Solutions for Tasks 1-11

ChE-437

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Task 1

You have prepared a preculture (100 mL) in the same medium as the batch medium you plan to use in the bioreactor. Ideally, the OD600 should be 0.1 (see lecture Part 1).

a) How much do you need to inoculate in the bioreactor that contains 2.5 L of sterile medium?
The final OD_{600} of the preculture is 4.1.

b) What is the percentile increase of the volume in the bioreactor because of the inoculation?

Solutions:
a) In general, the OD value can be used in analogy to the concentration:
(2500 mL + x mL)*0.1 = x mL*4.1
250 + 0.1x = 4.1x
x = 62.5 mL

b)
2.5%
(Note: Ideally the percentile increase should be in the range of 1 to max 10%. I usually target for 2%.)

Task 2

a) What will be the OD in the inoculated reactor after 10 h assuming you have a strain that finally grows exponentially with a μ_{max} = 0.69 h^{-1} after a lag phase at an OD600 = 0.1 for 4 h.

b) How much glucose would you have used up until 10 h when you know that the yield coefficient $Y_{OD(600)/Glucose}$ is 1.5 g^{-1}.

c) The strain produces propioniate during growth. What would be the propionate concentration when you know the yield coefficient for propionate is $Y_{Propionate/Glucose}$ = 0.1 g g^{-1}? (p_0 = 0 g L^{-1})

Solution:

a) $x = x_0 * e^{\mu*(t-t(lag))}$

x=OD600 = 6.28

Solution:

b) General equation:

$$\Delta s = \frac{x - x_0}{Y_{X/S}} = \frac{6.28 - 0.1}{1.5}$$

4.12 g L^{-1} of glucose

(4.19 g L^{-1} is wrong)

Alternatively, we could also use this equation:

$$\Delta s = \frac{x_0}{Y_{X/S}} * \left(e^{\mu*(t-t(lag)} - 1\right)$$

Solution:

c)

$$p = p_0 + \frac{Y_P}{Y_{\frac{X}{S}}} * x_0 * \left(e^{\mu*(t-t(lag)} - 1\right)$$

0.412 g L^{-1} of propionate

(0.419 g L^{-1} is wrong)

Task 3

a) What will be the specific growth rate of your strain (μ_{max} = 0.69 h^{-1}) when you know the Ks value is 0.2 g L^{-1} and you measure a glucose concentration in the supernatant that is 0.35 g L^{-1}?

b) What would be the substrate concentration when you determine a μ = 0.5 h^{-1} ?

$$\mu = \mu_{max} \frac{s}{s+Ks} = 0.44 \text{ h}^{-1}$$

Solution:

b) $$s = \frac{K_s * \mu}{\mu_{max} - \mu}$$

0.526 g L^{-1} of glucose

Task 4

Stoichiometric limitation:

a) What will be the maximum biomass (=OD600) that you can produce in a 2.5 L bioreactor with 40 g L^{-1} of glucose when you know the yields are $Y_{Propionate/Glucose}$ = 0.1 g g^{-1} and $Y_{OD(600)/Glucose}$ = 1.5 g^{-1}.

b) What would be the yield $Y_{Propionate/OD(600)}$?

Solution:

a)

$$x_{max} \approx Y_{\frac{X}{S}} * s_0 = 40 * 1.5$$

OD(600) = 60

Solution:

b)

$$Y_{Propionate/OD(600)} = \frac{Y_{Propionate/Glucose}}{Y_{OD(600)/Glucose}}$$

=0.067 g/OD(600)

Task 5

a) Analyze the graph to the right:
   - Growth phases
   - Limiting and non-limiting nutrients
   - Any products?
   - Where is PHA to be found (= Poly(3-hydroxyalkanoate), a bioplastic)?

b) Determine μ_{max}

c) Determine growth yield $Y_{X/N}$

d) Calculate the specific uptake rate for N (q_N) in g g h^{-1}

a) $\mu_{max} = 0.38\ h^{-1}$

b) $Y_{X/N} = 5.1\ g\ g^{-1}$ (=14 g/mM N)

