DEPARTEMENT OF CHEMICAL ENGINEERING

BIOCHEMCIAL ENGINEERING (CBI 310)

Tutorial 19 in test format

5 MARKS

Name:

Student number:

Script number: **C01**

This document will submitted for marking. Start by writing your name and student number in the blocks above!

Instructions:

- All numerical answers should be entered on Click-Up. The test fill-in form is under 'Tests'. You can only open this form once. **Only press save and submit when you are done!**
- For numerical inputs, use **3 significant numbers**! Here is some examples: 0.0633, 7.21, -0.831, 8.22, -0.250, 0.333. For negative answers use the minus sign. **Positive answers require no plus sign!**
- All required download files are in Click-Up under 'Tests'.
- All questions will be numbered numerically. There are two types of questions. The first type is a short question (type A) where only the numerical answer will be evaluated. The second type (type B) is a longer question where the method apply to get to the answer is evaluated. For type B questions a fill-in section is supplied as a block on this document. **All final numerical answers, type A and type B, must be entered onto the Click-Up form.**
- **Type A answers are all numerical and will only be evaluated electronically.** Make sure that your answer is saved on Click-Up after you have entered it. Once saved it won't get lost. For the unlikely scenario of computer troubles, you should request the official fill-in form from the invigilator. The fill-in form will only be used in case of **absolute emergency!** If you are the unlucky winner of a fill-in form you should be sure to put your name, signature **and most importantly your script number** on this form**.** Note that type A answers written directly on your script **won't be marked**.
- **Type B answers are numerical or text-based. Numerical answers should be entered into the Click-Up interface!** It will however be possible to earn partial marks for type B questions since the entries in the dedicated type B blocks will be marked manually. It is very important to plan your

hand-written entries into the blocks, since the space will be limited! **Do not use the blocks for rough-work!** Only your final and planned attempt should be entered into the type B blocks. If you run out of space you run out of marks, it is this simple. **No extra paper will be supplied so plan your hand-written entry.**

- Make sure to bring your own paper for all rough calculations, since no paper other than this one will be supplied.
- There is a dedicated script number at the top of your script. This number should be entered as the answer to Question 1. It will earn you a free mark, but make sure you **copy it correctly to the Click-Up interface.**

This is the last tutorial of CBI310. We'll return to the metabolic network of tutorial 14 for this question. We'll slightly change the yield based flux model of tutorial 14 to a rate based flux model. The model will be given as ipynb file but make sure you fully understand the model.

To recap, we are working with a modified *E.coli* with the gene coding for PEP carboxykinase inserted into the organism. All pathways to organic acids other than succinic acid has be deleted via gene deletion. Note that the organism is a facultative anaerobe. The metabolic pathway and flux model is given in the map below. The matrix based model is supplied as *Tut19.ipynb* under 'Tutorials'.

Node carbon balances
$V_0 = 1.1V_1 + V_2$
$V_2=1.5V_3+0.75V_4$
$V_4=V_5 + V_6$
$V_6 + V_3=V_7$
$V_9=V_5 + V_8$

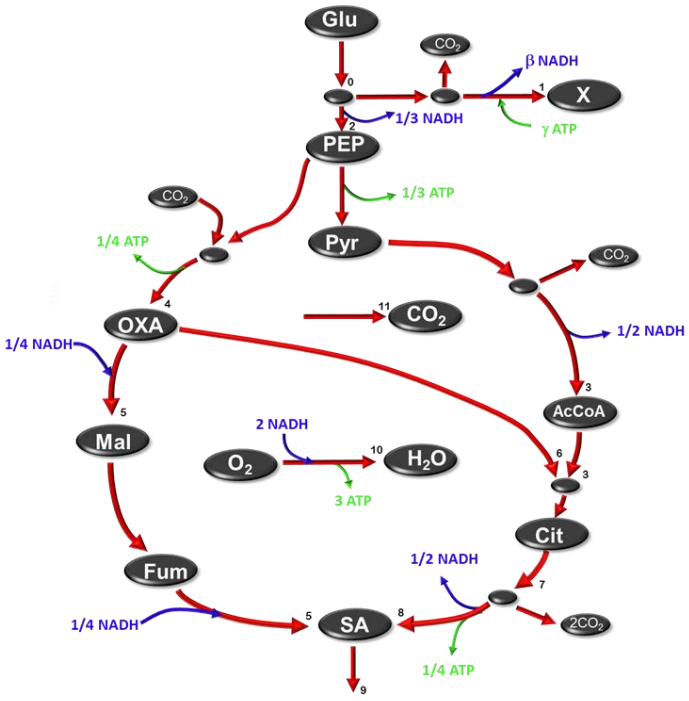
CO_2
$V_{11} = 0.1V_1 + 0.5V_3 + 0.25V_4 + (1/3)V_7$

Stoic balances
$V_6= 2V_3$
$V_8= 2/3V_7$

NADH balance
$\beta V_1 + (1/3)V_2 +0.5V_3 - 0.5V_5 + 0.5V_8 -2V_{10} = 0$

ATP balance
$-\gamma V_1 + 1/2V_3 + 0.25V_4 - 0.25V_8 +3V_{10} = \theta$

Rate spec
$V_1 = \mu$



We'll start the investigation by considering aerobic growth of the organism. For this scenario succinate excretion will be specified as zero. The following physiological parameters are given

X (elemental)	$CH_{1.8}O_{0.5}N_{0.2}$	
α	0.1	cmol CO_2/(cmol X)
γ	2.2	mol ATP/(cmol X)
μ_{max}	0.25	1/h
θ_{max}	0.08	mol ATP/(cmol X.h)

Question 1 *(type A, 1 free mark)*

What is the script number on page 1 of your script?

