a biostate. The chemo stat is more commonly used system because it has the advantage over biostate of not requiring complex control systems to maintain a steady state. However, the biostate may be advantageous in continuous enrichment culture in avoiding the total washout of the culture in the early stages.

24. Give two risks/defects given by Collins (1992)?

1. The known pathogenicity of the micro-organisms.

2. The virulence or pathogenicity of the micro-organisms is the diseases it causes mild orserious

3. The number of micro-organisms required to initiate an infection.

4. The routes of infection.

5. The known incidence of infection in the community, and the existence locally of vectorsand potential reserves.

6. The amounts and volumes of the organisms used in the fermentation process.

7. The techniques and processes used.

8. Ease of prophylaxis and treatments.

25. Write down four general requirements of fermenters / bioreactors?

There is no universal bioreactor. The general requirements of bioreactor are as follow:

1. The vessel should be capable of being operated aseptically for no. of days and should bereliable in long term operations and meet the requirements of containment regulations.

2. Adequate aeration and agitation should be provided to meet the metabolic requirements ofthe micro-organisms. However, the mixing should not cause any damage to the agitation.

3. Power consumption should be as low as possible.

4. A system of temperature control should be provided.

5. Sampling facilities should be provided.

6. A system of pH control should be provided.

7. Evaporation losses from fermenter should not be excessive.

8. The vessel should be designed to require minimal use of labor in operation, harvesting,cleaning and maintenance.

9. Ideally vessel should be suitable for range of processes, but this may be restricted becauseof containment regulations.

10. The vessel should be constructed to ensure smooth internal surface, using welds insteadof flange joints whenever possible.

11. The vessel should be of similar geometry to both smaller and larger vessels in the pilotplan or plan to facilitate scale-up.

12. The cheapest material should enable satisfactory results to be achieved should be used.

13. There should be adequate service provisions for individual plants.

26. Write down the characteristics of the standard fermenter?

1. Reliable aseptic seal is made between glass and glass, glass and metal or metal and metaljoints such as between a fermenter vessel and a detachable top or base plate.

2. With glass and metal, a seal can be made with a compressible gasket, a lip seal or an O'ring. With metal to metal, joints only an O' ring is suitable. This is placed in a groove, machined either in the end plate, the fermenter body or plate.

3. The seal ensures that a good liquid or gas-tight joint is maintained in spite of the glass ormetal expanding or contracting at different rates with change in temperature during a sterilization cycle or an incubation cycle.

4. Nitryl or butyl rubbers are normally used for these seals as they will withstand fermentation process conditions. These rubber seals have finite life and should be checkedregularly for damage or perishing.

27. Describe relationship between qp and u?

Product concentration in a fed batch system over the time of the fermentation will be dependant on the relationship between qp and u. If qp is growth related than it will change as u thus, the product concentration remains constant. If qp is constant and independent of uthan product concentration will start of the cycle when Dp is greater $q_p x$ but will rise with time as D decreases and $q_p x$ become greater than Dp.

28. Write two functions of agitation?

Agitation plays an important role in mixing and shearing fermentation process. It not only improves mass and oxygen transfer between different phases, but also maintains homogenous chemical and physical conditions in the medium by continuous mixing.

29. What is sterilization?

Removal of microbes from the fermentation media or equipment is called sterilization.

30. Calculate the del factor?

By combining together equations In ($N_{t\,/\,N_\circ}$) = -kt and k = $A^{-E/RT}$, the following expression may be derived for the heat sterilization of a pure culture at a constant temperature:

$$\text{In } N_{t\,/\,N_\circ} = A \cdot t \cdot e^{-E/RT},$$

Deindoerfer and Humphrey (1959) used the term In $N_{o/\,N_t}$ as a design criterion for sterilization, which has been variously called the del factor, Nabla factor and sterilization criterion represented by the term ∇. Thus, the Del factor is a measure of the fractional reduction in viable organism count produced by a certain heat and time regime. Therefore:

$$\nabla = In\ (N_\circ /\ N_t)$$

But $In\ (N_\circ /\ N_t)$ = kt

And kt = = $A \cdot t \cdot e^{-E/RT}$

Thus $\nabla = A \cdot t \cdot e^{-E/RT}$

On rearranging, above equation become

In t = E/RT + In (∇ / A)

31. What is freezing dry?

Freezing drying is an important operation in the production of many biologicals and pharmaceuticals. Material is first frozen and then dried by sublimation in a high vacuum. The great benefit of this technique is that it does not harm heat sensitive materials.

32. How sterilization produce exhaus air?

In many traditional fermentations the exhaust gas from the fermenter was vented without sterilization or vented through relatively inefficient depth filters. With the advent of the use of recombinant organisms and a greater awareness of safety and emission levels of allergic compounds the containment of exhaust air is more common (and in the case of recombinant organisms, compulsory). Fixed pore membrane modules are also used for this application but the system must be able to cope with the sterilization of water saturated air, at a relatively high temperature and carrying a large contamination level. Also, foam may overflow from the fermenter into the air exhaust line. Thus, some form of pretreatment of the exhaust gas is necessary before it enters the absolute filter. This pretreatment may

be a hydrophobic prefilter or a mechanical separator to remove water, aerosol particles and foam. The pretreated air is then fed to a 0.2µm hydrophobic filter. Again, it is important to appreciate that the filtration system must be steam sterilizable. Figures in next slides illustrate the prefilter and mechanical separator systems respectively.

33. Describe filter sterilization in detail?

As for the filter sterilization of liquids it is essential that a prefilter is incorporated up-stream of the absolute filter. The prefilter traps large particles such as dust, oil and carbon (from the compressor) and pipescale and rust (from the pipework). The use of a coalescing prefilter also ensures the removal of water from the air; entrained water is coalesced in the filter (air flow being from the inside of the filter to the outside) and is discharged via an automatic drain.

34. Discuss the batch culture and continuous culture?

Batch Culture: Batch culture is a technique used to grow micro-organisms under limited nutrients availability in a closed system.

Continuous Culture: Continuous cultures is a technique used to grow micro-organisms under optimum and continual supply of nutrients in an open system in industries.

35. Write factors consider when designing a fermenter?

Basic features of a bioreactor associated for monitoring, control & record.