c) $q_N = 0.075\ g\ g\ h^{-1}$

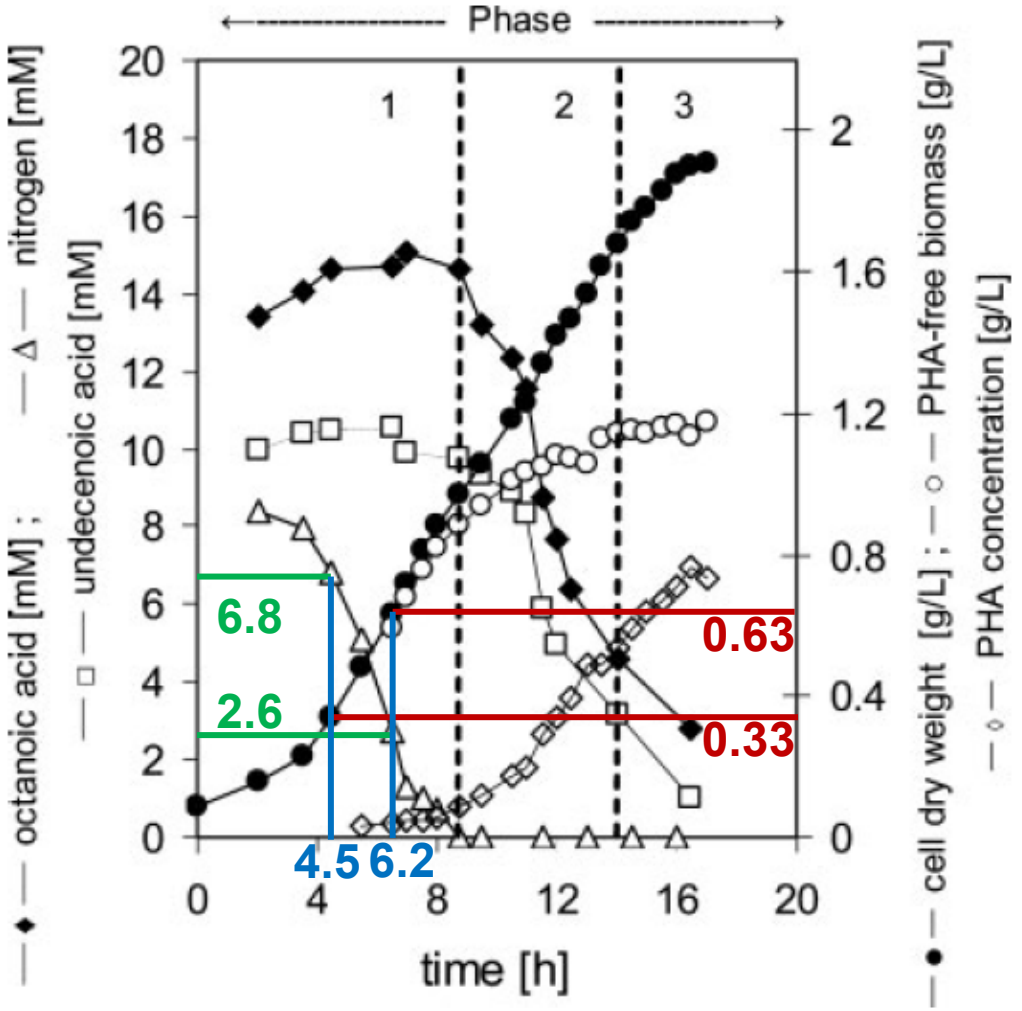


Figure 1. Accumulation of olefinic medium-chain-length polyhydroxyalkanoates (mclPHAs) by *P. putida* GPo1 during batch growth with a mixture of octanoate (15 mM) and 10-undecenoate (11 mM).

Hartmann, R., et al., *Tailor-made olefinic medium-chain-length poly[(R)-3-hydroxyalkanoates] by* Pseudomonas putida *GPo1: Batch versus chemostat production.* Biotechnol. Bioeng., 2006. **93**(4): p. 737-746.

Task 6

a) What will be the cell dry weight in the inoculated reactor after 10 h assuming you have a strain that finally grows exponentially with a μ_{max} = 0.69 h^{-1} i) normally and ii) after a lag phase at an x_0 = 0.1 g for 4 h.

b) How much glucose would you have used up until 10 h when you know that the yield coefficient $Y_{X/Glucose}$ is 0.5 g g^{-1}.

c) The strain produces propioniate during growth. What would be the propionate concentration after 10 h when you know the yield coefficient for propionate is $Y_{Propionate/Glucose}$ = 0.1 g g^{-1}? (p_0 = 0 g L^{-1})

Solution:

a)

$x = x_0 * e^{\mu * t}$

x = 99.23 g

$x = x_0 * e^{\mu * (t - t(lag))}$

x = 6.28 g

Solution:

b) $s = s_0 - \frac{x_0}{Y_{X/S}} * (e^{\mu * t} - 1)$

99.1 g => 198.26 g

12.36 g of glucose

Solution:

c) $p = p_0 + \frac{Y_{\frac{P}{S}}}{Y_{\frac{X}{S}}} * x_0 * (e^{\mu * t} - 1)$

19.826 g

1.236 g of propionate

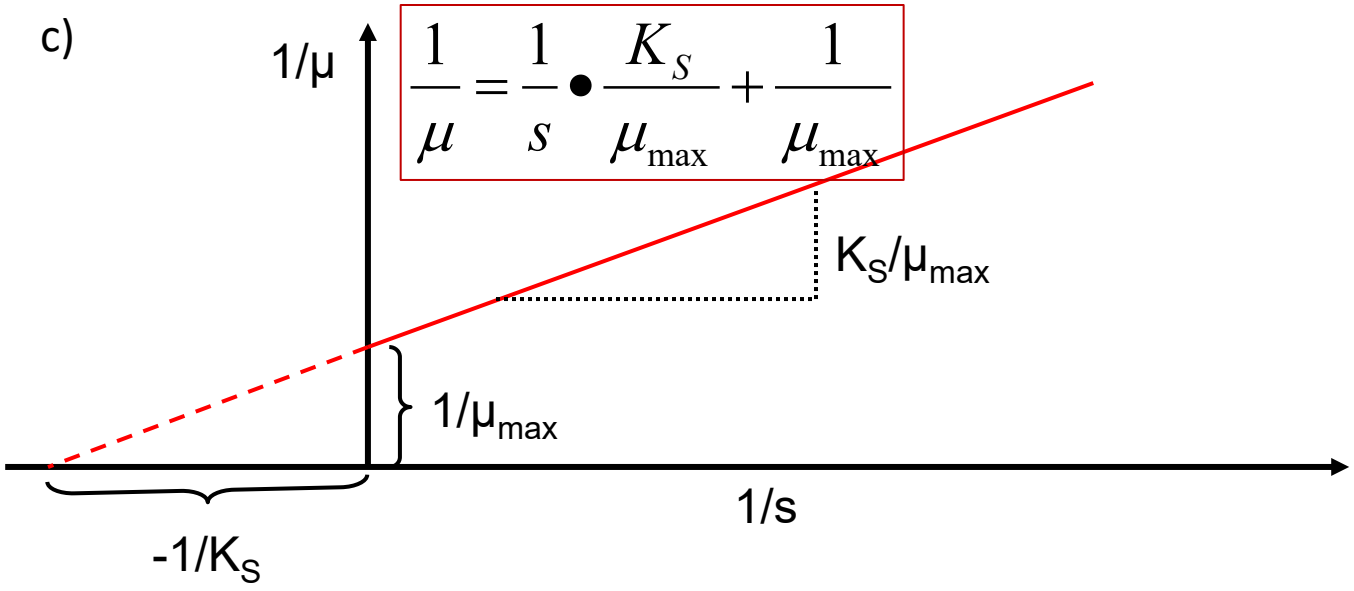
Task 7

a) How were the data obtained for the figure to the right?

b) Determine graphically the Ks and the μ_{max} values.

c) What other method could you use to determine these parameters?

a) Chemostat
b) Ks ≈ 0.12 mM (21.6 mg L^{-1}), μ_{max} ≈ 0.5 h^{-1}
c)



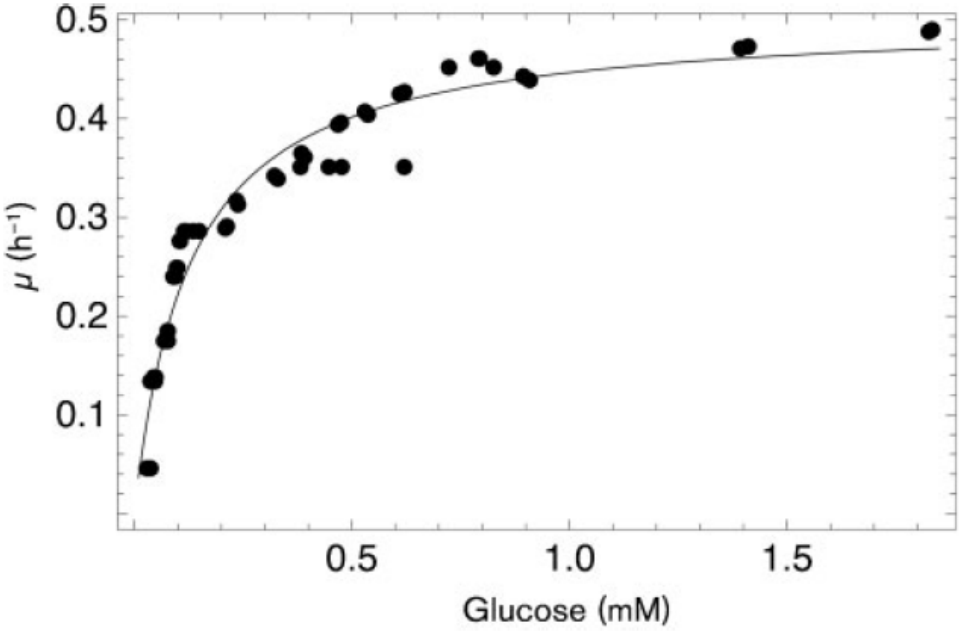


Fig. 2. μ as a function of the residual substrate concentration. *S. cerevisiae* was grown over a range of *D* values and steady-state residual glucose concentrations were determined. The Monod curve is fitted through the data points.

