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Fundamentals of Biological Engineering Interview Questions

INSIDE

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Reaction and enzyme kinetics

1. What does Km represent in Michaelis-Menten kinetics?

Km is the substrate concentration at which the reaction rate is half of V_{max}. It shows how much substrate the enzyme needs to work at half speed: a low Km means the enzyme is close to saturated at low substrate. This reading holds for a single substrate under the steady-state assumption for the enzyme-substrate complex.

2. What is the difference between competitive and uncompetitive inhibition?

A competitive inhibitor binds the free enzyme and competes with substrate, so V_{max} stays the same and the apparent K_m rises. High substrate can overcome it. An uncompetitive inhibitor binds only the enzyme-substrate complex, so both V_{max} and apparent K_m fall by the same factor, giving parallel lines on a double-reciprocal plot.

3. A first-order reaction in a batch vessel has a rate constant of 0.023 per minute. How long does it take to reach 90% conversion?

The time for 90% conversion is about 100 min.

WORKING

1. For first order, $\ln(C_0/C) = kt$.
2. At 90% conversion $C/C_0 = 0.1$, so $\ln(C_0/C) = \ln 10 = 2.303$.
3. $t = 2.303/0.023$.
4. $t \approx 100$ min.

Ideal reactors

4. What are the three ideal reactors, and what does each assume about mixing?

A batch reactor is closed and perfectly mixed, so composition changes with time but is uniform in space. A CSTR is perfectly mixed with continuous flow, so its contents equal its exit stream. A PFR has no axial mixing: each element moves through as a plug, so composition changes along the length at steady state.

5. Why does a plug flow reactor need a smaller volume than a CSTR for the same conversion?

A CSTR holds its whole volume at the exit concentration, which is the lowest, so the rate is lowest everywhere. In a PFR the concentration falls gradually, so most of the volume runs at a higher rate. This holds when the rate falls as conversion rises, as for positive-order kinetics without inhibition.

6. A CSTR carries out a first-order reaction with a rate constant of 0.5 per hour. The feed flows at 100 L/h. What reactor volume gives 80% conversion?

The CSTR volume is 800 L.

WORKING

1. For first order in a CSTR, $\tau = X/(k(1-X))$.
2. $\tau = 0.8/(0.5 \times 0.2) = 8$ h.
3. $V = Q\tau = 100 \times 8$.
4. $V = 800$ L.

Enzyme immobilisation and diffusion effects

7. Why is an enzyme immobilised on a support?

Immobilisation keeps the enzyme in the reactor, so it can be reused and run in continuous operation, and the product stream is free of enzyme. It often improves stability too. The cost is extra resistance to substrate transfer through the liquid film and the pores, and some activity may be lost on binding.

8. What do the Thiele modulus and the effectiveness factor tell you about an immobilised enzyme particle?

The Thiele modulus compares the reaction rate with the rate of diffusion inside the particle. The effectiveness factor is the actual rate divided by the rate if the whole particle sat at the surface concentration. For a first-order reaction in a slab, $\eta = \tanh\phi/\phi$: near 1 for small ϕ, about $1/\phi$ for large ϕ.

9. Why does internal diffusion make the apparent Km of an immobilised enzyme larger than the free enzyme's Km?

Substrate is consumed as it diffuses into the particle, so the concentration inside is lower than in the bulk liquid. The bulk concentration must therefore be higher to reach half the maximum rate. The measured K_m is only an apparent value: the enzyme's own affinity has not changed.

Cell growth, substrate utilisation and product formation

10. What does the Monod equation describe, and what does Ks mean?

The Monod equation, $\mu = \mu_{max} S/(K_s + S)$, gives the specific growth rate as a saturating function of one limiting substrate. K_s is the substrate concentration at which μ is half of μ_{max}. It is an empirical, unstructured model that assumes no inhibition and no other limiting nutrient.

11. What do growth-associated and non-growth-associated product formation mean?

For a growth-associated product, the formation rate follows the growth rate, as for some primary metabolites. For a non-growth-associated product it follows the cell mass, whether or not cells divide, as for many secondary metabolites. A mixed model combines both: $q_P = \alpha\mu + \beta$, where q_P is the specific production rate.

12. During exponential growth, the optical density of a culture rises from 0.2 to 1.6 in 6 hours. What is the specific growth rate?

The specific growth rate is about 0.35 per hour.

WORKING

1. During exponential growth, $\ln(X/X_0) = \mu t$.
2. $X/X_0 = 1.6/0.2 = 8$, so $\ln 8 = 2.079$.
3. $\mu = 2.079/6$.
4. $\mu \approx 0.35$ h^{-1}.

Batch, fed-batch and continuous bioreactors, and scale-up

13. In a chemostat at steady state, what fixes the specific growth rate of the cells?

The dilution rate does. At steady state the cell concentration is constant, so with a sterile feed and negligible cell death, cells leave as fast as they grow and $\mu = D$, where D is the flow rate divided by the culture volume. The operator therefore sets the growth rate by setting the feed flow, and the residual substrate adjusts to match.

14. Why does a chemostat wash out when the dilution rate is raised too far?

Cells are removed in the outflow at rate D per unit of cell mass, but they cannot grow faster than the saturating limit set by the feed substrate. Above the critical dilution rate, which is at most μ_{max}, removal exceeds growth, so the cell concentration falls steadily to zero.

15. Why is fed-batch operation used instead of adding all the substrate at the start?

Feeding substrate gradually keeps its concentration low. This avoids substrate inhibition and overflow metabolism, and it lets the operator control the growth rate and oxygen demand. It also allows a high final cell density. The volume rises during the run, so the balances must include the changing volume.

16. In a bioreactor at steady state, the oxygen uptake rate of the culture is 400 mg/(L h), kLa is 100 per hour and the saturation concentration of oxygen is 7.5 mg/L. What is the dissolved oxygen concentration?

The dissolved oxygen concentration is 3.5 mg/L.

WORKING

1. At steady state, oxygen transfer rate equals oxygen uptake rate.
2. $k_L a(C^* - C) = 400$.
3. $C^* - C = 400/100 = 4$ mg/L.
4. $C = 7.5 - 4 = 3.5$ mg/L.

Media formulation and sterilisation

17. What is the difference between a defined medium and a complex medium?

A defined medium is made from pure chemicals of known amounts, so it is reproducible and easy to analyse, but it costs more and may support slower growth. A complex medium uses raw materials such as yeast extract, peptone or molasses. It is cheaper and rich in growth factors, but its composition varies from batch to batch.

18. Why is continuous sterilisation done at a high temperature for a short time?

Spore death is much more sensitive to temperature than the loss of nutrients and vitamins. The z value of spores is small, about 10 °C, while that of many nutrients is larger. A higher temperature therefore kills spores far faster than it destroys the medium, so a short hold gives the same sterility with less damage.

19. A batch of medium contains 10^{12} spores. The D value at 121 °C is 1.5 min. How long must the batch be held at 121 °C to reach a survival level of 10^{-3} spores?

The holding time is 22.5 min.

WORKING

1. Decimal reductions needed $= \log_{10}(N_0/N)$.
2. $\log_{10}(10^{12}/10^{-3}) = 15$.
3. $t = 15 \times D = 15 \times 1.5$.
4. $t = 22.5$ min.

Downstream processing

20. What is the difference between ion exchange chromatography and gel filtration?

Ion exchange separates molecules by net charge: they bind a charged resin and are released by changing salt concentration or pH. Gel filtration separates by size: small molecules enter the pores and are delayed, while large ones are excluded and elute first. Gel filtration needs no binding, so there is no adsorption step.

21. Why does the flux of a membrane filtration fall during a run?

Rejected solute piles up at the membrane surface, which is concentration polarisation, and forms a gel or cake layer that adds resistance. Pore blocking and adsorption of foulants add to this. The flux therefore settles at a value below that of clean water, and it can be restored only partly by raising the cross-flow velocity or by cleaning.

22. A crude extract holds 500 mg of protein and 10000 units of enzyme activity. After a chromatography step the pool holds 20 mg of protein and 7000 units. What is the purification fold?

The purification fold is 17.5.

WORKING

1. Initial specific activity $= 10000/500 = 20$ U/mg.
2. Final specific activity $= 7000/20 = 350$ U/mg.
3. Fold $= 350/20 = 17.5$.
4. (Yield $= 7000/10000 = 70\%$.)

Measurement, dynamics and process control

23. What is the time constant of a first-order system?

The time constant τ is the time a first-order system takes to complete 63.2% of its response to a step change in input. After about 4τ the response is within 2% of its final value. A larger τ means a slower system, such as a large vessel with slow heat or mass transfer.

24. Why does proportional-only control leave an offset after a load change?

With only proportional action the controller output equals a bias plus K_c times the error. To hold the output at a new value after a load change, a nonzero error must remain. A larger K_c shrinks the offset but never removes it. Integral action removes it by acting until the error is zero.

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