211.   Project: Saving an ecosystem

What new skills will I possess after completing this laboratory?

- **Interpreting** an industrially relevant system in terms of specification diagrams
- **Describing** this specification in terms of differential equations
- **Generalising** these equations into a julia computer program
- **Developing** this program into a robust dynamical model
- **Evaluating** the correctness of this model with respect to a reference narrative
- **Applying** this model to solve an industrially relevant dynamical problem

Why do I need these skills?

Prof. Dr. Marcus Millitzer of the company *Biotensida* has sent us an urgent request. There has been a major oil-spillage in the Gulf of Riga, endangering the wildlife of several nature reserves along the neighbouring coastline, although the oil has not yet reached the coast. Biotensida has developed a bioremediation product *Oleoclear* based on a unique biocomplex of a glycolipid biosurfactant and an alginate biopolymer surfactant, which has achieved excellent results in the remediation of sea sands, birds and animals affected by ecological accidents. Oleoclear exhibits high emulsifying activity and is synthesised microbially from the bacterial strain *Pseudomonas sp. PS-17* (Shulga et al 2000).

Biotensida plans to use a 200t bioreactor that they own near Riga to produce enough Oleoclear to avert catastrophe in the Gulf of Riga; however, time is short: they have only thirty hours during which they can run the bioreactor. Professor Millitzer has asked us to implement a computer simulation that can calculate an optimum bioreactor feed schedule to achieve a maximum yield of Oleoclear during the 30h run.

What is the structure of the skills?

We wish to optimise a fed-batch bacterial fermentation. During this process, a **broth**, consisting of water and a mass concentration $c_{s,in} = 0.45$ of sugar **substrate**, is fed into a bioreactor whose maximum capacity is 200t. Initially, the bioreactor contains 100t of broth containing substrate at a *mass* concentration of 0.3, and **biomass** at a concentration of 0.01. This biomass consists of bacterial cells that convert substrate into new biomass and the **product** Oleoclear. The bioreactor initially contains no product.

The relevant reaction rates depend only on the substrate concentration c_s in the bioreactor. Sugar concentration determines the uptake rate of sugar into the cells, and this uptake rate in turn determines the amount of product and biomass that the cells produce per kilogram of sugar uptake. Clearly, the two cellular processes of growth and product formation must compete for the common resource of sugar. Therefore, if the sugar uptake rate is slow, more product is formed per kilogram of sugar. However, this slow uptake rate then also slows down the overall fermenter process, and generates lower levels of biomass available to produce the product.

Broth is pumped into the bioreactor according to a time-dependent infeed schedule $F_{in}(t)$, and is also extracted according to a time-dependent outfeed schedule $F_{out}(t)$. The infeed and outfeed schedules are our *only* policy variables, and we can adjust them in any way we choose. The following schedules provide a reference narrative for our bioreactor model:

$$F_{in}(t) = \begin{cases} 0 & (t < 10 \text{ h}) \\ 5 \text{ t/h} & (10 \text{ h} \leq t < 30 \text{ h}) \end{cases}$$

$$F_{out}(t) = 0$$

Under these conditions, the substrate in the bioreactor falls to 0.22t after around 12h, while the biomass increases to about 19t. Subsequently, biomass rises to about 21.3t after 30h, while substrate rises to about 0.382t. The total product formed over this entire 30h process is about 7.8t.

We use the following symbols to describe the stocks in the bioreactor: m_{br} (mass of broth), m_s (substrate mass), m_p (product mass), m_x (biomass). Using this notation, processes in the bioreactor lead to the balance equations, where the subscript i stands for the stocks s, p or x, and R_i is the rate of the reaction that generates stock i in the bioreactor:

$$\frac{dm_{br}}{dt} = F_{in} - F_{out}$$

$$\frac{dm_i}{dt} = F_{in,i} - F_{out,i} + R_i$$

$$= F_{in} \cdot c_{i,in} - c_i \cdot F_{out} + q_i \cdot m_x$$

$$= F_{in} \cdot c_{i,in} - m_i \cdot \left(\frac{F_{out}}{m_{br}}\right) + q_i \cdot m_x$$

