

**Subject: bio204
(Past Papers)**

CR: Muhammad Nouman

VU Medical Zone (biotechnologists)

**Admins: HAfiza Mubeen,
Muhammad Nouman, Tasha kHan,
Iqra Shaheen**

Q1).Write any Two Risks described by Collins? 2

ANS:Risk such as those given by Collins (1992):

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1. The known pathogenicity of the micro-organism.
2. The virulence or level of pathogenicity of the microorganism are the diseases it causes mild or serious?
3. The number of 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 vectors and potential reserves.
6. The amounts or volumes of organisms used in the fermentation process.
7. The techniques or processes used.
8. Ease of prophylaxis and treatment

Q3)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 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.

Q4). What is Sterilization? 2

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

Q5) Richards' RAPID METHOD FOR DESIGNING OF STERILIZATION CYCLES? 5

ANS: Sterilization: Richards' RAPID METHOD FOR DESIGNING OF STERILIZATION CYCLES

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

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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

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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..

Chemostat:

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 **chemostat**.

Q6). Discuss Oxygen delivery system and Form control system? 10

The oxygen delivery system consists of: a compressor, an inlet air sterilization system, an air sparger exit air sterilization system. Fermenter Design: Oxygen Delivery System-Air Sterilization System-1 Sterilization of the inlet air is undertaken to prevent contaminating organisms from entering the reactor.

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Q7)Fermenter Design: Oxygen Delivery System-Air Sterilization System-2

• 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. Fermenter Design: Oxygen Delivery System-Air Sterilization System-3

☐ ☐ 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 .

8)Requirements to run a fermenter.. ?

ANS:General Requirements ofFermenters/Bioreactors

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

1. The vessel should be capable of being operated aseptically for a number of days and should 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 of the 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 restricted because of containment regulations.
 10. The vessel should be constructed to ensure smooth internal surfaces, using welds instead of flange joints whenever possible.
 11. The vessel should be of similar geometry to both smaller and larger vessels in the pilot plant or plant to facilitate scale-up.
 12. The cheapest materials which enable satisfactory results to be achieved should be used.
- There should be adequate service provisions for individual plants

Batch and continuous..

The relative merits of batch and continuous sterilization may be summarized as follows:

☐ *Advantages of continuous sterilization over batch sterilization*

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- i. Superior maintenance of medium quality.
 - ii. Ease of scale-up - discussed later.
 - iii. Easier automatic control.
 - iv. The reduction of surge capacity for steam.
 - v. The reduction of sterilization cycle time.
 - vi. Under certain circumstances, the reduction fermenter corrosion.
- Advantages of batch sterilization over continuous sterilization*
- i. Lower capital equipment costs.
 - ii. Lower risk of contamination - processes require the aseptic transfer of the sterile broth to the sterile vessel.
 - iii. Easier manual control.
 - iv. Easier to use with media containing a high proportion of solid matter.
- The early continuous sterilizers were constructed as plate heat exchangers and these were unsuitable on two accounts:
- i. Failure of the gaskets between the plates resulted in the mixing of sterile and unsterile streams.
 - ii. Particulate components in the media would block the heat exchanger.
- Parts of fermenter..

9)How sterilized product exhaust air..Aisa he tha..

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

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4. How sterilized product exhaust air? 3

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.

11) Discuss batch and continuous sterilization process, filter Sterilization of Media, Sterilization of the Fermenter, Feeds and of Liquid Wastes. 10

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

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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.

12)WRITE A NOTE on Batch and continuous fermenter. ?

ANS:

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❑ *Advantages of continuous sterilization over batch sterilization*

- i. Superior maintenance of medium quality.
- ii. Ease of scale-up - discussed later.
- iii. Easier automatic control.
- iv. The reduction of surge capacity for steam.
- v. The reduction of sterilization cycle time.
- vi. Under certain circumstances, the reduction fermenter corrosion.

