

BPL_CHO_Fedbatch - demo

This notebook deals with CHO fedbatch cultivation and recombinant protein production is included. First we make a check of the model by comparing a simulation result with corresponding published diagram. Then we take a closer look at the start-up strategy to keep the by-product formation low. After that we investigate at a whole cultivation and see the impact of feeding strategy on both cell growth and protein production where a trade-off is needed in this case.

The model used takes its inspiration from the microbial bottleneck models as described in the original papers [1] and [2] and reformulated and studied in [3]. The laboratory cultures used for model validation in [1] did produce MAb (against part of IgG) but no MAb-data was presented. The paper focus on viable and non-viable cell concentrations only. The original model is here expanded with the classical empirical Luedeking-Piret model recombinant protein production, see [4]. In this way can get more insight into choice of feeding profile.

Interaction with the compiled model as FMU is mainly through the simplified commands: `par()`, `init()`, `newplot()`, `simu()` etc. The last simulation is always available in the workspace and called 'sim_res'. The command `describe()` brings mainly up description information from the actual Modelica code from the FMU but is complemented with information given in the dedicated Python setup-file.

The idea is to demonstrate how simulations and varying conditions can provide some process insight that can support the experimental work. I hope that at the end of this session you are ready to formulate your own questions you want to address with simulations - and you can just go on in this notebook! Just press the field "+Code" in the upper left part of notebook interface and you get a new "cell" where you write your own code. You can copy and paste from cells above using `ctrl-c` and `ctrl-v` as usual and edit the cell. When you are ready to execute the cell just press the "play button" to the left in the cell or press `shift-enter` as in "ordinary" Jupyter notebooks.

After a session you may want to save your own notebook. That you can do on your Google Drive account and I refer to Colab instructions for how to do this. It is easy.

Good luck!

```
In [1]: run -i BPL_CHO_fedbatch_explore.py
```

Linux - run FMU pre-compiled JModelica 2.4

Model for bioreactor has been setup. Key commands:

- `par()` - change of parameters and initial values
- `init()` - change initial values only
- `simu()` - simulate and plot
- `newplot()` - make a new plot
- `show()` - show plot from previous simulation
- `disp()` - display parameters and initial values from the last simulation
- `describe()` - describe culture, broth, parameters, variables with values / units

Note that both `disp()` and `describe()` takes values from the last simulation

Brief information about a command by help(), eg help(simu)
Key system information is listed with the command system_info()

```
In [2]: plt.rcParams['figure.figsize'] = [30/2.54, 24/2.54]
```

1 About the process model

We can get information about the process and liquid phase by the command describe(). Here is no gas-phase included. This command can also be used to bring up information about a specific variable or parameter. However, you should use describe() after a simulation to get the valued used during the simulation.

```
In [3]: describe('culture'); print(); describe('liquidphase')
```

Reactor culture CHO-MAb - cell line HB-58 American Culture Collection ATCC

Reactor broth substances included in the model

Cells viable	index = 1	molecular weight = 24.6 Da
Cells dead	index = 2	molecular weight = 24.6 Da
Glucose	index = 3	molecular weight = 180.0 Da
Glutamine	index = 4	molecular weight = 146.1 Da
Lactate	index = 5	molecular weight = 90.1 Da
Ammonia	index = 6	molecular weight = 17.0 Da
Protein	index = 7	molecular weight = 150000.0 Da

The molecular weight of the recombinant protein (MAb) is somewhat arbitrarily chosen and then value not used in the simulations.

