BBT065 Industrial Biotechnology

Simulation Assignment 2024

Production of Baker's Yeast

You, the consultant, have been asked by our customer, the company LivePods, to give advice on the best way to design and operate a large-scale bioreactor system for production of baker's yeast. You do not have access to laboratory equipment. However, a detailed kinetic model is available, which describes the growth and product formation of *Saccharomyces cerevisiae*. Therefore, you can motivate your advice based on simulated results.

LiveProds have access to reactors according to the table below.

Reactor volume	**10 L**	**100 L**	**1000 L**	**10 m^3**	**100 m^3**
Number of reactors	2	2	2	2	4
Minimum liquid volume	2 L	20 L	300 L	2000 L	20 000 L
Maximum liquid volume	7.5 L	75 L	750 L	7500 L	75 000 L
K_La (h^{-1})	1000	600	400	$=350+(7500-V_L)/30$	$=250+(75000-V_L)/300$

You can assume that upstream (medium and inoculum preparation) and downstream (separation and packaging) processes are in place and that transfer of cell suspensions between reactors can be done flexibly. The available raw material will be beet molasses which can be mixed with minerals and water to provide a growth medium corresponding to a 1M (180 g/l) glucose solution.

Assignment:

A) Evaluate different modes of operation in terms of productivity and yields in the 100 L reactor.

B) Evaluate different options for operating the whole set of reactors.

Deliverable: You should deliver a report which contains a motivated suggestion for how to operate and control the bioreactors to obtain maximum economic gain. The report should include an analysis that assesses the risk of errors in predicted yields and productivities.

A full techno-economic evaluation is *not* required, reasonably motivated assumptions are good enough in this case.

A draft report may be submitted to the customer's representative Calle, via Canvas, no later than Monday, May 6, 18.00. The project group may then address any questions or objections that the customer raises.

The final report is due on Friday, May 17, 18.00 and should be submitted via Canvas.

General instructions and educational aspects.

The simulation assignment deals with mass transfer and physiologically based control of bioreactors, and with biochemically structured models for microbial growth and product formation. Students must submit a report on these simulation exercises, which answers to the assignment on the previous page and can be developed stepwise using the B3 and B4 exercises, and the exercises that follow in the pages below.

The exercises and report may be done in groups of three people. You are allowed to discuss the exercises with other students but each group must write their own solutions, programs and report. Direct copying of code segments from other groups is not allowed.

Each student must be able to individually explain the group's simulation procedures and results, and motivate the suggestion given to the customer. Therefore, I strongly recommend that each student runs your own matlab application. However, please work together on all the tasks and help each other within the group before asking for help!

To pass the course, students must hand in an acceptable report and be able to discuss it in a 45 minute follow-up meeting. If the report is submitted before **Friday, May 17, 18.00**, the reports will also contribute to the final grade with a maximum of 30 points, according to the following continuous scale:

Grade:	Acceptable		Good		Excellent
Points awarded:	10	15	20	25	30

The follow-up meetings will be conducted according to a special schedule on May 22-26.

You have the chance to submit a draft report no later than Monday, May 6, 18.00. You will receive simple feedback on the draft so that you can improve the analysis and discussion in the final report.

The following points contribute to an excellent report:

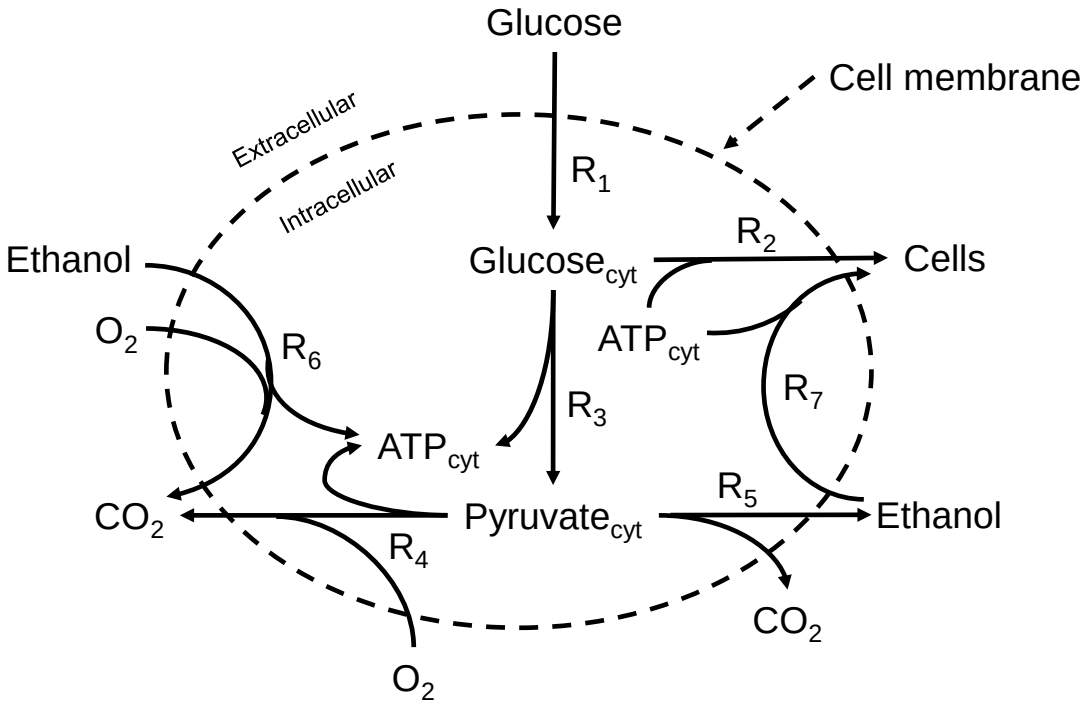
- Overall impression / Structure, language and style:
  - The report is a brief and concise document of a maximum of 8 pages, using good style and format for both text and figures. A front page, potential reference list, and appendices (containing program code and potentially other supporting material), are not included in the 8 pages. The text is thoroughly checked for errors in spelling and grammar.
  - There is a clear and concise executive summary of results and recommendations, in the beginning of the report.
  - The simulated results are appropriately selected to motivate your recommendations, and are clearly presented using clear graphs and text.
  - Results are presented using past tense; the text describes what happened in the experiments (simulations) rather than what the graphs look like. Conclusions, general knowledge, and generalizations of your specific findings are presented using present tense.

- Sources of required additional data or background are properly referred to.

- Content and understanding
  - The main point is that you show that you understand the physiology of the yeast, the dynamics of the bioreactor, the feed rate control, the simulation procedures, the use of several bioreactors as a set, and implications of large scale production.
  - The problem is clearly and concisely described (including aims of the study, strategies for simulations and control). Extensive background and unnecessary information is avoided – background should be approprately selected to match the overall content of the report.
  - The investigated modes of operation and control strategies, including choice of analytical method for monitoring the controlled variable, are clearly explained and motivated based on microbial physiology and likely physicochemical conditions in the bioreactors.
  - Results are interpreted based on the physiology of the microorganism and the physicochemical conditions in the simulated bioreactor.
  - Differences in results from different simulations (e.g. batch vs controlled fed-batch) are concisely discussed and interpreted. The implications of these differences for the recommendations are discussed. For example: Why, or under what circumstances, is either mode of operation better than the other?
  - Preferably, economic considerations are discussed (e.g. how the choice of operations influences capital investment, time for cleaning, productivity, total cost of raw material; i.e. general effects of the chosen operating modes on expected fixed costs and operating costs). However, no quantitative technoeconomic analysis is expected.
  - Limitations or drawbacks with the chosen control strategy in the lab and large scales are discussed, as well as potential ways to overcoming these in real experiments and large scale operations.
  - Well-structured Matlab code used for simulations is clearly presented in an appendix. Programs are clearly and extensively commented such that the purpose of each part is explained for someone that has not written the program themselves but knows about Matlab programming in general.

