

Subject: bio204

(past papers)

CR: Muhammad Nouman

VU Medical Zone (biotechnologists)

Admins:

**Hafiza Mubeen,
Muhammad Nouman, Tasha Khan,
Iqra Shaheen**

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1) Differentiate between quasi steady state and steady state of chemostat also write the effect on the value of Y , U_{max} , K_s . ?

The major difference between the steady state of a chemostat and the quasi steady state of a fed-batch culture is that μ is constant in the chemostat but decreases in the 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 the fermentation will

be dependent on the relationship between qp and μ (hence D).

Quasi steady state:

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

Steady state:

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. If medium is 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 vessel.

The kinetic characteristics of an organism (and, therefore, its behavior in a chemostat) are described by the numerical values of the constants Y , μ_{max} and K_s . The value of Y affects the steady-state biomass concentration; the value of μ_{max} affects the maximum dilution rate that may be employed and the value of K_s 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 angled pitch of the agitators coupled with the thin trailing edges of the impeller blades reduce formation of eddies in the wake of the moving blades. Axial flow impeller blades are pitched at an angle and thus direct the liquid flow towards the base of the tank. Examples of axial flow impellers are marine impellers and hydrofoil impellers. Axial flow mixing is considerably more energy efficient than radial flow mixing. They are also more effective at lifting solids from the base of the tank.

Radial flow:

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

3) Write down four accessories of fermentation process?

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

4) Covert monod equation into linear equation?

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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 three parameters:

1. The concentration of growth limiting substrate, S
2. The maximum specific growth rate, μ_{\max}
3. A substrate-specific constant, K_s

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

Taking the reciprocal values in the Monod equation and rearranging it:

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

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

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

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

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

This equation is fitted in linear regression i.e. $Y = a + bx$

Now we consider $a = \frac{1}{\mu_{\max}}$, $b = \frac{K_s}{\mu_{\max} S}$ and $\frac{1}{\mu}$ is the yielded which is represented by Y

Hence this is we got linear equation

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

5) What is the situation of quasi steady state?

Thus, $(ds/dt) \approx 0$. Although the total biomass in the culture (X) increases with time, cell concentration (x) remains virtually constant, that is $(dx/dt) \approx 0$ and therefore $\mu \approx D$. This situation is termed a 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 inoculum development, slants or flask cultures can be utilized.

• Pilot-scale Fermenters:

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In-situ sterilization but seed fermenter is not required for inoculums development, slants or flask cultures can be utilized.

• **Industrial-scale Fermenters:**

In-situ sterilization and seed fermenter is required for inoculums development.

7) **What is Agitators radial flow and axial flow characteristics?**

Agitators are classified as having radial flow or axial flow characteristics.

- ☐ With radial flow mixing, the liquid flow from the impeller is initially directed towards the wall of the reactor; i.e. along the radius of the tank.
- ☐ Radial flow impellers are primarily used for gas-liquid contacting (such as in the mixing of sparged bioreactors) and blending processes.
- ☐ Radial flow impellers contain two or more impeller blades which are set at a vertical pitch:
- ☐ Radial flow mixing is not as efficient as axial flow mixing. For radial flow impellers, a much higher input of energy is required to generate a given level of flow.
- ☐ Radial flow impellers do and are designed to, generate high shear conditions. This is achieved by the formation of vortices in the wake of the impeller.
- ☐ With axial flow mixing, the liquid flow from the impeller is directed downwards towards the base of the reactor, i.e. in the direction of the axis of the tank.
- ☐ 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 agitator?**

Top entry agitators:

The impeller 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 the shaft will need to be structurally supported.