Advantages of batch sterilization over continuous sterilization

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- iii. Easier manual control.
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- ii. Particulate components in the media would block the heat exch

Parts of fermenter..

13) write a note on Chemostat(10)?

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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 chemostat.

Originally Chemostat was the name given to a device that enables people to grow and harvest bacteria continuously, in contrast to the older batch culture in which a fixed quantity of nutrient was supplied and bacteria were harvested after a chosen growth period. In general, the chemostat is operated (as long as possible) in a steady state (for example in a logarithmic growth phase of the bacterial population). As Dr. Murugesan Muthu says, now chemostat is used in a wider sense to refer to a continuous flow bio reactor operated in steady state. Now a days 'Chemostat' used to grow microorganism in fixed quantity of a particular substrate rather than all supplements and only that quantity of particular substrate is maintained and cells were harvested along with product.

OR

A **chemostat** (from *chemical* environment is *static*) is a bioreactor to which fresh medium is continuously added, while culture liquid containing left over nutrients, metabolic end products and microorganisms are continuously removed at the same rate to keep the culture volume constant.^{[2][3]} By changing the rate with which medium is added to the bioreactor the specific growth rate of the microorganism can be easily controlled within limits.

The yield substrate by $Y_{x/s}$, a ratio of the respective concentrations. Yields can be easily calculated by determining the production of dry cell mass over a given period of the cultivation and dividing by mass of carbon substrate consumed.

$Y_{x/s} = \text{g dry cells produced / g substrate consumed}$

$Y_{x/ATP} = \text{g dry cells produced / moles of ATP formed}$

$Y_{x/O_2} = \text{g dry cells produced / moles of } O_2 \text{ consumed}$

Yield with respect to ATP synthesis or oxygen consumption is relatively constant for many organisms, e.g. the yield of cells per mole of ATP synthesized under conditions of energy substrate limitation and high growth rate is approximately $10 + 2 \text{ g cells}$. Yields of cells per mole of ATP is not constant for all bacteria and is variable if the energy substrate is not growth limiting or the growth rate is lower than the maximum rate.

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

Example: In a 2-litre chemostat with flow rate of (inlet and outlet) $F = 0.5 \text{ L/h}$, and with dilution rate of $F/V = D = 0.25 \text{ h}^{-1}$

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❑ Establishment of Steady-State Condition in a Chemostat

The time necessary for 4 changes of medium volume = $4/D$ or $4/0.25h^{-1} = 16h$

Steady-state will be established after 16 h of changing the growth conditions of a chemostat. Only then the overall growth yield can be determined as below:

$$Y_{x/s} = (X - X_0) / (S_0 - S)$$

X = g cells dry weight per liter of effluent medium.

X_0 = g cells dry weight per liter of influent medium i.e. zero in sterile medium,

S = g substrate per liter of effluent medium,

S_0 = g substrate per liter of influent medium

Overall growth Yield is related maintenance and growth requirement for limiting substrates that act as energy sources:

$$1/Y_{x/s} = (m/m) + (1/Y_G)$$

m = specific rate of substrate uptake for cellular maintenance,

$Y_{x/s}$ = overall yield,

Y_G = growth specific yield,

m and Y_G can be estimated by plotting $(1/Y_{x/s})$ vs. $(1/\mu)$ on rectangular paper. If this gives a straight-line relationship,

The intercept = $1/Y_G$; Slope of the line = m

($\mu = D$, in simple chemostat without cell recycle).

Q14) Factors consider when designing a fermenter?

Factors consider when designing a fermenter..

Therefore, basic features/accessories of a bioreactor associated for monitoring, control & record:

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

Q14 Rofler 5 technique for the recovery purification of the product..?

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Recovery and purification..

The extraction and purification of fermentation products may be difficult and costly. Ideally, one tries to obtain a high-quality product as quickly as possible at an efficient recovery rate using minimum plant investment operated at minimal costs.