Physiologically based control of aerobic growth and product formation of *S. cerevisiae*

Baker's yeast is produced by respiratory growth of *Saccharomyces cerevisiae* on sugar beet or cane molasses under aerobic conditions. The metabolism can be modelled as aerobic growth and production formation by the following simplified structured model:



The stoichiometry and rate equations for the seven reactions of the biochemically structured model are summarized in Table 1, together with parameter values. All maximum specific rates are given per g dry cell weight (g DW).

NOTE: The m-file "Kinetics_and_parameters_2024.m" contains the stochiometric coefficients, parameter values, and kinetic expressions (rate equations) listed in Table 1. The m-file is provided on the course homepage. Please use that m-file in your code, to avoid simple typing errors!

Table 1. Stoichiometry, kinetics and parameter values, 2024. In the stoichiometric equations X, S_{ec}, E, O_2, and CO_2 indicate cell biomass, extracellular glucose, ethanol, O_2, and CO_2, respectively. The intracelluar concentrations of E, O_2, and CO_2 are assumed equal to the extracellular concentrations. G, ATP, and Pyr indicate intracellular glucose, ATP and pyruvate, respectively.

Stoichiometry and rate equation	Parameter values	Variable and parameter description
R_1: Glucose uptake $-S_{ec} + G = 0$ $q_1 = \frac{q_1^{max} \cdot S_{ec}}{(K_{1,Sec} + S_{ec})}$	q_1^{max} = 14 mmol (g DW) $^{-1}$ h^{-1} $K_{1,Sec}$ = 1 mM	q_1 specific glucose uptake rate q_1^{max} maximum specific glucose uptake rate $K_{1,Sec}$ Glucose saturation constant (Michaelis-Menten constant)
R_2: Cell growth on glucose $-G - g_{21}ATP + y_{21} X = 0$ R_2, continued. $q_2 = \frac{q_2^{max} \cdot G \cdot ATP}{(K_{2,G} + G)(K_{2,ATP} + ATP)}$	y_{21} = 0.15 g mmol^{-1} g_{21} = 10 mmol mmol^{-1} q_2^{max} = 2.7 mmol (g DW) $^{-1}$ h^{-1} $K_{2,G}$ = 0.05 mM $K_{2,ATP}$ = 0.20 mM	g_{21} Stoichiometric coefficient, ATP consumed per glucose consumed for growth y_{21} Stoichiometric coefficient, "True" biomass yield on glucose q_2 specific rate of glucose consumption due to growth (biomass formation) q_2^{max} maximum specific rate of glucose consumption due to growth $K_{2,G}$ Glucose saturation constant $K_{2,ATP}$ ATP saturation constant
R_3: Glycolysis $-G + 2Pyr + 2ATP = 0$ $q_3 = \frac{q_3^{max} \cdot G \cdot ATP}{(K_{3,G} + G)(K_{3,ATP} + ATP)\left(1 + \frac{ATP}{K_{3,I,ATP}}\right)}$	q_3^{max} = 60 mmol (g DW) $^{-1}$ h^{-1} $K_{3,G}$ = 0.8 mM $K_{3,ATP}$ = 0.5 mM $K_{3,I,ATP}$ = 1 mM	q_3 maximum specific rate of glycolysis q_3^{max} maximum specific glycolytic rate $K_{3,G}$ Glucose saturation constant $K_{3,ATP}$ ATP saturation constant $K_{3,I,ATP}$ ATP inhibition constant
R_4: Respiration of pyruvate $-3 O_2 - Pyr + 6 ATP + 3 CO_2 = 0$ $q_4 = \frac{q_4^{max} \cdot Pyr \cdot O_2}{(K_{4,Pyr} + Pyr)(K_{4,O_2} + O_2)\left(1 + \frac{S_{ec}}{K_{4,I,S_{ec}}}\right)}$	q_4^{max} = 10 mmol (g DW) $^{-1}$ h^{-1} $K_{4,Pyr}$ = 0.2 mM $K_{4,O2}$ = 0.02 mM $K_{4,I,S_{ec}}$ = 1 mM	q_4 specific respiration rate q_4^{max} maximum specific rate of respiration K_{4,O_2} Oxygen saturation constant $K_{4,Pyr}$ Pyruvate saturation constant $K_{4,I,S_{ec}}$ Inhibition constant, glucose repression