Bottom entry agitators:

Bottom entry agitators tend to require more maintenance than top entry impellers 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 the term fed-batch culture to describe batch cultures which are fed continuously, or sequentially, with medium, without the removal of culture fluid. A fed-batch culture is established initially in batch mode and is then fed according to one of the following feed strategies:

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- i. The same medium used to establish the batch culture is added, resulting in an increase in volume.
- ii. A solution of the limiting substrate at the same concentration as that in the initial medium is added, resulting in an increase in volume.
- iii. A concentrated solution of the limiting substrate is added at a rate less than in (i) and (ii), resulting in an increase in volume.
- iv. A very concentrated solution of the limiting substrate is added at a rate less than in (i), (ii) and (iii), resulting in an insignificant increase in volume.

10) Why glass use in fermenter?

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

11) 2. What is the appropriate containment requirements can be applied for hazardous group?

- 12) Once the organism has been allocated to a hazard group, the appropriate containment requirements can be applied (*as mentioned in the Table*). p, prevent release. No detectable contamination during work should be found in the air, working surfaces and personnel. Unless required for product quality, , not required; +, required

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Procedures	GILSP [*]	Containment Category		
		1	2	3
<i>Secondary containment: Facilities</i>				
Protective clothing appropriate to the risk category	+	+	+	+
Changing/washing facility	+	+	+	+
Disinfection facility	—	+	+	+
Emergency shower facility	—	—	+	+
Airlock and compulsory shower facilities	—	—	—	+
Effluents decontaminated	—	—	+	+
Controlled negative pressure	—	—	—	+
HEPA filters in air ducts	—	—	+	+
Tank for spilled fluids	—	—	+	+
Area hermetically sealable *	—	—	—	+

13) Define is 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 13. system is described as a chemostat.

14) Give biomass relation μ_{max} ?

The final biomass concentration produced when $s = 0$ may be described as x_{max} and, provided \approx that x_0 is small compared with x_{max} and provided \approx that x_0 is small compared with x_{max} : $x_{max} Y$. SR. If, at the time when $x = x'$ a medium feed is started such that the dilution rate is less than μ_{max} , virtually all the substrate will be consumed as fast as it enters the culture

15) What are important things to consider for the formation of fermenter?

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

16) G and Y stands for in the equation $Dt/dt = GY$?

Where G is the substrate feed rate ($g\ dm^{-3}\ h^{-1}$) and Y is the yield factor.

17) The productivity of biological products depends on which factors?

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Better productivity of Biological products depends on:

- Strain improvement
- Media development
- Process Design & Control

18) Derive the relationship equation for change in biomass and change in time?

Cell density (X_t) after time 't' will be:

$$X_t = X_0 e^{\mu t} = X_0 2^{t/t_d}$$

$$X \propto t$$

$$dX \propto dt$$

change in biomass change in time

$$dX = \mu \cdot X \cdot dt$$

$$dX/dt = \mu \cdot X$$

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 ($dX/dt = \mu \cdot X$) becomes:

$X_t = x_0 e^{\mu t}$ X_0 = original biomass concentration

X_t = biomass concentration after the time interval t hours

e = base of the natural logarithm

To convert equation $x_t = x_0 e^{\mu t}$ into linear equation, take natural logarithm by which this equation becomes:

$$\ln X_t = \ln X_0 + \mu t (\ln e)$$

$$\ln X_t = \ln X_0 + \mu t, \text{ because } \ln e = 1$$

This equation fit to linear regression equation:

$$Y = a + bX$$

19) Can change in result find in chemostate experiment and predicted/ foregoing theory?

The results of chemostat experiments may differ from those predicted by the foregoing theory. The reasons for these deviations may be anomalies associated with the equipment or the theory not predicting the behavior of the organism under certain circumstances. Practical anomalies include imperfect mixing and wall growth. Imperfect mixing would cause an increase in the degree of heterogeneity in the fermenter when some organisms being subject to nutrient excess whilst others are under severe limitation.

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This phenomenon is particularly relevant to very low dilution rate system when the flow of medium is likely to be very intermittent. This problem may be overcome by the use of feedback system. Wall growth is another commonly encountered practical difficulty in which the organism adheres to the inner surface of the reactor resulting, again, in an increase in heterogeneity.

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

Cell growth is also measured by counting total cell number of the microbes present in that sample. Total cells (both live and dead) of liquid sample are counted by using a Haemocytometer/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 cells would be formed which would create problem in exact counting of bacterial cells.

The immobilized cells are not subject 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 μ according to fed batch profile.

Pirt (1979) described the product balance in a fixed volume fed-batch system as:

$$dp/dt = qp \cdot x$$

but substituting for x from equation gives:

$$dp/dt = qp(x_0 + GYt)$$

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 $qp = YP/x \cdot \mu$

22) Why the bottom entry agitators tends to require more maintenance than top entry impellers?

A reactor with bottom entry impeller however will need higher maintenance due to damage of the seal by particulates 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?

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An alternative type of continuous culture to the chemostat is the turbidostat, where the concentration of cells in the culture is kept constant by controlling the flow of medium such that the turbidity of the culture is kept within certain, narrow limits. This may be achieved by monitoring the biomass with a photoelectric cell and feeding the signal 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 CO₂ concentration or pH in which case it would be more correct to term the culture a biostat.

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

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

1. The known pathogenicity of the micro-organism
2. The virulence or level of pathogenicity of the microorganism is 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

25) Write down the four General Requirements of Fermenters/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.

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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

26) Write down the characteristics standard fermenter?

- Reliable aseptic seal is made between glass and glass, glass and metal or metal and metal joints such as between a fermenter vessel and a detachable top or base plate.
- 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.
- 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 during a sterilization cycle or an incubation cycle.
- Nitril 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 checked regularly for damage or perishing.

27) Growth → Change in **state, number, size, volume All**

28) **Doubling time (td)** is defined as the time required for **cell mass** to double.

29) $\ln X_t = x_0 e \mu t$, X_0 = **original biomass concentration**

30) Specific growth rate is independent of **substrate concentration** as long as excess substrate is present.

31) Multi blade impeller is discovered by **Jackson (1958)**

32) Parts of Fermenter are : **Detachable stirrer motor, Vessel pH/O2 Electrodes Exhaust Gas Condenser Dialysis Unit (All)**

33) There is **no universal** bioreactor.

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- 34) The function of the fermenter or bioreactor is to provide a suitable **environment**
- 35) Nitril or butyl rubbers are normally used for these **seals**.
- 36) If medium is 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 vessel.
- 37) If q_p is strictly growth related then it will change as μ with D and, thus, the product concentration **remain constant**.
- 38) Pirt (1979) described the kinetics of fixed volume fed-batch culture as follows. Consider a batch culture in which the growth of the process organism **depleted** the limiting substrate to a limiting
- 39) μ_{max} = is the maximum Specific Growth Rate **per unit cell mass**
- 40) The kinetics of (variable) volume in fed-batch culture have been developed by Dunn and Mor **1975** and Pirt **1974, 1975, 1979**.
- 41) **An alternative** type of continuous culture to the chemostat is the turbidostat
- 42) What increase the turbulence of fermenter Shaft, baffles, Vessle Confirm
- 43) Axial flow impellers provide more gentle but efficient mixing and are used for reactions involving shear sensitive **cells and particles**
- 44) The same medium used to establish the batch culture is **added**, resulting in an **increase** in volume.
- 45) AISI Grade **316** steels which contain **18%** chromium, **10%** nickel and **2-2.5% molybdenum** are now com mainly **used** in fermenter construction.
- 46) Storage phase is equivalent to the **late exponential phase** where the increase in mass is due to the accumulation of lipid and carbohydrate
- 47) Pressure of **Sterile compressed air (at 1.5 to 3.0 atmospheres)** ☐ air compressed in fermenter
- 48) The agitation consists of **agitator** and baffles.
- 49) Which of the following is included in parts of Parts of fermenter a **Body vessel, Agitator, Sparger all**
- 50) Nitril or butyl rubbers are normally used for these **seals**.