2 Simulation reproducing the original paper

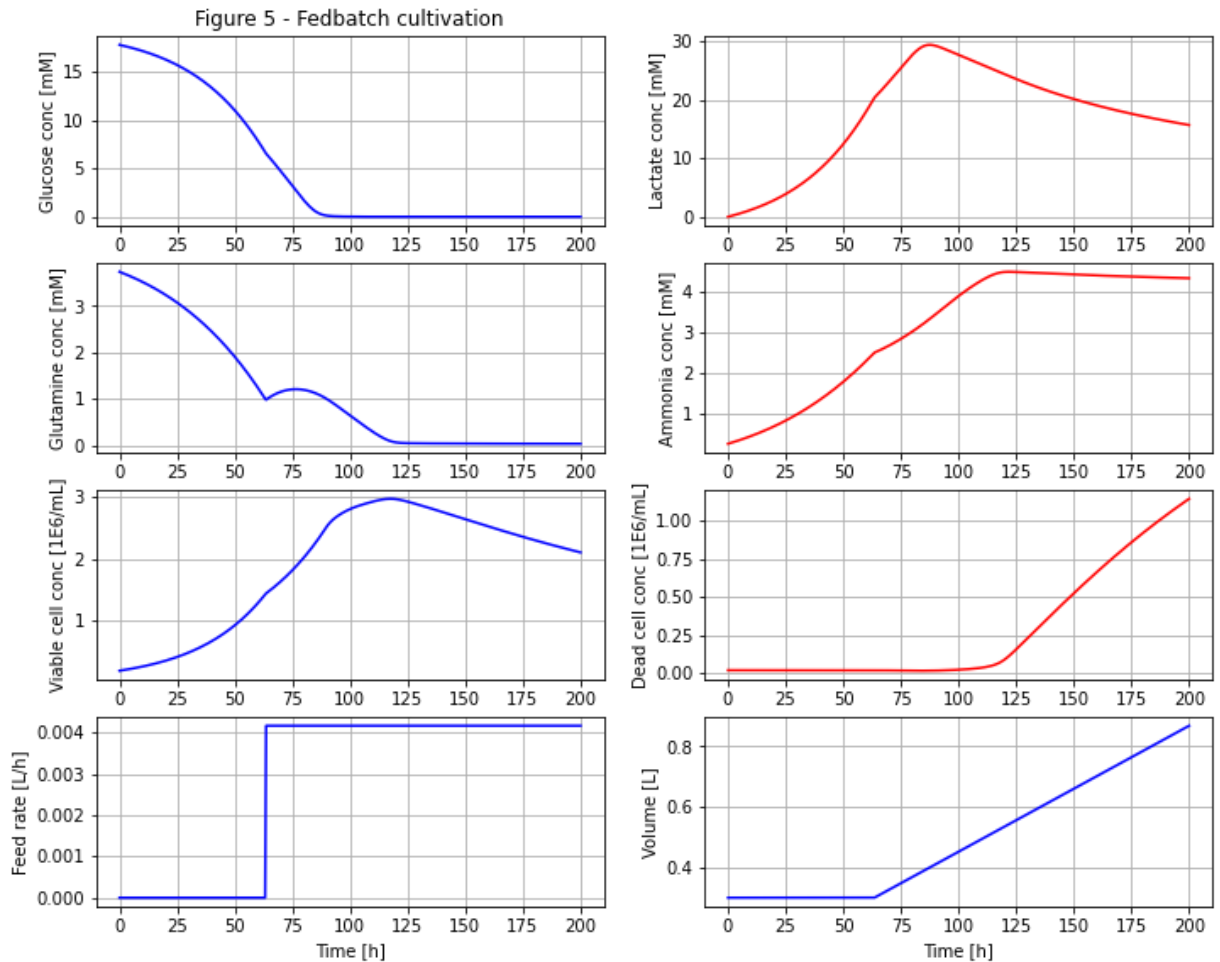
```
In [4]: # Data from Table 1 and 2 for experiment 4 shown in Figure 5 in paper [1]
# -culture parameters taken from Table 5 identified parameters for cultures

# Initial process conditions
V_0=0.30
init(V_0=V_0, VXv_0=V_0*0.172, VXd_0=V_0*0.020)
init(VG_0=V_0*17.83, VGn_0=V_0*3.74, VL_0=V_0*0.12, VN_0=V_0*0.24)

# Feeding
Feed=0.1/24
par(G_in=15, Gn_in=9.3)
par(t0=0, F0=0, t1=63.5, F1=Feed, t2=300, F2=Feed)

# Simulation
newplot(title='Figure 5 - Fedbatch cultivation')
simu(200)
```

Simulation interval : 0.0 - 200.00000000000003 seconds.
Elapsed simulation time: 0.03819744999964314 seconds.