R_5: Fermentation $- Pyr + CO_2 + E = 0$ $q_5 = \frac{q_5^{max} \cdot Pyr}{(K_{5,Pyr} + Pyr)}$	$q_5^{max} = 40$ mmol (g DW) $^{-1}$ h^{-1} $K_{5,Pyr} = 5$ mM	q_5 Specific fermentation rate q_5^{max} Maximum specific fermentation rate $K_{5,Pyr}$ Pyruvate saturation constant
R_6: Respiratory ethanol consumption incl glyoxylate shunt $- E - 4\ O_2 + 2\ CO_2 + 8\ ATP = 0$ $q_6 = \frac{q_6^{max} \cdot E \cdot O_2}{(K_{6,E} + E)(K_{6,O_2} + O_2)\left(1 + \frac{S_{ec}}{K_{6,I,S_{ec}}}\right)}$	$q_6^{max} = 6$ mmol (g DW) $^{-1}$ h^{-1} $K_{6,E} = 3$ mM $K_{6,O_2} = 0.02$ mM $K_{6,I,S_{ec}} = 0.5$ mM	q_6 specific catabolic ethanol consumption rate q_6^{max} maximum specific ethanol consumption rate $K_{6,E}$ Ethanol saturation constant K_{6,O_2} Oxygen saturation constant $K_{6,I,S_{ec}}$ Inhibition constant, glucose repression
R_7: Cell growth on ethanol $- E - g_{71} ATP + y_{71} X = 0$ $q_7 = \frac{q_7^{max} \cdot E \cdot ATP}{(K_{7,E} + E)(K_{7,ATP} + ATP)\left(1 + \frac{S_{ec}}{K_{7,I,S_{ec}}}\right)}$	$y_{71} = 0.025$ g mmol^{-1} $g_{71} = 12$ mmol mmol^{-1} $q_7^{max} = 2$ mmol (g DW) $^{-1}$ h^{-1} $K_{7,E} = 0.5$ mM $K_{7,ATP} = 0.5$ mM $K_{7\,I,S_{ec}} = 0.5$ mM	g_{21} Stoichiometric coefficient, ATP consumed per ethanol consumed for growth y_{21} Stoichiometric coefficient, "True" biomass yield on ethanol q_7 specific ethanol consumption rate for growth on ethanol q_7^{max} maximum specific ethanol consumption rate $K_{7,E}$ Ethanol saturation constant $K_{7,ATP}$ ATP saturation constant $K_{7,I,S_{ec}}$ Inhibition constant, glucose repression

To successfully address this assignment, the following solution procedure is suggested:

A) Formulate the stoichiometry of the seven reactions according to the general matrix framework for stoichiometry of cellular reactions: $AS + BP + \Gamma X_{macro} + GX_{met} = 0$.

Express the specific production rates of all compounds (extracellular and intracellular) as functions of the reaction rates $q_1 - q_7$.

NOTE: The matrix notation may be used in the matlab program but it is not necessary. It may actually be easier to spell out each row in the differential equations. However, the matrix formulation helps you identify different characteristics of the compounds and helps you structure the model correctly.

B) As a reference case, simulate a **batch culture** of *S. cerevisiae* in the 100 L reactor, using the following additional data:

Initial glucose conc 1000 mmol L^{-1}

Initial intracellular glucose 0.1 mM

Initial intracellular ATP 1 mM

Initial intracellular pyruvate 0.5 mM

Initial biomass conc 0.1 g L^{-1} (7.5 g in 75 L)

Cell density 500 g DW (L cell)$^{-1}$

Liquid volume 75 L

Total volume 100 L

K_La = 600 h^{-1}

Inlet gas is air (20.95% O_2, 0.05% CO_2)

Gas flow rate Q = 1 VVM @ 20 °C and 1 atm.

Henry's constant He = 0.790 atm L $mmol^{-1}$ for oxygen.