- An agitation system
- An oxygen delivery system
- A foam control system
- A temperature control system
- A pH control system
- A sterilization and cleaning system
- A sump and dump line system (only on pilot and industrial scale)

36. What is Moist heat sterilization technique?

The destruction of microorganisms by steam (moist heat) may be described as a first-order chemical reaction and, thus, may be represented by the following equation:

$$-dN / dt = kN$$

Where N = no. of viable organism present

t = time of sterilization treatment

k = reaction rate constant of the reaction or 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 (in previous slide) the following expression is obtained:

$$In\ (N_{\circ} / N_t) = \text{-kt}$$

37. What are the consequences of any contamination in fermentation process?

Containment guidelines were initiated during the 1970s. To establish the appropriate degree of containment which will be necessary to grow a micro- organism, it, and in fact the entire process, must be carefully assessed for potential hazards that could occur should there be accidental release. Different assessment procedures are used depending on whether or not the organism contains foreign DNA (genetically engineered). Once the hazards are assessed, an organism can be classified into a hazard group for which there is an appropriate level of containment.

38. Write the types of reaction contributed during sterilization to loss the quality of material?

Interactions between nutrient components of the medium: A common occurrence during sterilization is the Maillard- type browning reaction which results in discoloration of the medium as well as loss of nutrient quality.

Degradation of heat labile components: Certain vitamins, amino acids and proteins may be degraded during a steam sterilization regime. In extreme cases, such as the preparation of media for animal- cell culture, filtration may be used.
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41. Write effluent of disposal method?

1. The effluent is discharged to land, river or sea in an untreated state.
2. The effluent is removed and disposed of in a landfill site or is incinerated.
3. The effluent is partially treated on site prior to further treatment or disposal by one of theother routes indicated.
4. Part of the effluent is untreated and discharged as in 1 or 2, the remainder is treated at asewage works or at the site before discharge.
5. All of the effluent is sent to the sewage works for treatment, although there might be reluctance by the sewage works to accept it, possibly resulting in some preliminary on-sitetreatment being required, and discharge rates and effluent composition defined.
6. All the effluent is treated at the factory before discharge.

42. What the effect if low speed of impeller?

Another consequence of too slow impeller speed is a flooded impeller. ▪Under these conditions, the bubbles will accumulate and coalesce under the impeller, leading to the formation of large bubbles and poor oxygen transfer rates.A similar phenomenon will happen when aeration rate is too high. In this case, the oxygen transfer efficiency will be low.

43. Common modification of chemostat?

The basic chemostat may be modified in a number of ways, but the most common modifications are the addition of extra stages (vessels) and the feedback of biomass into the vessel.

44. Why filtration is used for animal cell culture media instead of autoclaving?

Media may be sterilized by filtration, radiation, ultrasonic treatment, chemical treatment or heat. However, for practical reasons, steam is used almost universally for the sterilization of fermentation media. The major exception is the use of filtration for the sterilization of

media for animal-cell culture - such media are completely soluble and contain heat labile components making filtration the method of choice. Filtration techniques will be considered in later modules.
Degradation of heat labile components. Certain vitamins, amino acids and proteins may be degraded during a steam sterilization regime. In extreme cases, such as the preparation of media for animal- cell culture, filtration may be used.

45. PH control system in fermenter?

The pH control system consists of: a pH probe, alkali delivery system & acid delivery system. The pH probe is typically steam sterilizable. The pH control system (and indeed all

other fermenter control systems) are designed to have a dead-band. A dead-band is used to prevent excessive alkali and acid addition. The set-point is the pH at which the fermenter is being attempted to be controlled at. For example, if the fermentation is to be run at a constant pH of 6.5, then the set-point is set to 6.50.
If for example, a 5% dead-band is used, then the upper dead-band limit will be
1.05 x 6.5 = 6.83
and the lower dead-band limit will be
0.95 x 6.5 = 6.18
If the dead-band is too small, then it is possible that pH will often overshoot and undershoot the dead-bands leading to excessive alkali and acid addition. The trade off is that a wide dead-band will lead to less precise pH control.

46. Five techniques roffer et al 1984?
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48. Five techniques roffer et al 1984?

1. Vacuum and flash fermentations for the recovery of ethanol from fermentation broths. 2.
2. Extractive fermentation (liquid-liquid and phase aqueous) for the recovery of organicacids and toxin produced by Clostridium tetani.

3. Adsorption for the recovery of ethanol and cycloheximide.

4. Ion-exchange in the extraction of salicylic acid and antibiotics.

5. Dialysis fermentation in the selective recovery of lactic acid, salicylic acid and cycloheximide.

49. Advantages of spiral heat exchanger?

1. The two streams of medium and cooling liquid, or medium and steam, are separated by acontinuous stainless steels barrier with gasket seals being confined to the joints with the endplates. This makes cross contamination between the two streams unlikely.

2. The spiral route traversed by the medium allows sufficient clearances to be incorporated for the system to cope with suspended solids. The exchanger tends to be self-cleaning whichreduces the risk of sedimentation, fouling and 'burning- on'.

50. What are types of sterilization?

There are two types of continuous sterilizer which may be used for the treatment of fermentation media: the indirect heat exchanger and the direct heat exchanger (steam injector).

51. How many times liquid volume changed?
In terms of cell and substrate concentrations, steady- state condition can be ensured by allowing at least 4 changes of fermenter liquid volume.

52. What is batch fermentation?
Batch fermentation is a process where all the substrate and nutrients are added at zero time or soon after inoculation takes place, and the vessel is allowed under a controlled environment to proceed until maximum end product concentration is achieved.

53. Describe positive Pressure?
During sterilization the concept of "maintaining positive pressure" is often used. Maintaining positive pressure means that during sterilization, cooling and filling and if appropriate, the fermentation process, air must be pumped into the reactor. In this way the reactor is always pressurized and thus aerial contaminants will not be "sucked" into the reactor. It is very important that positive pressure is maintained when the bioreactor is cooled following sterilization. Without air being continuously pumped into the reactor, a vacuum will form and contaminants will tend to be drawn into the reactor.

54. Fermentation and its steps?
Fermentation: It is a metabolic process that produces chemical changes in organic substrate through the action of enzymes. In biochemistry, it is narrowly defined as the extraction of energy from carbohydrates in the absence of oxygen.
Steps: Fermentation consists of four stages including:
1. Inoculum preservation
2. Inoculum Build-up
3. Pre-fermenter culture
4. Production fermentation

55. What is streaking?
In microbiology, streaking is a technique used to isolate a pure strain from a single species of microorganism, often bacteria. Samples can then be taken from the resulting colonies and a microbiological culture can be grown on a new plate so that the organism can be identified, studied, or tested.