3 Simulation of different start-up feeding strategies

```
In [5]: DeltaVG = 15*0.1*0.4
print(DeltaVG)
```

```
0.6000000000000001
```

```
In [6]: DeltaVG_0=(1-0.4)*0.30*17.8
print(DeltaVG_0)
```

```
3.204
```

```
In [7]: describe('bioreactor.inlet[1].F')
```

```
Flow rate : 0.004 [ L/h ]
```

```
In [8]: # Figur 5
V_0=0.30
init(V_0=V_0, VXv_0=V_0*0.172, VXd_0=V_0*0.020)
init(VG_0=V_0*17.83, VGn_0=V_0*3.74, VL_0=V_0*0.12, VN_0=V_0*0.24)

# Feeding
Feed=0.1/24
par(G_in=15, Gn_in=9.3)
par(t0=0, F0=0, t1=63.5, F1=Feed, t2=300, F2=Feed)

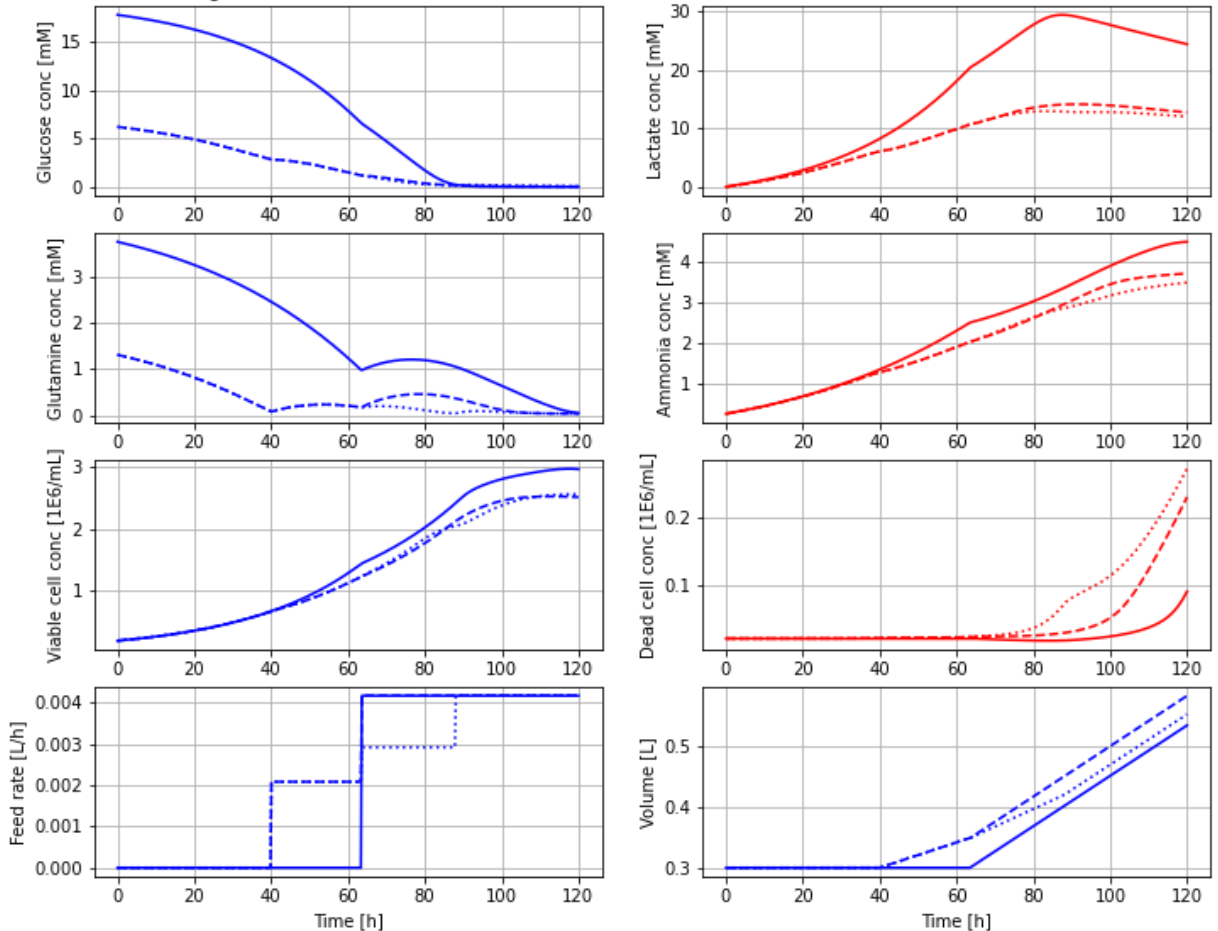
newplot(title='Figure 5 - Fedbatch cultivation')
simu(120)

init(VG_0=0.35*V_0*17.83, VGn_0=0.35*V_0*3.74)
par(t0=0, F0=0, t1=40.0, F1=0.5*Feed, t2=63.5, F2=Feed, t3=300, F3=Feed)
simu(120)
```

```
init(VG_0=0.35*V_0*17.83, VGn_0=0.35*V_0*3.74)
par(t0=0, F0=0, t1=40.0, F1=0.5*Feed, t2=63.5, F2=0.7*Feed, t3=88.0, F3=Feed,
simu(120))
```