Here, $c_i \equiv m_i/m_{br}$ is the mass concentration of component i in the bioreactor, $r_{out} \equiv F_{out}/m_{br}$ is the specific broth outfeed, and q_i is the production rate of component i by the biomass. The following structure-process diagram clarifies the causal structure of these bioreactor processes:
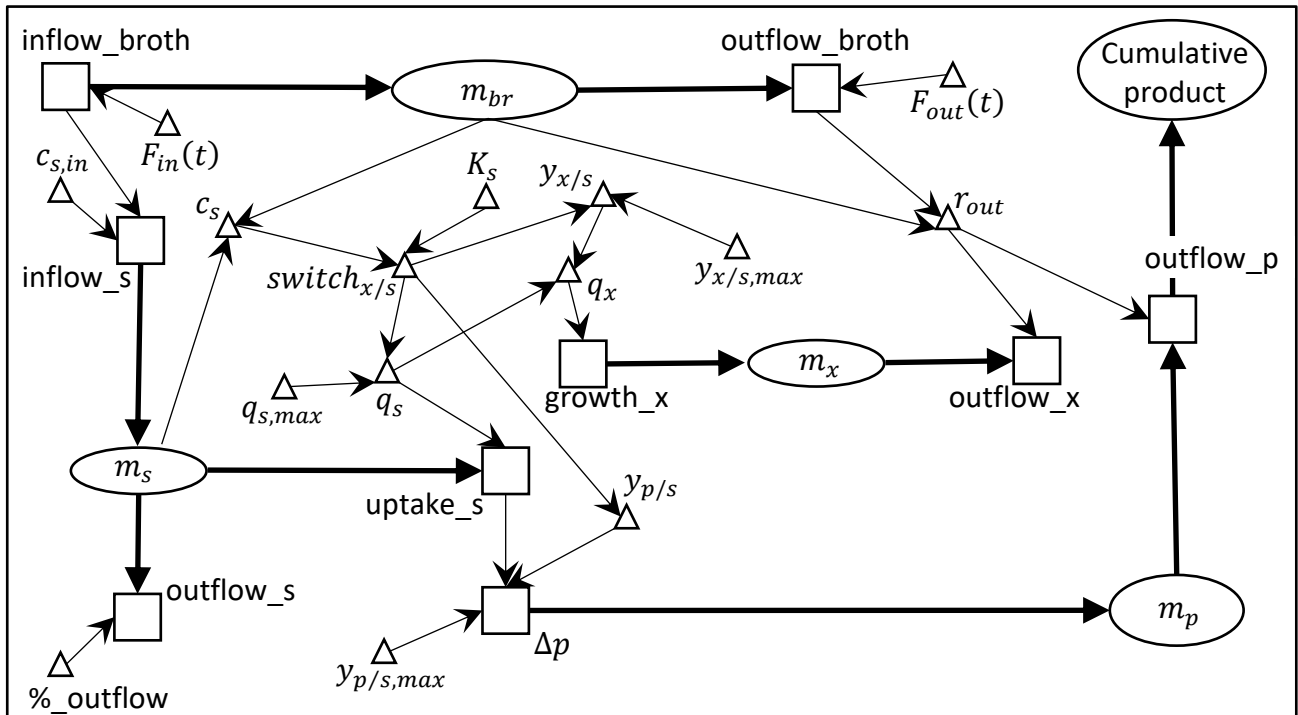
Further relevant symbols and equations in this diagram are:

- $y_{p/s,max} = 0.2$: Maximum possible yield of production per unit consumption of substrate.
- $y_{x/s,max} = 0.6$: Maximum possible yield of biomass per unit consumption of substrate.
- $q_{s,max} = 1.14$: Maximum production rate of substrate per unit biomass.
- $y_{i/j}$: Yield of component i per unit consumption of component j.
- $switch_{x/s}$: Hill function that switches on biomass growth and the uptake of substrate. $switch_{x/s}$ also inhibits formation of the product Oleoclear.
- $K_s = 0.006$: Half-saturation constant for $switch_{x/s}$.
- $q_s = q_{s,max} \cdot switch_{x/s}$: Production rate of substrate per unit biomass.
- $q_x = q_s \cdot y_{x/s}$: Specific growth rate of biomass.
- $\Delta_p = y_{p/s,max} \cdot y_{p/s} \cdot uptake_s$: Formation rate of product Oleoclear.

How can I extend my skills?

The steps of our task are:

(i)   Build a dynamical model in julia of the above bioreactor system. Professor Millitzer has made clear that Biotensida attaches *extreme importance to the accessibility of our program code* to the non-programmer biotechnologists who will need to check its logic.

(ii)   Prove that our model fulfils the given reference narrative.

(iii) Prove the robustness of this model by modifying the policy variables and demonstrating that this leads to realistic output results.

(iv)   Use our dynamical model to find a feed schedule that optimises the quantity of Oleoclear formed over 30h. Although the reference narrative forms Oleoclear within the fermenter, in practice, we need to isolate the product downstream from the bioreactor during the 30h period of the fermentation process. We therefore need to maximise the level of the stock "Cumulative product" that is isolated from F_{out} by standard downstream processes.

(v)   We will provide two deliverables: a paper and a demonstration program. The paper is presented in APA (2022) style; it will clearly explain to non-programmers the results and logical reasoning behind our optimum proposed feed schedule. The program deliverable will demonstrate this optimum schedule graphically, compared with other potential schedules that we ended up discarding in favour of the optimum schedule.

How can I deepen my practice of the skills?

(vi)   Professors Millitzer and Palfreyman are available as advisors throughout the project. They have negotiated with Biotensida that the team that *achieves and justifies* formation of the greatest mass of cumulative product over the 30-hour run will receive a bar of chocolate!

(vii) The paper should not exceed 3000 words of clear, understandable English, and should contain the following components:

- **Title**, **authors**, **contact** details and **date**.
- **Abstract**: Summarises our work and results in not more than 150 words.
- **Introduction**: Defines the context of our work in practice and in the existing literature.
- **Material and methods**: Describes and justifies the structure of our dynamical model. This section may refer to specific aspects of the program deliverable.
- **Results**: Describes the results obtained from the model on which we will base our conclusions.
- **Analysis**: Describes any statistical or error analysis required to bring our results into a form that is easily comprehended by our (biotechnological) readership.
- **Discussion**: Explains the meaning and implications of our results for the wider context of bioreactor engineering.
- **Conclusions**: Describes further work in this area that is made possible by the work we have presented earlier in the paper.
- **References**: Contains references for all citations within the text of the paper.

References

APA (2022). https://apastyle.apa.org/style-grammar-guidelines/paper-format/sample-papers (accessed 7/5/2024).

Shulga, A., Karpenko, E., Vildanova-Martsishin, R., Turovsky, A. & Soltys, M. (2000). Biosurfactant-enhanced Remediation of Oil-contaminated Environments. *Adsorption Science & Technology*, 18(2).