Question 2 *(type A, 2 marks)*

Determine the yield of oxygen on glucose for aerobic growth (zero succinate formation) in mole oxygen per cmol glucose.

Question 3 *(type B, 6 marks)*

Provide a simple flux model (in drawing and equations) for aerobic growth (zero product formation) by using a single carbon flux for respiration. Assume that μ and θ is at its maximum values and determine the biomass yield in gram biomass per gram glucose.

Question 4 *(type A, 2 marks)*

Repeat the calculation above but only use the supplied metabolic map (with zero succinate excretion). Determine the biomass yield in gram biomass per gram glucose.

Question 5 *(type B, 3 marks)*

The supplied metabolic map does not represent the conventional TCA cycle due to the insertion of PEP carboxykinase that adds to the energy efficiency of the cycle (with pyruvate carboxinase more ATP is required to form oxaloacetate). Examine the difference between the answers in question 3 and 4 and explain your findings.

Question 6 *(type A, 2 marks)*

In this question we'll consider the effect of oxygen limitation on aerobic growth. Ignore succinate formation for this question (also Q7-Q9) since only biomass will be formed as product. The Monod constant for growth is known to be 50 mg/L, while the maintenance Monod constant is 5 mg/L. In this question a batch fermentation is performed and it is specified that the glucose concentration in the fermenter varies between 2 and 140 g/L. What is the maximum percentage that μ_{max} will decrease by because of substrate limitation only?

Question 7 *(type B, 5 marks)*

Ignore succinate formation like in Question 6 and assume batch fermentation. The fermenter is sparged with air and the k_La value of oxygen transfer is known to be 200 h^{-1}. The saturation concentration of pure oxygen in the fermenter broth (at operating temperatures) is known to be 31

mg O_2/L. Determine at what concentration of biomass in the fermenter (in g/L) will oxygen supply limit the growth rate? Perform the calculation without any integration!

Question 8 *(type A, 3 marks)*

Ignore succinate formation like in Question 6 and assume batch fermentation. At a certain time in the batch fermentation the biomass concentration reaches a value of 8 g/L, while the substrate concentration remains within the boundaries defined in Q6. What is the value of the μ at this time in h^{-1}?

Question 9 *(type A, 2 marks)*

Ignore succinate formation like in Question 6 and assume batch fermentation. Wat is the glucose expenditure on maintenance at the time in Q8. Give your answer in gram glucose per gram biomass per hour.

Question 10 *(type A, 2 marks)*

For the remainder of the questions succinate formation will be considered in a batch fermenter unless specified otherwise. The glucose ranges specified in Q6 and mass transfer conditions still apply. Note that the anaerobic pathway of succinate formation is only used when the oxygen supply limits the growth rate. The value of μ and θ are not dependant on aerobic, partial aerobic or anaerobic conditions. However μ is inhibited by the succinate concentration in the broth via the following inhibition function:

$$f_{PI} = 1 - \frac{C_{SA}}{C_{SA}^{max}}$$

The maximum succinic acid concentration is known to be 35 g/L. The maintenance metabolism is not affected by the succinate concentration.

Determine at what concentration of biomass in the fermenter (in g/L) will succinate will start forming. The substrate ranges of Q6 still applies. No integration required.

Question 11 *(type A, 2 marks)*

At a certain time in the fermentation the biomass concentration is 12 g/L, while while the succinic concentration is 14 g/L. What is the growth rate at this point in time? No integration required.

Question 12 *(type A, 4 marks)*

Given the conditions in Q11, what will be instantaneous yield of succinate on glucose in cmol per cmol. No integration required.

Question 13 *(type B, 5 marks)*

At the condition in Q11 the k_La value is suddenly changed to 150 1/h. Assume that the response of the organism is immediate and determine the ratio of the new succinate formation rate relative to the rate achieved in Q12 ($\frac{r_{Q13}}{r_{Q12}}$).

Question 14 *(type A, 3 marks)*

Given an biomass concentration of 15 g/L and a succinate concentration is excess of 35 g/L, what is the minimum value of k_La that will keep the organisms alive?

Question 15 *(type A, 3 marks)*

For the conditions in Q14 at the minimum k_La, what is the instantaneous yield of succinate on glucose in cmol succinate per cmol glucose?

Question 16 *(type A, 2 marks)*

For the conditions in Q14, what is the rate of CO_2 formation in mol CO_2 per cmol biomass per hour.

More preparation?

Repeat the calculations in chapter 7 part 2 using the 'new' formulation from chapter 7 part 3. See how you generate the same answers with much less work!