Simulation interval : 0.0 - 119.99999999999999 seconds.
 Elapsed simulation time: 0.03299655800037726 seconds.
 Simulation interval : 0.0 - 119.99999999999999 seconds.
 Elapsed simulation time: 0.02843777499947464 seconds.
 Simulation interval : 0.0 - 119.99999999999999 seconds.
 Elapsed simulation time: 0.032088834001115174 seconds.

Figure 5 - Fedbatch cultivation



4 Simulation of optimal feed profile for cell growth

At the end of the original paper [1], section 5, the derived model is used to find an optimal feeding profile for high final cell concentration. The optimization is first done using simulation with first a step-wise increase in the feed rate and after that with an exponential structure of the feed rate. The simulation here just show the derived exponential step-wise increase in the feed rate, Figure 12 in [1].

```
In [9]: # Culture parameters taken from Table 5 identified parameters for cultures 1,
# Data chosen
V0=0.35
init(V_0=V0, VXv_0=V0*0.20, VXd_0=V0*0.0)
init(VG_0=V0*18.0, VGn_0=V0*2.4, VL_0=V0*0, VN_0=V0*0)

# Feeding
par(G_in=15, Gn_in=4.0)
par(t0=0, F0=0, t1=50, F1=0.0012, t2=64, F2=0.0020, t3=78.0, F3=0.0040)
par(t4=92.0, F4=0.0080, t5=106, F5=0.012, t6=150, F6=0.012)

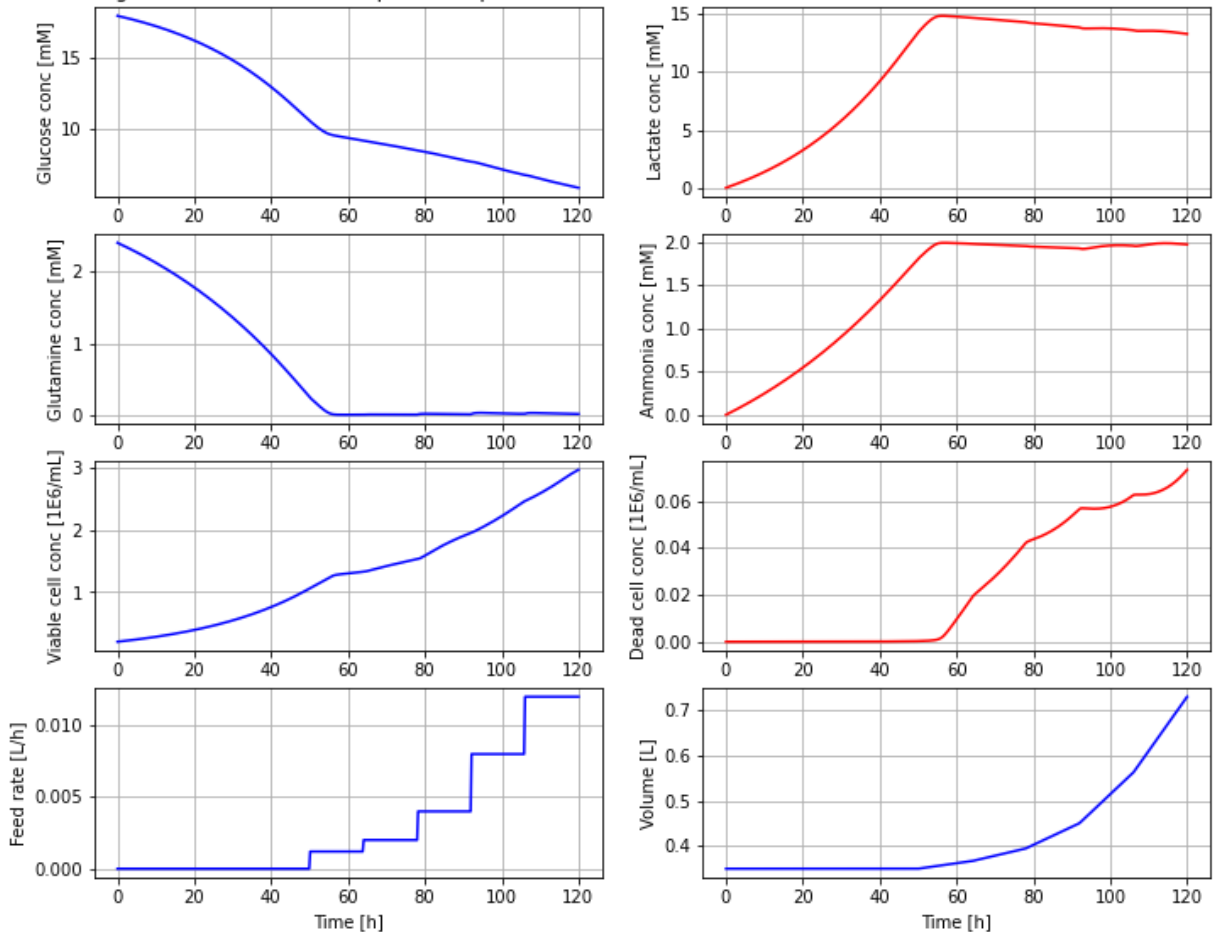
# Simulation
```

```
newplot(title='Figure 12 - Fedbatch with optimal step-wise feed')
simu(120)

# Reset feeding parameters since the table need time in strict increasing val
par(t3=1004, t4=1005, t5=1005, t6=1006)
```

Simulation interval : 0.0 - 119.99999999999999 seconds.
 Elapsed simulation time: 0.031516820999996833 seconds.

Figure 12 - Fedbatch with optimal step-wise feed



5 Simulation of different feed profiles to increase recombinant protein production

In this section we take a closer look at recombinant protein production. The original model is extended with the empirical model for specific protein production, see chapter 5 in [4]