R = $8.206 \cdot 10^{-5}$ atm L $mmol^{-1}$ K^{-1}

Hints for simulations:

a) The seven reactions lead to six mass balances (ODEs) for extracellular compounds (incl. O_2 and CO_2 in the gas phase, and O_2 in the liquid phase), three balances for intracellular compounds, and for later application also one for the volume, i.e. 10 coupled ODEs.

b) Make sure that the units are correct in all terms of the mass balances! Please use the following suggested units: Time: h; volume: L; concentrations of extracellular substrates and products: mmol/L; concentrations of intracellular metabolites: mmol / L cell volume; concentration of dissolved oxygen, micromol/L, mmol/L, or dissolved oxygen tension DOT (% of air saturation, also often called pO_2); for gas phase components, mole fraction (-) or %.

NOTE: Get this part right before you do any other simulations! Check with Calle!

C) Try to improve the biomass yield in the 100 L reactor by simulating a fed-batch instead of a batch. Start by simulating a constant feed rate, then try to control the feed rate by feed-back control of the dilution rate, so that overflow metabolism is largely avoided. Choose a control strategy and try to implement it using simulations. Refer to lectures on overflow metabolism and physiology-based control.

Use the same initial intracellular metabolite concentrations and gas-related data as in the batch simulation. In addition, use the following starting values, which corresponds to the same total amount of glucose being added to the reactor in the batch and fed-batch cases:

Initial glucose conc 1000 mmol L^{-1}

Initial biomass conc 0.375 g L^{-1} (7.5 g in 20 L)

Initial liquid volume 20 L

Final liquid volume 75 L

Total volume 100 L

The feed medium solution contains 1000 mmol L^{-1} glucose (180 g/L)

D) Check the performance of the smaller and larger reactors.

E) Test different combinations of reactors – how would you operate the whole set of reactors as a production facility?

Try to further improve the overall biomass yield per added glucose, increase the productivity, or minimize expected costs in other ways. Feel free to test ‘anything’. What variables can you play around with? What trade-offs will you have to take into consideration when giving your recommendation?

Make sure you talk to Calle and Adolf along the way, to discuss ideas, results and interpretations.

Some **hints** for analysing and describing the results

(Note: you *do not have to* specifically answer each question in the report!)

a) What is the highest average productivity of biomass that you can reach in the batch and the controlled fed-batch?

b) What is the overall (final) yield of biomass on consumed glucose after both glucose and ethanol are finally consumed?

c) How do productivity and yield influence the process economy? How do they relate to each other?

d) What limits the rate of biomass growth?

e) Can you provoke oscillations in the feed rate and ethanol concentration? Why / why not? What can you do to avoid oscillations?

f) When comparing fed-batch results and batch results, it is important to remember that in fed-batch, the liquid volume increases and glucose is fed into the reactor. So it is better to compare the total amounts in the bioreactor and total added glucose instead of just concentrations.

g) How long time does it take? Shorter or longer than the batch cultivation? How does this influence the process economy?

h) Under what circumstances would you recommend a customer to run controlled fed-batch cultivations rather than batch cultivations, and vice versa, given the simulation results above?

i) The *S. cerevisiae* simulations are not quite correct in all details compared to experimental results, but some of the major characteristic features are represented. What typical features of an aerobic yeast batch cultivation are not simulated by the model? Can you suggest ways how to include these features in the model? How would that improve the predictions?

If you have time and enjoy this, here are some additional activities:

D) Try to take the inhomogeneities and non-ideal mixing of a 100 m3 reactor into account by splitting it into two, three or more volume compartments, each of which is ideally stirred and interconnected. Substrate addition would be done into only one of the compartments and they would be filled up gradually. You will need to decide on exchange flows between these compartments. With very high exchange flows the system becomes ideally mixed, while at low exchange flows, it takes on a very different character of poor mixing. Test the residence time distribution by making a step change in the inlet flow concentration of a compound, without cells in the reactor!

E) Add a slow random variation in the kinetics to simulate variation in the metabolism of the yeast. Check if the control system can track the changes and keep the yeast in respiratory growth on glucose!