Snoep, J.L., et al., Microbiology (2009), 155, 1699–1707

Task 8

How much of the nitrogen source do you have to add to a carbon-limited growth medium when you want to reach a maximum biomass of 50 g L^{-1} cell dry weight and the nitrogen source (NH_4Cl) should only be 1.1-fold in excess? How much nitrogen source do you have to add if you use KNO_3 instead of NH_4Cl?

Answer:

- Required N for 50g of dry biomass L^{-1} = 50 g L^{-1} / $Y_{X/N}$ = 50 / 8 = 6.25 g N L^{-1}

- This corresponds to (6.25 g N L^{-1} / Mw N) x Mw NH_4Cl = 6.25 g N L^{-1} / 14) x 53.5 = **23.88 g L^{-1} NH_4Cl**

- N has to be 1.1-times in excess: 23.7 g NH_4Cl x 1.1 = **26.27 g NH_4Cl L^{-1}**

- If nitrate is used instead of ammonium chloride: (6.25 g N L^{-1} / Mw N) x MG KNO_3 =
(6.25 g N L^{-1} / 14) x 101.1 = **45.13 g KNO_3 L^{-1}**

- With the excess factor of 1.1: **49.65 g KNO_3 L^{-1}**

Task 9

M9 is probably the most widely used mineral medium to grow *E. coli*. It contains per litre:
Glucose 2 g, Na_2HPO_4 6 g, KH_2PO_4 3 g, NaCl 0.5 g, NH_4Cl 1 g, $MgSO_4 \cdot 7H_2O$ 0.25 g, $CaCl_2$ 7.6 mg.
Analyse and comment.

Answer:

- The medium lacks trace elements, particularly iron.

- We calculate the possible biomass that can theoretically be formed from the different elements,
  e.g. for **carbon**: (2 g glucose L^{-1} / MG glucose) x MG C x 6 x 1 = **0.8 g DW L^{-1}**
  from P: 66.3 g DW L^{-1}
  from K: 86 g DW L^{-1}
  from N: 2.08 g DW L^{-1}
  from Mg: 4.93 g DW L^{-1}
  from S: 3.25 g DW L^{-1}
  from Ca: 0.275 g DW L^{-1}

This shows you that P and K are far in excess, N, Mg, S are roughly O.K. (3-5 times in excess) but **Ca is probably low** (increase at least 10-times).
The element with the lowest excess factor is N, hence by increasing glucose in the medium one is very quickly nitrogen-limited (already by adding 5 g L^{-1} glucose).

Task 10

You have to treat wastewater from a fruit juice producing factory. It contains on average 30 g L^{-1} of easily degradable carbon compounds (mainly sugars $C_6H_{12}O_6$). The concentration of easily accessible nitrogen compounds in this wastewater is on average 0.5 g L^{-1}. What are the conclusions you draw from your analysis? Suggest solutions!

Answer:

- From 30 g L^{-1} of sugar one will obtain 12.0 g L^{-1} of DW (30x(72/180)x1)
- From the nitrogen that is easily available we can obtain 4.0 g L^{-1} of DW (0.5x8)

- This implies that the **carbon is far in excess** and will not be degraded because the treatment **plant is running nitrogen-limited**. The consequences are usually that this leads to the formation of filamentous bacteria that float on the surface and form so-called “bulking sludge”. As a solution one can **add a nitrogen-rich wastewater**, e.g. from a slaughter house.

- You can now also predict how much additional nitrogen you would have to add.

Task 11

You grow an obligate aerobic bacterial strain with a water soluble but volatile carbon source in closed glass bottles (volume 100 ml) with a mineral medium that allows cell densities of up to 5 g DW L^{-1}. The total amount of carbon supplied is 0.5 g L^{-1} and the total amount of growth medium in the bottle is 20 ml. The headspace of the bottle is filled with O_2. Is there sufficient oxygen in the headspace such that all carbon will be utilized? Assume a yield factor for molecular oxygen of 30 g DW (mol O_2)$^{-1}$. (Please ask if you need more information?)

Answer:

- In 20 ml of medium the dry biomass that will be produced from the carbon is
  0.5 gC L^{-1} x (20ml/1000ml) x 1 = **0.01 g DW**
- From the oxygen available one can form the following amount of dry biomass:
  (1 mol O_2/ 22'400 mL) x 80 ml x 30 g DW/mol O_2 = **0.1086 g DW**

Hence, there is **sufficient oxygen** in the headspace and all the carbon can be degraded by the culture.