$$q_P = \alpha \cdot \mu + \beta$$

Here we choose a negative value of growth-associated protein production α while keeping the non-growth associated β positive. The culture produced recombinant protein in the form of monoclonal antibodies for a specific IgG1 molecule, see section 2 in [1]. However, no experimental results were given. The only information we have is that feed rate was kept constant at a low level during fedbatch production and indicates that the growth-associated protein production is negative. The consequence of this observation for the feed profile we take a look at there by simulation.

```
In [10]: # Slide 3
newplot('CHO fedbatch cultivation - protein expression', plotType='Textbook_3')

# Data from Table 1 and 2 for experiment 3
```

```

V_0=0.35
init(V_0=V_0, VXv_0=V_0*0.29, VXd_0=V_0*0.010)
init(VG_0=V_0*17.17, VGn_0=V_0*3.02, VL_0=V_0*1.12, VN_0=V_0*0.29)

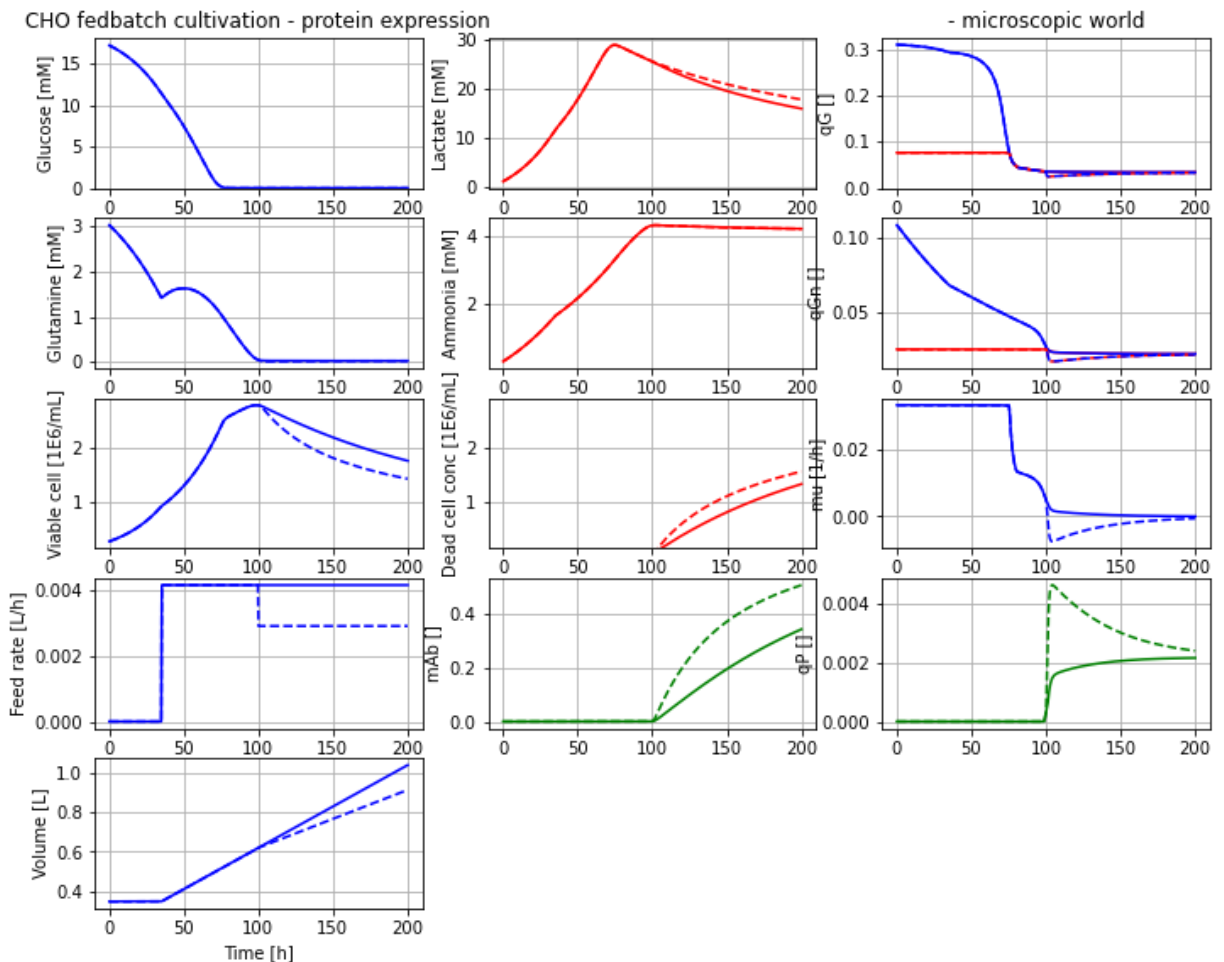
# Feeding
Feed=0.1/24
par(G_in=15, Gn_in=9.3)
par(t0=0, F0=0, t1=35, F1=Feed, t2=100, F2=Feed, t3=300, F3=Feed)

# Culture parameters
par(alpha=-1.0, beta=0.01)

# Simulation
simu(200)
par(t2=100, F2=0.7*Feed, t3=300, F3=0.7*Feed); simu(200)
par(F2=Feed, F3=Feed)