- Unfortunately, recovery costs of microbial products may vary from as low as 15% to as high as 70% of the total manufacturing costs.
- If a fermentation broth is analysed at the time of harvesting it will be discovered that the specific product may be present at a low concentration in an aqueous solution that contains intact micro-organisms, cell fragments, soluble and insoluble medium components and other metabolic products.
- The product may also be intracellular, heat labile and easily broken down by contaminating micro-organisms. All these factors tend to increase the difficulties of product recovery.
- To ensure good recovery or purification, speed of operation may be the over-riding factor because of the labile nature of a product.

Recovery and Purification of Fermentation Products-2

The processing equipment must therefore be of the correct type and also the correct size to ensure that the harvested broth can be processed within a satisfactory time limit.

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

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

The main objective of the first stage for the recovery of an extracellular product is the removal of large solid particles and microbial cells usually by centrifugation or filtration (as shown in Fig. on next slide).

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

In the next stage, the broth is fractionated or extracted into major fractions using ultrafiltration, reverse osmosis, adsorption/ ion-exchange/gel filtration or affinity chromatography, liquid- liquid extraction, two phase aqueous extraction or precipitation.

Afterwards, the product-containing fraction is purified by fractional precipitation, further more precise chromatographic techniques and crystallization to obtain a product which is highly concentrated and essentially free from impurities. Other products are isolated using modifications of this flow-stream.

Recovery and Purification of Fermentation Products-4

Drying of any product (including biological products) is often the last stage of a manufacturing process. It involves the final removal of water from a heat-sensitive material ensuring that there is minimum loss in viability, activity or nutritional value. Drying is undertaken because:

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- i. The cost of transport can be reduced.
- ii. The material is easier to handle and package.
- iii. The material can be stored more conveniently in the dry state.

It is important that as much water as possible is removed initially by centrifugation or in a filter press to minimize heating costs in the drying process.

Diagram of Counter-current spray drier

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.

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.

Recovery and Purification of Fermentation Products-5

Crystallization is an established method used in the initial recovery of organic acids and amino acids, and more widely used for final purification of a diverse range of compounds. In citric acid production, the filtered broth is treated with Ca(OH)₂ so that the relatively insoluble calcium crystals will be precipitated from solution.

The concept of recovering a metabolite directly from an unfiltered fermentation broth is of considerable interest because of its simplicity, the reduction in process stages and the potential cost savings. It may also be possible to remove the desired fermentation product continuously from a broth during fermentation so that inhibitory effects due to product formation and product degradation can be minimized throughout the production phase.

Roffleret *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.

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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.

15)PARTS OF FERMENTAR?

AIM:

To study the construction & control system of a fermenter.

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Theory:

A fermenter is a system consisting of a few pieces of equipments which provide controlled environmental conditions for the growth of microbes (and/or production of specific metabolites) in liquid culture while preventing entry and growth of contaminating microbes from the outside environment. Major components of a fermenter:

Base components including drive motor, heater, pumps, gas control, etc.

Vessel and accessories.

Peripheral equipments such as reagent bottles.

Instrumentation and sensors. These components combine to perform the following functions

Facilitates the growth of a wide range of organisms.

Provide operations free from contamination.

Maintain a specific temperature.

Provide adequate mixing and aeration.

Control the pH of the culture.

Allow monitoring and/or control of dissolved oxygen.

Allow feeding of nutrients solutions and reagents.

Provide access points for inoculation and sampling.

Body Construction:

For a small scale (1 to 30 dm³) glass and/or stainless steel is used because it can withstand repeated steam sterilization cycles.

Two basic types are used:

A glass vessel with a round or flat bottom and top flanged carrying plates.

A glass cylinder with stainless steel top and bottom plates. This fermenter may be sterilized in situ. (AISI graded steel are now commonly used in fermenter construction.)

Peripheral parts: Reagents pumps:

Q16) function of agitation system (3)

ANS: The function of the agitation system is to provide good mixing and thus increase mass transfer rates—through the bulk liquid and bubble boundary layers. provide the appropriate shear conditions required for the—breaking up of bubbles. The agitation system consists of the agitator and the baffles. The baffles are used to break the liquid flow to increase turbulence and mixing efficiency.

17) Relatio btwn qp and according to fed batch cultr ?

Ans: • Pirt (1979) has expressed the change in product concentration in variable volume fed-batch culture $dp/dt = q_p x - Dp$ • Thus, product concentration changes according to the balance between production rate and dilution by the feed. • Fed-batch quasi steady state change over the time of the fermentation. • Product concentration in a fed-batch system over the time of the fermentation will be dependent on the relationship between q_p and μ (hence D). • If q_p is strictly growth related then it will change as μ with D and, thus, the product concentration remain constant. • However, if q_p is constant and independent of μ , then product concentration will the start of the cycle when Dp is greater than $q_p x$, but will rise with time as D decreases and $q_p x$ become greater than Dp . • If q_p is related to μ in a complex manner then product concentration

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will vary according relationship. Thus, the feed strategy of a system would be optimized according to the ship between q_p and μ .

18) $X_t/X_o = e^{-kt}$, define x_t and kt .

Batch Culture-3 Cell density (X_t) after time 't' will be: $X_t = X_o e^{-kt}$ $n = X_o e^{-kt}$ $t/d \times \alpha \times t \frac{dX}{dt} \propto dt$ change in biomass \propto change in time $dX = \mu \cdot X \cdot dt$ $\frac{dX}{dt} = \mu \cdot X$ Where, X = concentration of microbial biomass t = time, (mostly in hrs) μ =is the Specific Growth Rate per unit cell mass On applying integration equation this equation ($\frac{dX}{dt} = \mu \cdot X$) becomes:

$X_t = x_o e^{\mu t}$ X_o = original biomass concentration X_t = biomass concentration after the time interval t hours e = base of the natural logarithm To convert equation $x_t = x_o e^{\mu t}$ into linear equation, take natural logarithm by which this equation becomes: $\ln X_t = \ln X_o + \mu t$ ($\ln e$) $\ln X_t = \ln X_o + \mu t$, because $\ln e = 1$ 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, $\ln X$ 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 μ . By using this relationship: $\ln X_t = \ln X_o + \mu t$, we can calculate doubling time by putting $X_t = 2X_o$ $\ln 2X_o = \ln X_o + \mu t_d$ $\ln 2X_o - \ln X_o = \mu t_d$ $\ln (2X_o / X_o) = \mu t_d$ $\ln (2) = \mu t_d$ $\ln 2 / \mu = t_d$.

19) Difference between batch culture and continuous culture ?

ANS: The key difference between batch culture and continuous culture is that **batch culture is a technique used to grow microorganisms under limited nutrient availability in a closed system while continuous culture is a technique used to grow microorganisms under optimum and continual supply of nutrients in an open system in industries.**

20) . How can we calculate both live and dead cells?

ANS: If both live and dead cell counts have been recorded for each set of 16 corner squares, an estimate viability can be calculated.

1. Add together the live and dead cell count to obtain a total cell count.
2. Divide the live cell count by the total cell count to calculate the percentage viability.

Example:

- Live cell count: 2,337,500 cells/mL
- Dead cell count: 50,000 cells/mL
- $2,337,500 + 50,000 = 2,387,500$ cells
- $2,337,500 \div 2,387,500 = 97.9\%$ viability

21) Convert Monod equation to linear equ ?

To convert Monod equation into Linear equation we can write it as: $1/\mu = 1/\mu_{max} + K_s/\mu_{max}S$ $Y = a + bx$ The plot of $1/\mu$ against $1/S$ produces a straight line with intercept on the y axis $1/\mu_{max}$ and slope equals to: K_s / μ_{max}

22) what is Sterilization and its technique?

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

A) Moist heat methods.

B) Dry heat methods.