56. How we sterilized large Volume fermenter?

A spray drier is most widely used for drying of biological materials when the starting material is in the form of a liquid or paste. The material to be dried does not come into contact with the heating surfaces, instead, it is atomized into small droplets through for example a nozzle or by contact with a rotating disc. Spray driers are the most economical available handling large volumes, and it is only at feed below 6 kg min^{-1}that drum driers become economic.

57. Which technique are use to separate the solid from liquid?

Suspended solids maybe separated from a fluid during filtration by the following mechanisms:

i. Inertial impaction.
ii. Diffusion.
iii. Electrostatic attraction.
v. Interception.

58. Write factors that are investigate during industrial operations of economical waste?

1. Daily flow rate
2. Fluctuations in daily, weekly and seasonal flow
3. BOD/COD
4. Suspended
5. Turbidity
6. pH range
7. Temperature range
8. Odours and tastes
9. Hardness
10. Color
11. Detergents
12. Radioactivity
13. Presence of specific toxins and inhibitors

59. Write parts of fermemter?

1. An agitation system
2. An oxygen delivery system
3. A foam control system
4. A temperature control system
5. A pH control system
6. A sterilization and cleaning system
7. A sump and dump line system (only on pilot and industrial scale)

60. What is Foaming control process?

Foam control is an essential element of the operation of a sparged bioreactor. The following photograph shows the accumulation of foam in a 2 liter laboratory reactor. Excessive foam formation can lead to blocked air exit filters and to pressure build up in the reactor. The latter can lead to a loss of medium, damage to the reactor and even injury to operating personnel. Foam is typically controlled with aid of antifoaming agents based on silicone or on vegetable oils. Excessive antifoam addition can however result in poor oxygen transfer rates.

61. Describe air exust filtration?

In many traditional fermentations the exhaust gas from the fermenter was vented without sterilization or vented through relatively inefficient depth filters. With the advent of the use of recombinant organisms and a greater awareness of safety and emission levels of allergic compounds the containment of exhaust air is more common (and in the case of recombinant organisms, compulsory). Fixed pore membrane modules are also used for this application but the system must be able to cope with the sterilization of water saturated air, at a relatively high temperature and carrying a large contamination level. Also, foam may overflow from the fermenter into the air exhaust line.
Thus, some form of pretreatment of the exhaust gas is necessary before it enters the absolute filter. This pretreatment may be a hydrophobic prefilter or a mechanical separator to remove water, aerosol particles and foam.
The pretreated air is then fed to a 0.2µm hydrophobic filter. Again, it is important to appreciate that the filtration system must be steam serializable. Figures in next slides illustrate the prefilter and mechanical separator systems respectively.

62. Modify broth medium?
Broth medium are liquid cultures used to grow bacteria in laboratories. To create a broth culture, a scientist being with a sterile liquid growth medium. The medium is inoculated with bacteria and placed in an incubator at the appropriate temperature.

63. Lag and log phase?
Lag phase is the period when the bacteria are adjusting to the environment. Following, the lag phase is the log phase, in which population grows in a logarithmic fashion.

64. What is clearing and sterilization?
Small scale reactors are taken apart and then cleaned before being re-assembled, filled and then sterilized in an autoclave. However, reactors with volumes greater than 5 liters cannot be placed in an autoclave and sterilized. These reactors must be cleaned and sterilized "in place". This process is referred to "Clean in Place".
CIP involves the complete cleaning of not only the fermenter but also all lines linked to the internal components of the reactor. Steam, cleaning and sterilizing chemicals, spray balls and high pressure pumps are used in these processes. The process is usually automated to minimize the possibility of human error.

65. **Write four mechanisms of sterilization of solid form liquid fermentation?**
Suspended solids maybe separated from a fluid during filtration by the following mechanisms:

i. Inertial impaction.
ii. Diffusion.
iii. Electrostatic attraction.
v. Interception.

66. Importance of sterilization of inlet air from fermentation?
Sterilization of the inlet air is undertaken to prevent contaminating organisms from entering the reactor. The exit air on the other hand is sterilized not only to keep contaminants from entering but also to prevent organisms in the reactor from contaminating the air. A common method of sterilizing the inlet and exit air is filtration. For small reactors (with volumes less than 5 litres), disk shaped hydrophobic Teflon membranes housed in a polypropylene housing are used. Teflon is tough, reusable and does not readily block. Sterilization of the inlet and

exit air in large bioreactors (>**10,000** liters) can present a major design problem. Large scale

membrane filtration is a very expensive process. The filters are expensive as they are difficult to make and the energy required to pass air through a filter can be quite considerable.

67. Why chemostateis used?

The chemostate is the more commonly used system because it has the advantage over the biostate of not requiring complex control systems to maintain a steady state. However, the biostate may be advantageous in continuous enrichment culture in avoiding the total washout of the culture in its early stages.

68. What are inlet and exhaust system?

Inlet System:

Sterilization of the inlet air is undertaken to prevent contaminating organismsfrom entering the reactor. The exit air on the other hand is sterilized not only to keep contaminants from entering but also to prevent organisms in the reactor from contaminating the air. A common method of sterilizing the inlet and exit air is filtration. For small reactors (with volumes less than 5 litres), disk shaped hydrophobic Teflon membranes housed in a polypropylene housing are used. Teflon is tough, reusable and does not readily block.

Exhaust System:

In many traditional fermentations the exhaust gas from the fermenter was vented without sterilization or vented through relatively inefficient depth filters. With the advent of the use of recombinant organisms and a greater awareness of safety and emission levels of allergic compounds the containment of exhaust air is more common (and in the caseof recombinant organisms,

compulsory). Fixed pore membrane modules are also used for this application but the system must be able to cope with the sterilization of water saturated air, at a relatively high temperature and carrying a large contamination level. Also, foam may overflow from the fermenter into the air exhaust line.

69. Define Batch biomass?

Biomass is organic, meaning it is made of material that comes from living organisms, such as animals and plants. The most common biomass materials are used for energy are plants, wood and waste. These are called biomass feedbacks. Biomass energy can also be a non-renewable energy source.

70. What is advantage of anaerobic treatment of organic waste material?

Sludge conditioning and disposal; physical, chemical and biological methods. Anaerobic digestion is often used to condition (make it more amenable to dewatering) the sludge produced in previous stages. Following dewatering (e.g. by centrifugation using a decanter centrifuge) the sludge can then be disposed of by incineration, landfilling, etc. The basic activated-sludge process consists of aerating and agitating the effluent in the presence of a flocculated suspension of micro-organisms on particulate organic matter - the activated sludge. This process in most widely used biological treatment process for both domestic and industrial wastewaters. A number of modifications of the basic process can be used to improve treatment efficiency, or for a more specific purpose such as denitrification. Tapered aeration and stepped feed aeration are used to balance oxygen demand with the amount of oxygen supplied.

71. Define contamination and contaminant?

Contamination: Contamination is the presence of a constituent, impurity, or some other undesirable element that spoils, corrupts, infects, make unfit or makes inferior a material, physical body, natural environment or workplace.

Contaminant: Any physical, chemical, biological or radiological substance that makes any matter impure is known as contaminants.

72. What are chemical methods to disrupt cell?

Chemically, cells are disrupted by using following methods;

1. Detergents
2. Osmotic Shock
3. Alkali Treatment
4. Enzyme Treatment

73. What is end product of fermentation?

The products produced by the fermentation: Secreted proteins or nucleic acids released as a result of cell death and hydrolysis have detergent like properties.

74. Expain Richard fast method of sterilization cycling?

Richards (1968) proposed a rapid method for the design of sterilization cycles avoiding the time consuming graphical integrations. The method assumes that all spore destruction occurs at temperatures above 100°C and that those parts of the heating and cooling cycle above 100°C are linear. Both these assumptions reasonably valid and the technique loses very little in accuracy and gains considerably in simplicity.

Furthermore, based on these assumptions, Richards has presented a table of Del factors for *B. stearothermophilus* spores which would be obtained in heating and cooling a broth up to (and down from) holding temperatures of 101- 130°C, based on a temperature change of 1°C per minute.

75. What is main object of first stage all process of fermentation?

The main objective of the first stage for the recovery of an extracellular product is the removal of large solid particles and microbial cells usually by centrifugation or filtration

76. Define trophase and idiophase?

Tropophase: (Refers to exponential phase- stage where primary metabolites are produced).
Idiophase: (Usually refers to stationary or death phase- where secondary, tertiary or other metabolites are produced).

77. What is working and principal of Autoclave?

An autoclave: The entering steam forces the air out of the bottom (blue arrows). The automatic ejector valve remains open as long as an air-steam mixture is passing out of the waste line. When all the air has been ejected, the higher temperature of the pure steam closes the valve, and the pressure in the chamber increases. A holding vessel for the batch sterilization of waste. Whichever method is employed the effluent must be cooled to below 60°C before it is discharged to waste. The sterilization processes have to be validated and are designed using the Del factor approach. However, the kinetic characteristics used in the calculations would be those of the process organism rather than of *B. stearothermophilus*. Also, the N_t value used in the design calculations would be smaller than 10^{-3} which is used for medium sterilization and would depend on the assessment of the hazard involved should the organism survive the decontamination process. Thus, the sterilization regime used for destruction of the process organism will be different from that used in sterilizing the medium.

78. Write techniques of sterilization?

1. Moist heat method
2. Dry heat method

79. Richards rule briefly explain?

Richards (1968) proposed a rapid method for the design of sterilization cycles avoiding the time consuming graphical integrations. The method assumes that all spore destruction occurs at temperatures above 100°C and that those parts of the heating and cooling cycle above 100°C are linear. Both these assumptions reasonably valid and the technique loses very little in accuracy and gains considerably in simplicity. Furthermore, based on these assumptions, Richards has presented a table of Del factors for B. stearothermophilus spores which would be obtained in heating and cooling a broth up to (and down from) holding temperatures of 101- 130°C, based on a temperature change of 1°C per minute.

80. What is the first stage recovery of fermentation?

The main objective of the first stage for the recovery of an extracellular product is the removal of large solid particles and microbial cells usually by centrifugation or filtration

81. Characteristics of fermenter seal?

1. Reliable aseptic seal is made between glass and glass, glass and metal or metal and metaljoints such as between a fermenter vessel and a detachable top or base plate.
2. With glass and metal, a seal can be made with a compressible gasket, a lip seal or an ‘O'ring. With metal to metal joints only an ‘O' ring is suitable. This is placed in a groove, machined in either the end plate, the fermenter body or both.
3. This seal ensures that a good liquid-and/or gas-tight joint is maintained in spite of the glass or metal expanding or contracting at different rates with changes in temperature

duringa sterilization cycle or an incubation cycle.

4. Nitryl or butyl rubbers are normally used for these seals as they will withstand fermentation process conditions. These rubber seals have a finite life and should be checkedregularly for damage or perishing.

82. Richard sterilization cycle?

Richards (1968) proposed a rapid method for the design of sterilization cycles avoiding the time consuming graphical integrations. The method assumes that all spore destruction occurs at temperatures above 100°C and that those parts of the heating and cooling cycle above 100°C are linear. Both these assumptions reasonably valid and the technique loses very little in accuracy and gains considerably in simplicity.

83. Write function of compressor?

A compressor forces the air into the reactor. The compressor will need to generate sufficient pressure to force the air through the filter, sparger holes and into the liquid. Air compressors used for large scale bioreactors typically produce air at **250** kPa. The air should be dry and oil free so as to not block the inlet air filter or contaminate the medium. It is very important that an "instrument air" compressor is not used. Instrument air is typically generated at higher pressures but is aspirated with oil. Instrument air compressors are used for pneumatic control.

Long questions

1. Briefly discuss the batch and continuous culture

Batch Culture:

In batch processing, a batch of culture medium in a fermenter is inoculated with a microorganism (the 'starter culture'). Fermentation proceeds for a certain duration (the 'fermentation time' or 'batch time'), and the product is harvested at the. It is a closed system which contains an initial limited amount of nutrient. We put all the things at one time here and 's' (concentration of substrate) will remain same. Minor change in volume.

Continuous Culture:

Exponential growth in batch culture may be prolonged by the addition of fresh medium to the vessel. Provided that the medium has been designed such that growth is substrate limited (i.e. by some component of the medium), and not toxin limited, exponential growth will proceed until the additional substrate is exhausted. This exercise may be repeated until the vessel is full. However, if an overflow device were fitted to the fermenter such that the added medium displaced an equal volume of culture from the vessel then continuous production of cells could be achieved.

2. What is spiral heat exchanger and what is the advantage of using it

The most suitable indirect heat exchangers are of the double-spiral type which consists of two sheets of high grade stainless steel which have been curved around a central axis to form a double spiral. Spiral heat exchangers are also used to cool the medium after passing through the holding coil. Incoming unsterile medium is used as the cooling agent in the first cooler so that the incoming medium is partially heated before it reaches the sterilizer and, thus, heat is conserved. The major advantages of the spiral heat exchanger are:

i. The two streams of medium and cooling liquid, or medium and steam, are separated by acontinuous stainless steel barrier with gasket seals being confined to the joints with the endplates. This makes cross contamination between the two streams unlikely.

ii. The spiral route traversed by the medium allows sufficient clearances to be incorporated for the system to cope with suspended solids. The exchanger tends to be self-cleaning whichreduces the risk of sedimentation, fouling and 'burning on.

3. Explain filter sterilization in detail

FILTER Sterilization

Suspended solids maybe separated from a fluid during filtration by the following mechanisms:

i. Inertial impaction.

ii. Diffusion.

iii. Electrostatic attraction.

iv. Interception.

Inertial Impaction

Suspended particles in a fluid stream have momentum. The fluid in which the particles are suspended will flow through the filter by the route of least resistance. However, the particles, because of their momentum, tend to travel in straight lines and may therefore become impacted upon the fibers where they may then remain. Inertial impaction is more significant in the filtration of gases than in the filtration of liquids.

Diffusion

Extremely small particles suspended in a fluid are subject to Brownian motion which is random movement due to collisions with fluid molecules. Thus, such small particles tend to deviate from the fluid flow pattern and may be come impacted upon the filter fibres. Diffusion is more significant in the filtration of gases than in the filtration of liquids

Electrostatic attraction

Charged particles may be attracted by opposite charges on the surface of the filtration medium.

Interception

The fibres comprising a filter are interwoven to define openings of various sizes. Particles which are larger than the filter pores are removed by direct interception. However, a significant number of particles which are smaller than the filter pores are also retained by interception. This may occur by several mechanisms – more than one particle may arrive at a pore simultaneously, an irregularly shaped particle may bridge a pore, once a particle has been trapped by a mechanism other than interception the pore may be partially occluded enabling the entrapment of smaller particles. Interception is equally important a mechanism in the filtration of gases and liquids. Filters have been classified into two types – those in which the pores in the filter are smaller than the particles which are to be removed and those in which the pores are larger than the particles which are to be removed.

4. Design of continuous sterilization

Continuous sterilization cycle:

The design of continuous sterilization cycles may be approached in exactly the same way as for batch sterilization systems. The continuous system includes a time period during which the medium is heated to the sterilization temperature, a holding time at the desired temperature and a cooling period to restore the medium to the fermentation temperature. The temperature of the medium is elevated in a continuous heat exchanger and is then maintainedin an insulated serpentine holding coil for the holding period. The length of the holding period is dictated by the length of the coil and the flow rate of the medium. The hot medium is then cooled to the fermentation temperature using two sequential heat exchangers - the firstutilizing the in-coming medium as the cooling source (thus conserving heat by heating-up theincoming medium) and the second using cooling water. The major advantage of the continuous process is that a much higher temperature may be utilized, thus reducing the holding time and reducing the degree of nutrient degradation. The required Del factor may beachieved by the combination of temperature and holding time which gives acceptably small degree of nutrient decay. There are two types of continuous sterilizer which may be used for the treatment of fermentation media: the indirect heat exchanger and the direct heat exchanger (steam injector).

5. Separate medium of batch sterilization advantages air disadvantages

Advantages:

The major advantages of a separate medium sterilization vessel may be summarized as:

i. One cooker may be used to serve several fermenters and the medium may be sterilized asthe fermenters are being cleaned and prepared for the next fermentation, thus saving time between fermentations.

ii. The medium may be sterilized in a cooker in a more concentrated form than would be usedin the fermentation and then diluted in the fermenter with sterile water prior to inoculation. This would allow the construction of smaller cookers.

iii. In some fermentations, the medium is at its most viscous during sterilization and the power requirement for agitation is not alleviated by aeration as it would be during the fermentation proper. Thus, if the requirement for agitation during in situ sterilization were removed, the fermenter could be equipped with a less powerful motor. Obviously, the sterilization kettle would have to be equipped with a powerful motor, but this would providesterile medium for several fermenters.

iv. The fermenter would be spared the corrosion which may occur with medium at hightemperature.

Disadvantages:

The major disadvantages of a separate medium sterilization vessel may be summarized as:

I. The cost of constructing a batch medium sterilizer is much the same as that for the fermenter.

ii. If a cooker serves a large number of fermenters complex pipework would be necessary totransport the sterile medium, with the inherent dangers of contamination.

iii. Mechanical failure in a cooker supplying medium to several fermenters would render allthe fermenters temporarily redundant. The provision of contingency equipment may be prohibitively costly.

6. What use for the stop of waste production in fermentation?

Every fermentation plant utilizes raw materials which are converted to a variety of products. Depending on the individual process, varying amounts of a range of waste materials are produced. Typical wastes might include unconsumed inorganic and organic media components, microbial cells and other suspended solids, filter aids, waste wash water from cleansing operations, cooling water, water containing traces of solvents, acids, alkalis, human sewage, etc. Historically, it was possible to dispose of wastes directly to a convenient area of land or into a nearby watercourse. This cheap and simple method of disposal is now very rarely possible nor is it environmentally desirable. Water authorities and similar bodies have become more active in combating pollution caused by domestic and industrial wastes.

Legislation in all developed countries now regulates the discharge of wastes. With liquid wastes, it may be possible to dispose of untreated effluents to a municipal sewage treatment works (STW). Obviously, much will depend on the composition, strength and volumetric flow rate of the effluent. STWs are planned to operate with an effluent of a reasonably constant composition at a steady flow rate. Thus, if the discharge from an industrial process is large in volume and intermittently produced it may be necessary to install storage tanks on site to regulate the effluent flow. Normally, fermentation effluents do not contain toxic materials which directly affect the aquatic flora or fauna.

7. How to avoid contamination in fermentation process?

Avoidance of contamination

It may be achieved by:

I. Using a pure inoculum to start the fermentation.

ii. Sterilizing the medium to be employed.

iii. Sterilizing the fermenter vessel.

Iv. Sterilizing all materials to be added to the fermentation during the process.

v. Maintaining aseptic conditions during the fermentation.

8. Spiral heat exchanger

They are modern continuous sterilizers use double spiral heat exchangers in which the two streams are separated by a continuous steel division. Also, the spiral exchangers are far less susceptible to blockage. But a major limitation to the adoption of continuous sterilization was the precision of control necessary for its success. This precision has been achieved with the development of sophisticated computerized monitoring and control systems resulting in continuous sterilization being very widely used and it is now the method of choice. To achieve sterilization temperatures steam is passed through one spiral and medium through the other in counter current streams. Spiral heat exchangers are also used to cool the medium after passing through the holding coil. Incoming unsterile medium is used as the cooling agent in the first cooler so that the incoming medium is partially heated before it reaches the sterilizer and, thus, heat is conserved. The major advantages of the spiral heat exchanger are:

I. The two streams of medium and cooling liquid, or medium and steam, are separated by a continuous stainless steel barrier with gasket seals being confined to the joints with the end plates. This makes cross contamination between the two streams unlikely. ii. The spiral routetraversed by the medium allows sufficient clearances to be incorporated for the system to cope with suspended solids. The exchanger tends to be self- cleaning which reduces the risk of sedimentation, fouling and 'burning- on'.

9. Positive pressure

During sterilization the concept of positive pressure" is often used.

✓ Maintaining positive pressure means "maintaining that during sterilization, cooling andfilling and if appropriate, the fermentation process, air must be pumped into the reactor.

✓ In this way the reactor is always pressurized and thus aerial contaminants will not be"sucked" into the reactor.

✓ It is very important that positive pressure is maintained when the bioreactor is cooled following sterilization. Without air being continuously pumped into the reactor, a vacuumwill form and contaminants will tend to be drawn into the reactor. Maintaining positive pressure at all stages of the fermentation setup and operation is an important aspect of reducing the risk of contamination.

10. Importance of sterilization of inlet air from fermentation

Sterilization of the inlet air is undertaken to prevent contaminating organisms from entering the reactor. The exit air on the other hand is sterilized not only to keep contaminants from entering but also to prevent organisms in the reactor from contaminating the air. A common method of sterilizing the inlet and exit air is filtration. For small reactors (with volumes less than 5 litres), disk shaped hydrophobic Teflon membranes housed in a polypropylene housing are used. Teflon is tough, reusable and does not readily block. During the emptying of a fermenter, it is important that the air feed valve is closed. This will minimize the contamination of the inlet air line.

11. Five techniques roffer et al 1984

Roffler et al. (1984) reviewed the use of a number of techniques for the in-situ recovery of fermentation products:

1. Vacuum and flash fermentations for the recovery of ethanol from fermentation broths.

2. Extractive fermentation (liquid-liquid and phase aqueous) for the recovery of organic acidsand toxin produced by Clostridium tetani.

3. Adsorption for the recovery of ethanol and cycloheximide.

4. Ion-exchange in the extraction of salicylic acid and antibiotics.

5. Dialysis fermentation in the selective recovery of lactic acid, calicylic acid and cycloheximide.

12. PH control system in fermenter

Neutralizing Agents:

The neutralizing agents used to control pH should be non- corrosive. They should also be non-toxic to cells when diluted in the medium. Potassium hydroxide is preferred to NaOH, as potassium ions tend to be less toxic to cells than sodium ions. However KOH is more expensive than NaOH. Sodium carbonate is also commonly used in small scale bioreactor

systems. Hydrochloric acid should never be used as it is corrosive even to stainless steel. Likewise, sulphuric acid concentrations should not be between 10% and 80% as between this range, sulphuric acid is most corrosive. For laboratory fermenters, a peristaltic pump is used to add the pH adjusting agents. Silicone tubing is often used. However, note that silicone tubing will decay in the presence of high alkali concentrations. Thick-walled silicone tubing should be used. Alternatively, Tygon or Neoprene tubing can be used. Tygon is not autoclavable but can be sterilized by passing the NaOH through the tubing for about 1 hour. Neoprene is autoclavable but is not transparent or translucentasis Tygonor silicone. For fermentations that produce large amount of acids, for example lactic acids fermentation using media containing high sugar concentrations, high concentrations of alkali (4 M and above) are preferred. This will prevent dilution of the medium due to the addition of excessive addition of the alkali solution. The pH control system consists of: a pH probe, alkali delivery system & acid delivery system. The pH probe is typically steam sterilizable. The pH control system (and indeed all other fermenter control systems) are designed to have a dead-band. A dead-band is used to prevent excessive alkali and acid addition.

13. Del factor

Deindoerfer and Humphrey (1959) used the term ln No/ Nt as a design criterion for sterilization, which has been variously called the Del factor, Nabla factor and sterilization criterion represented by the term . Thus, the Del factor is a measure of the fractional reduction in viable organism count produced by a certain heat and time regime. Therefore:

$\nabla = \ln (N0/Nt)$, Thus, a plot of the natural logarithm of the time required to achieve a certain value against the reciprocal of the absolute temperature will yield a straight line, the slope of which is dependent on the activation energy, This kinetic description of bacterial death enables. The design of procedures (giving certain factors) for the sterilization of fermentation broths. By choosing a value for Nt, procedures may be designed having a certain probability of achieving sterility, based upon the degree of risk that is considered acceptable.

14. Filter sterilization in detail

Suspended solids maybe separated from a fluid during filtration by the following mechanisms:

I. Inertial impaction.

ii. Diffusion.

iii. Electrostatic attraction.

iv. Interception.

Inertial Impaction

Suspended particles in a fluid stream have momentum. The fluid in which the particles are suspended will flow through the filter by the route of least resistance. However, the particles, because of their momentum, tend to travel in straight lines and may therefore become impacted upon the fibres where they may then remain. Inertial impaction is more significant in the filtration of gases than in the filtration of liquids.

Diffusion

Extremely small particles suspended in a fluid are subject to Brownian motion which is random movement due to collisions with fluid molecules. Thus, such small particles tend to deviate from the fluid flow pattern and may be come impacted upon the filter fibres. Diffusion is more significant in the filtration of gases than in the filtration of liquids.

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15. **Accessories for a Fermenter**:

Accessories for a Fermenter:

- Baffle
- Sterile compressed air (at 1.5 to 3.0 atmospheres)
- Chilled water (12 to 15°C)
- Cold water (4°C)
- Hot water
- Steam (high pressure)
- Steam condensate
- Electricity
- Stand-by generator
- Drainage of effluents
- Motors
- Storage facilities for media components

16. Sterilization of fermenter exhaust air

In many traditional fermentations the exhaust gas from the fermenter was vented without sterilization or vented through relatively inefficient depth filters. With the advent of the use of recombinant organisms and a greater awareness of safety and emission levels of allergic compounds the containment of exhaust air is more common (and in the case of recombinant organisms, compulsory). Fixed pore membrane modules are also used for this application but the system must be able to cope with the sterilization of water saturated air, at a relatively high temperature and carrying a large contamination level. Also, foam may overflow from the fermenter into the air exhaust line. Thus, some form of pre- treatment of the exhaust gas is necessary before it enters the absolute filter. This pre-treatment may be a hydrophobic pre-filter or a mechanical separator to remove water, aerosol particles and foam. The pre-treated air is then hydrophobic filter. Again, it fed to a 0.2μm is important to appreciate that the filtration system must be steam sterilizable. Figures in next slides illustrate the pre-filter and mechanical separator systems respectively.

17. Requirements to run a fermenter

There is no universal bioreactor. The general requirements of the bioreactor are as follow:

1. The vessel should be capable of being operated aseptically for a number of days andshould be reliable in long-term operation and meet the requirements of containment regulations.

2. Adequate aeration and agitation should be provided to meet the metabolic requirements ofthe micro-organism. However, the mixing should not 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 should not be excessive.

8. The vessel should be designed to require the minimal use of labour in operation,harvesting, cleaning and maintenance.

9. Ideally the vessel should be suitable for a range of processes, but this may be restrictedbecause of containment regulations.

10. The vessel should be constructed to ensure smooth internal surfaces, using welds insteadof flange joints whenever possible.

11. The vessel should be of similar geometry to both smaller and larger vessels in the pilotplant or plant to facilitate scale-up.

12. The cheapest materials which enable satisfactory results to be achieved should be used.

13. There should be adequate service provisions for individual plants.

18. What the effect if low speed of impeller

The shear forces that an impeller generates play a major role in determining bubble size. If the impeller speed is to slow then the bubbles will not be broken down. In addition, if the impeller speed is too slow, then the bubbles will tend to rise directly to the surface due to the another consequence of too slow impeller speed is a flooded impeller.

▪Under these conditions, the bubbles will accumulate and coalesce under the impeller,leading to the formation of large bubbles and poor oxygen transfer rates.

▪A similar phenomenon will happen when aeration rate is too high. In this case, the

oxygentransfer efficiency will be low.

19. Write effluent of disposal method

The effluent disposal procedure which is finally adopted by a particular manufacturer is obviously determined by a number of factors, of which the most important is the control exercised by the relevant authorities in many countries on the quantity and quality of the waste discharge and the way in which it might be done. The range of effluent-disposal methods which can be considered is:

1. The effluent is discharged to land, river or sea in an untreated state.

2. The effluent is removed and disposed of in a landfill site or is incinerated.

3. The effluent is partially treated on site prior to further treatment or disposal by one of theother routes indicated.

4. Part of the effluent is untreated and discharged as in 1 or 2, the remainder is treated at asewage works or at the site before discharge.

5. All of the effluent is sent to the sewage works for treatment, although there might be reluctance by the sewage works to accept it, possibly resulting in some preliminary on-sitetreatment being required, and discharge rates and effluent composition defined.

6. All the effluent is treated at the factory before discharge.

20. Foaming control process

▪ Antifoam requirement will depend on:

I. The nature of the medium: Media rich in proteins will tend to foam more readily than simple media.

ii. The products produced by the fermentation: secreted proteins or nucleic acids released as aresult of cell death and hydrolysis have detergent like properties.

iii.The aeration rate and stirrer speed: Increasing the aeration rate and stirrer speed increasesfoaming problems.

iv.The use of mechanical foam control devices: Foam control devices such as mechanical and ultrasonic foam breakers help to reduce the antifoam requirement. v. The head space volume:The larger headspace volume, then the greater the tendency for the foam to collapse under its own weight. For example, for fermentations in which high levels of foam is produced, a 50%headspace volume may be required.

vi. Condenser temperature: In laboratory scale reactors, a cold condenser temperature can help to control the foam. The density of the foam increases when it moves from the warm headspace volume to the cold condenser region. This causes the foam to collapse. Foam is typically detected using two conductivity or "level" probes. One probe is immersed in the fermentation liquid while the other placed above the liquid level. When the foam reaches the upper probe, a current is carried through the foam. The detection of a current by the foam controller results in the activation of a pump and the antifoam is then added until the foam subsides.

21. What is Sterilization OF FERMENTER EXHAUST AIR

In many traditional fermentations the exhaust gas from the fermenter was vented without sterilization or vented through relatively inefficient depth filters. With the advent of the use of recombinant organisms and a greater awareness of safety and emission levels of allergic compounds the containment of exhaust air is more common (and in the case of recombinant organisms, compulsory). Fixed pore membrane modules are also used for this application but the system must be able to cope with the sterilization of water saturated air, at a relatively high temperature and carrying a large contamination level. Also, foam may overflow from the fermenter into the air exhaust line. Thus, some form of pre-treatment of the exhaust gas is necessary before it enters the absolute filter. This pre-treatment may be a hydrophobic pre-filter or a mechanical separator to remove water, aerosol particles and foam. The pre-treated air is then fed to a 0.2um hydrophobic filter. Again, it is important to appreciate that the filtration system must be steam sterilizable. Figures in next slides illustrate the pre-filter and mechanical separator systems respectively.

22. Richards' RAPID METHOD FOR DESIGNING OF STERILIZATION CYCLES

Sterilization: Richards' RAPID METHOD FOR DESIGNING OF STERILIZATIONCYCLES

Richards (1968) proposed a rapid method for the design of sterilization cycles avoiding the time consuming graphical integrations. The method assumes that all spore destruction occurs a temperatures above 100°C and that those parts of the heating and cooling cycle above 100°C are linear. Both these assumptions reasonably valid and the technique loses very little in accuracy and gains considerably in simplicity. Furthermore, based on these assumptions, Richards has presented a table of Del factors for B. stearothermophilus spores which would be obtained in heating and cooling a broth up to (and down from) holding temperatures of 101-130°C, based on a temperature change of 1°C per minute. This information is presented in Table (on next slide), together with the specific death rates for B. stearothermophilus spores over the temperature range.

OR

Richard sterilization cycle. Richards (1968) proposed a rapid method for the design of sterilization cycles avoiding the time consuming graphical integrations. The method assumes that all spore destruction occurs at temperatures above 100°C and that those parts of the heating and cooling cycle above 100°C are linear. Both these assumptions reasonably valid and the technique loses very little in accuracy and gains considerably in simplicity. Furthermore, based on these assumptions, Richards has presented a table of Del factors for B. stearothermophilus spores which would be obtained in heating and cooling a broth up to (and down from) holding temperatures of 101-130°C, based on a temperature change of 1°C per minute. This information is presented in Table (on next slide), together with the specific death rates for B. stearothermophilus spores over the temperature range. If the rate of temperature change is 1° per minute, the Del factors for heating and cooling may be read directly from the table; if the temperature change deviates from 1° per minute, the Del factors may be altered by simple proportion. For example, if a fermentation broth were heated from 100° to 121°C in 30 minutes and cooled from 121° to 100° in 17 minutes, the Del factors for the heating and cooling cycles may be determined as follows: From Table (in previous two slides), if the change in temperature had been 1° per minute, the Del factor for both the heating and cooling cycles would be 12.549. But the temperature change in the heating cycle was 21° in 30 minutes; therefore, and the temperature change in the cooling cycle was 21° in 17 minutes, therefore, Having calculated the Del factors for the heating and cooling periods the holding time at the constant temperature may be calculated as before. Chemo stat: The growth of the cells in a continuous culture of this type is controlled by the availability of the growth limiting chemical component of the medium and, thus, the system is described as a chemo stat.

23. Discuss Oxygen delivery system and Form control system

The oxygen delivery system consists of: a compressor, an inlet air sterilization system, an air sparger exit air sterilization system. A compressor forces the air into the reactor. The compressor will need to generate sufficient pressure to force the air through the filter, sparger holes and into the liquid. Air compressors used for large scale bioreactors typically produce air at 250 kPa. The air should be dry and oil free so as to not block the inlet air filter or contaminate the medium. It is very important that an "instrument air" compressor is not used. Instrument air is typically generated at higher pressures but is aspirated with oil. Instrument air compressors are used for pneumatic control. Sterilization of the inlet air is undertaken to prevent contaminating organisms from entering the reactor. The exit air on the other hand is sterilized not only to keep contaminants from entering but also to prevent organisms in the reactor from contaminating the air. A common method of sterilizing the inlet and exit air is filtration. For small reactors (with volumes less than 5 liters), disk shaped hydrophobic Teflon membranes housed in a polypropylene housing are used. Teflon is tough, reusable and does not readily block. For larger laboratory scale fermenters (up to 1000 liters), pleated membrane filters housed in polypropylene cartridges are used. By pleating the membrane, it is possible to create a compact filter with very large surface area for air filtration. Increasing the filtration area decreases the pressure required to pass a given volume of air through the filter.

24. Discuss batch and continuous sterilization process, filter Sterilization of Media, Sterilization of the Fermenter, Feeds and of Liquid Wastes.

Sterilization of the FERMENTER, FEEDS, and of LIQUID WASTES

Sterilization of the Fermenter If the medium is sterilized in a separate batch cooker, or is sterilized continuously, then the fermenter has to be sterilized separately before the sterile medium is added to it. This is normally achieved by heating the jacket or coils of the fermenter with steam and sparging steam into the vessel through all entries, apart from the air outlet from which steam is allowed to exit slowly. Steam pressure is held at 15 psi in the vessel for approximately 20 minutes. It is essential that sterile air is sparged into the fermenter after the cycle is complete and a positive pressure is maintained; otherwise a vacuum may develop and unsterile air be drawn into the vessel.

Sterilization of the Feeds A variety of additives may be administered to a fermentation during the process and it is essential that these materials are sterile. The sterilization method depends on the nature of the additive, and the volume and feed rate at which it is administered. If the additive is fed in large quantities, then continuous sterilization may be desirable. Batch sterilization of feed liquids normally involves steam injection into the material held in storage vessels. Whatever the sterilization system employed it is essential that all ancillary equipment and feed pipework associated with the additions are sterilizable.

Sterilization of the Liquid Wastes Process organisms which have been engineered to produce 'foreign' products and therefore contain heterologous genes are subject to strict containment regulations. Thus, waste biomass of such organisms must be sterilized before disposal. Sterilization may be achieved by either batch or continuous means but the whole process must be carried out under contained conditions. Batch sterilization involves the sparging of steam into holding tanks, whereas continuous processes would employ the type of heat exchangers. An autoclave. The entering steam forces the air out of the bottom (blue arrows). The automatic ejector valve remains open as long as an air-steam mixture is passing out of the waste line. When all the air has been ejected, the higher temperature of the pure steam closes the valve, and the pressure in the chamber increases

25. Factors consider when designing a fermenter

Factors consider when designing a fermenter. Therefore, basic features/accessories of a bioreactor associated for monitoring, control & record:

- An agitator system
- An oxygen delivery system
- A foam control system
- A temperature control system
- A pH control system
- A cleaning and sterilization system
- A sump and dump line system (only on pilot and Industrial Scale)

26. Relationship between qp and according to fed batch culture

Pirt (1979) has expressed the change in product concentration in variable volume fed batch culture dp/dt =qpx - Dp

• Thus, product concentration changes according to the balance between production rate anddilution by the feed. • Fed-batch quasi steady state change over the time of the fermentation.

• Product concentration in in a fed-batch system over the time of the fermentation will bedependent on the relationship between qp and u (hence D).

• If qp is strictly growth related then it will change as u with D and, thus, the product concentration remain constant. However, if qp is constant and independent of u, then product concentration will the start of the cycle when Dp is greater than qpx, but will rise with time asD decreases and qpx become greater than Dp. If qp is related to u in a complex manner then product concentration will vary according relationship. Thus, the feed strategy of a system would be optimized according to the ship between qp and u.

Xt/Xo = e-kt, define xt and kt.

Batch Culture-3 Cell density (Xt) after time 't' will be: Xt=Xo2 n=X02 t/td xatdX« dt change in biomass a change in time dX = u.X.dt dX/dt = u.X Where, X=concentration of microbial biomass t=time, (mostly in hrs) u=is the Specific Growth Rate per unit cell mass On applying integration equation this equation (dX/dt = u.X)becomes:

Xt=xo e ut Xo= original biomass concentration Xt= biomass concentration after the time interval t hours e=base of the natural logarithm To convert equation xt = xo e ut into linear equation, take natural logarithm by which this equation becomes: InXt=lnXo+ut (In e) InXt=lnXo+ut, because In e=1 This equation fit to linear regression equation: Y=a+ bx Where:

- Y= Vertical axis (dependent variable)
- X=Horizontal axis (independent variable)
- a= intersect point on plot
- b=slope of line (specific growth rate)

•If, InX is taken along Y-axis & T is taken on X-axis, a plot of the natural logarithm of biomass concentration against time should yield a straight line, the slope of which would equal u.

•By using this relationship: InXt=lnXo+ut , we can calculate doubling time by puttingXt=2Xo

In 2Xo=lnXo+utd In 2Xo -InXo= utd

In (2Xo Xo)= utd

In (2)= utd

In2/u =td.

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