```

Simulation interval : 0.0 - 200.00000000000003 seconds.
 Elapsed simulation time: 0.033549985999343335 seconds.
 Simulation interval : 0.0 - 200.00000000000003 seconds.
 Elapsed simulation time: 0.03378108600009 seconds.



The simulation results show that actually a decrease in the feed rate can lead to an increase in recombinant protein produced, although the cell concentration is a bit lower. This is a result due to the fact that growth-associated protein production here is set to a negative value. The main point is that the model can capture this phenomena.

6 Summary

In short we have done the following:

- The model was checked by comparing the simulation results with one of the published diagrams [1].
- The common startup-procedure with 3 days batch cultivation can be questioned. We found that by shorten it to 2 days, and giving smaller feed rate day 3, byproduct formation can be kept lower at the prize of just a bit lower cell concentration. Similar idea was shown in section 2.1 in [3].
- In the original paper the experimental feeding strategy was to keep the substrate feed at a constant lower level. The authors made a point of that the optimal feeding strategy should be exponential for maximal cell production. This is an insight derived from the bottle-neck model and they showed that through simulation optimization [1]. However, there was no experimental support to confirm the results. The optimial cell growth feedprofile simulation was just reproduced here.
- To optimize recombinant protein production we must include production in the model. Here we do that with the empirical model that distinguish between growth-associated and non-growth-associated protein production, see chapter 5 in [4]. For a class of CHO-processes the recombinant prtein productivity is acutally negatively affected by cell growth. Simulation of the original model extended with such a protein production model shows that keeping the substrate feed rate constant as the cell culture grows, giving less and less feed per cell, actually can give higher protein production than an increaeing feed rate. Simulation confirms this idea. The results gives some possible background to why the constant feed rate was used experimetnally in the original paper [1].

7 References

- [1] Amribt, Z., Niu, H. and Bogaerts P.: "Macroscopic modelling of overflow metabolism and model based optimization of hybridoma cell fed-batch cultures.", Biochem. Eng. Journal, 2013.
- [2] Niu,H., Amribt, Z., Fickers, P., Tan, W. and Bogaerts P.: "Metabolic pathway analysis and reduction for mammalian cell cultures - towards macroscopic modelling", Chem. Eng. Science, 2013.
- [3] Axelsson, J. P.: "Simplified model of CHO-cultivation in Bioproses Library for Modelica - some experience", conference paper 22nd NPCW Lyngby, Denmark, August 22-23, 2019.
- [4] Hu, W-S: "Cell culture bioprocess engineering", 2nd edition, CRC Press, 2020.

Appendix

```
In [11]: # List of components in the process setup and also a couple of other things
describe('parts')
```

```
['bioreactor', 'bioreactor.broth_decay', 'bioreactor.culture', 'dosagescheme', 'feedtank', 'liquidphase', 'MSL']
```

```
In [12]: describe('MSL')
```

```
MSL: 3.2.2 build 3 - used components: RealInput, RealOutput, CombiTimeTable, Types
```

In [13]: `system_info()`

System information

- OS: Linux
- Python: 3.8.2
- PyFMI: 2.7.4
- FMU by: JModelica.org
- FMI: 2.0
- Type: FMUModelCS2
- Name: BPL_CHO.Fedbatch
- Generated: 2022-09-13T18:58:35
- MSL: 3.2.2 build 3
- Description: Bioprocess Library version 2.1.0 beta
- Interaction: FMU-explore ver 0.9.3

In []: