

## Problems in Cell Cultivations

References: Lee JM, Biochemical Engineering

Shuler & Kargi, Bioprocess Engineering

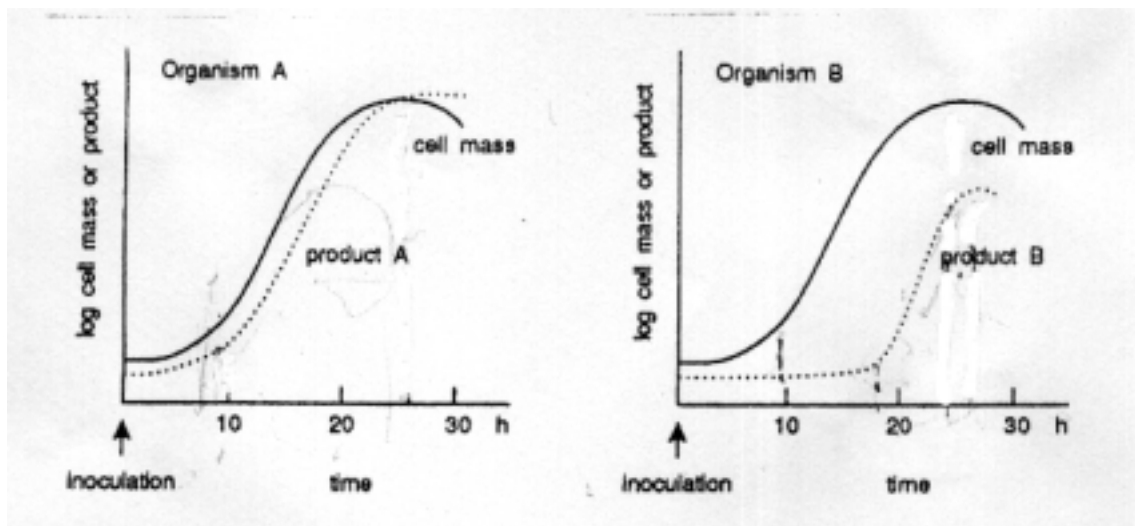
Biotol, In vitro cultivation of micro-organism

1. You are growing two microorganisms(A and B) separately in a chemostat, with 0.2% glucose as limiting substrate. Both organisms have the same maximum growth rate( $0.5 \text{ h}^{-1}$ ) but a different  $K_s$  for glucose( $10^{-4}$  and  $10^{-2} \text{ mol/l}$ , respectively). At which values for  $D$  will these organisms wash out?

(Molecular mass of glucose = 180)

2. Below are the growth cycles of batch cultures of two organisms. Examine these carefully and answer the following questions. Products A and B are extracellular products.

- What is the duration of the idiophase in each culture?
- What is the duration of the trophophase in each culture?
- Which product is probably a primary metabolite and which a secondary metabolite?



3. The specific growth rate of a culture in exponential phase is  $0.2 \text{ h}^{-1}$  and the growth yield coefficient( $Y_s$ ) for limiting nutrient in  $0.4 \text{ g biomass}(\text{g}^{-1} \text{ substrate})$ . Determine the metabolic quotient for limiting nutrient in suitable units? Would you expect  $q$  to change during exponential growth?

4. For a bacterial strain grown on glucose:

a) Determine the growth yield coefficient ( $Y_s$ ) from the following data.

Glucose conc. (g/l)	Dry weight biomass produced (g/l)
0.5	0.25
1.5	0.75
2.5	1.25
3.5	1.75

Assume all of the substrate is used.

b) If the metabolic quotient for glucose ( $q_{\text{glucose}}$ ) is  $2.5 \text{ h}^{-1}(\text{g}^{-1} \text{ biomass})$ , what is the specific growth rate in suitable units?

5. Complete the table using the words 1)Constant, 2)Variable, 3)Increasing, 4)Decreasing.

	Exponential phase of batch culture	Chemostat operating in steady-state
Growth rate of culture		
Specific growth rate of culture		
Biomass		
Available nutrient		
Culture volume		
Toxic metabolites		

6. A strain of mold was grown in a batch culture on glucose and the following data were obtained.

Time(h)	Cell Conc.(g/l)	Glucose Conc.(g/l)
0	1.25	100
9	2.45	97
16	5.1	90.4
23	10.5	76.9
30	22	48.1
34	33	20.6
36	37.5	9.38
40	41	0.63

- a) Calculate the maximum growth rate.
- b) Calculate the substrate yield.
- c) What is the maximum cell conc. one could expect if 150 g of glucose was used with the same size inoculum?

7. A simple batch fermentation of an aerobic bacterium growing on methanol gave the results shown in the table. Calculate:
- a) Maximum specific growth rate
  - b) Yield on substrate
  - c) Mass doubling time
  - d) Saturation constant
  - e) Specific growth rate at  $t=10$  h

Time(h)	Cell Conc.(g/l)	Substrate Conc.(g/l)
0	0.2	9.23
2	0.211	9.21
4	0.305	9.07
8	0.98	8.03
10	1.77	6.8
12	3.2	4.6
14	5.6	0.92
16	6.15	0.077
18	6.2	0

8. A bacterial culture containing 100 cells has a generation time of 15 minutes. How long will it take for this culture to reach a population of one million cells?

9. In a period of 5 hours the number of cells in a batch culture increases from  $10^3$  to  $10^6$ . Determine the value of the doubling time( $t_d$ ).

10. A bacterial culture containing 100 cells increased in population to one billion cells( $10^9$ ) in 10 hours. Determine

- a) the number of generations( $n$ )
- b) the generation time( $t_g$ )
- c) the specific growth rate( $\mu$ )
- d) the growth rate at the end of the incubation