VU Medical Zone

BIO 204 Current Final Term Spring 2021

MCQ

1-Chemostat is also known as_____. **Steady State**

2- A spectrophotometer is used to determine_____. **Turbidity**

3- Spectrophotometer measuring the amount of_____. **Light**

4-Inlet and exist Air sterilize through _____. **Filtration**

5- Foam is detected by_____. **Single probe level**

6- Which is not pH system part? **Alkali delivery**

7- How much volume of pleated membrane filter are used in larger laboratory scale fermenter?

Up to 1000 liters

8- Removal or destruction of all living organisms. **Sterilization**

9- An ideal way sterilization media for animal culture? **Filtration**

10- Part of fermenter?

All

pH/ O_2 electrode

Detachable stirrer motor

Exhaust gas condenser

11- Biology oxygen demand is abbreviated as

BOD

bod

Bod

BoD

Q1. How sterilization the fermenter exhaust air? 2 Marks

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.

Q2. Which strategy adopts to remove thermal destruction of fermenter? 3 Marks

The thermal destruction of essential media components conforms approximately with first order reaction kinetics and, therefore, may be described by equations similar to those derived for the destruction of bacteria:

$$x_t/x_0 = e^{-kt}$$

where x_t is the concentration of nutrient after a heat treatment period, t,
x_0 is the original concentration of nutrient at the onset of sterilization,
k is the reaction rate constant.

Therefore, the ideal technique would be to heat the fermentation medium to a high temperature, at which it is held for a short period, before being cooled rapidly to the fermentation temperature.

However, it is obviously impossible to heat a batch of many thousands of litres of broth in a tank to a high temperature, hold for a short period and cool without the heating and cooling periods contributing considerably to the total sterilization time.

Q3. What is spiral heat exchanger? Write its advantages? 5 Marks

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.

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 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 which reduces the risk of sedimentation, fouling and 'burningon'.

Q4. How many types reactions are involved in filtration during sterilization? 5 Marks

Two types of reaction contribute to the loss of nutrient quality during sterilization:

i. 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. These reactions are normally caused by the reaction of carbonyl groups, usually from reducing sugars, with the amino groups of amino acids and proteins. An example of the effect of sterilization time on the availability of glucose in a corn-steep liquor medium is shown in Table (in next slide). Problems of this type are normally resolved by sterilizing the sugar separately from the rest of the medium and recombining the two after cooling.

ii. 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.

Q5. Applications of condenser? 2 Marks

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.

Q6. How we sterilize large volume fermenter? 3 Marks

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.

Q7. Factors investigate during industrial operation of economical waste? 3 Marks

Factors to investigate in a site survey

Daily flow rate
Fluctuations in daily, weekly and seasonal flow
BOD/COD
Suspended solids
Turbidity
pH range
Temperature range
Odours and tastes
Colour
Hardness
Detergents
Radioactivity
Presence of specific toxins or inhibitors (e.g. heavy metals, phenolics etc.)

Q8. Why we sterilize the exhaust air? 3 Marks

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).

Q9. Define Sterilization. 2 Marks

Sterilization is the removal or destruction of all living microorganisms. Heating is the most common method used for killing microbes, including the most resistant forms, such as endospores.

Q10. What techniques can be employed for the purification of fermentation product after removal biomass? 2 Marks

Chromatographic techniques and crystallization to obtain a product which is highly concentrated and essentially free from impurities.

Q11. How we can modify the characteristics of fermentation broth for making it purification easy and fast? 3 Marks

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

1. Selection of a micro-organism which produce pigments or undesirable metabolites.
2. Modification of the fermentation reduce the production of metabolites.
3. Precise timing of harvesting.
4. pH control after harvesting.
5. Temperature treatment after harvesting.

6. Addition of flocculating agents.

7. Use of enzymes to attack cell walls.

Q12. How we sterilize the media for fermentation? 3 Marks

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. Before the techniques which are used for the steam sterilization of culture media are discussed it is necessary to discuss the kinetics of sterilization.

Q13. Techniques by Roffler et al. 5 Marks

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 acids 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.

Q14. What consequences may occur if the fermentation is invaded by a foreign microorganism? 5 Marks

If the fermentation is invaded by a foreign microorganism, then the following consequences may occur:

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

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

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

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

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

vi. Contamination of a bacterial fermentation with phage could result in the lysis of the culture.

Q15. What is Freeze drying and write its advantage. 2 Marks

Freeze 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.

Q16. How Avoidance of contamination may be achieved? 3 Marks

Avoidance of contamination may be achieved by:

i. Using a pure inoculum to start the fermentation.

ii. Sterilizing the medium to be employed.

iii. Sterilizing the fermenter vessel.

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

v. Maintaining aseptic conditions during the fermentation.

Q17. Which techniques are used to separate the solid from liquid? 2 Marks

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

i. Inertial impaction.

ii. Diffusion.

iii. Electrostatic attraction.

iv. Interception.

Q18. How Del factor calculates? 2 Marks

Deindoerfer and Humphrey (1959) used the term ln No / Nt as a design criterion for sterilization, which has been variously called the Del factor.

$$\nabla = \ln (N_0/N_t)$$

A frequently adopted risk of contamination is 1 in 1000, which indicates that Nt should equal 10-3 of a viable cell.

Q19. How risk of contamination can be minimized in fermentation? 2 Marks

Maintaining positive pressure at all stages of the fermentation setup and operation is an important aspect of reducing the risk of contamination.

Q20. Filtration system for the sterilization of animal cell culture fermentation? 3 Marks

An ideal filtration system for the sterilization of animal cell culture media must fulfill the following criteria:

i. The filtered medium must be free of fungal, bacterial and mycoplasma contamination.

ii. There should be minimal adsorption of protein to the filter surface.

iii. The filtered medium should be free of viruses.

iv. The filtered medium should be free of bacterial endotoxins.

Q21. Which information must be available for the design of a batch sterilization according Deindoerfer and Humphrey? 3 Marks

Deindoerfer and Humphrey (1959) presented a method to assess the contribution made by the heating and cooling periods. The following information must be available for the design of a batch sterilization process:

i. A profile of the increase and decrease in the temperature of the fermentation medium during the heating and cooling periods of the sterilization cycle.

ii. The number of micro-organisms originally present in the medium.

iii. The thermal death characteristics of the 'design‘ organism. As explained earlier this may be Bacillus stearothermophilus or an alternative organism relevant to the particular fermentation.

Q22. Is overheating dangerous for media and its components during sterilization? 2 Marks

Yes, overheating is dangerous for media and its components during sterilization, because some components are heat sensitive and thermolabile.

Q23. Write all details about all 4 steps for treatment process of fermentation waste? 5 Marks

Treatment processes may also be described in the following manner:

1. Primary treatment; physical and chemical methods, e.g. sedimentation, coagulation etc.

2. Secondary treatment; biological methods (e.g. activated sludge) conducted after primary treatment.

3. Tertiary treatment; physical, chemical or biological methods (e.g. microstrainers, sand filters and grass plot irrigation) used to improve the quality of liquor from previous stages.

4. 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.

Q24. Write a detail note on phenomena of positive pressure? 5 Marks

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.

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