# BPL_CHO_Fedbatch - demo

This notebook deals with CHO fedbatch cultivation and recombinant protein production is included. We look first at start-up strategy to keep the by-product formation low. After that we look 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 [1]. The original model is here expanded with the classical empirical Luedeking-Piret model recombinant protein production, see [4].

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 infomration 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 varyiing conditions can provide some process insight that can support the experimetnal 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 ctrol-c and ctrl-p as usual and edit the cell. When your 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-comiled 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 simulati
on
 - 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.

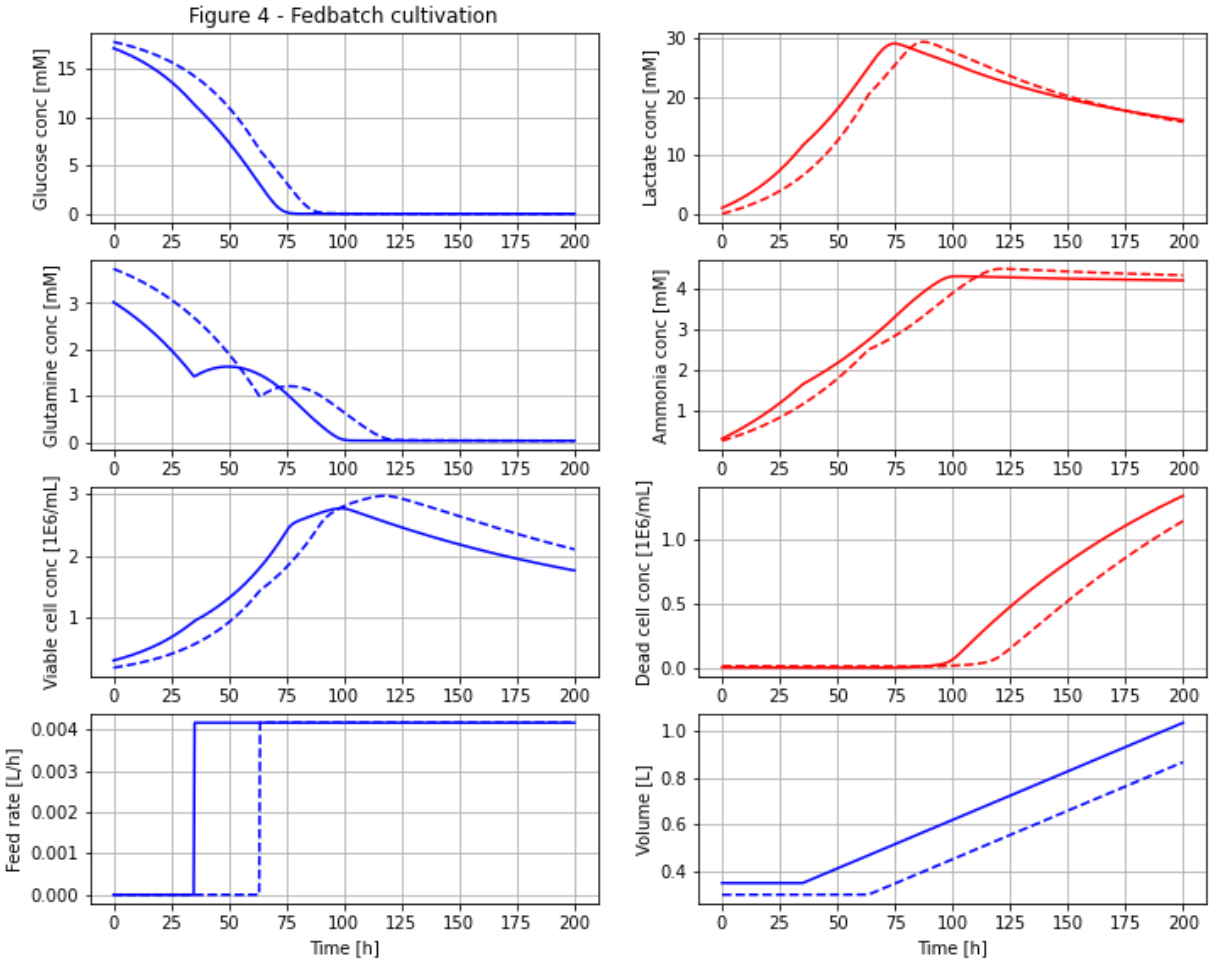
In [3]:
```
describe('culture'); print(); describe('liquidphase')
```

```
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
```

# 2 Simulation reproducing the original paper

In [4]:
```
# 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=300, F2=Feed)

# Culture parameters taken from Table 5 identified parameters for cultures 1,

# Simulation
newplot(title='Figure 4 - Fedbatch cultivation')
simu(200)

# 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)
simu(200)
```

```
Simulation interval    : 0.0 - 200.00000000000003 seconds.
Elapsed simulation time: 0.03971077000096557 seconds.
Simulation interval    : 0.0 - 200.00000000000003 seconds.
Elapsed simulation time: 0.03149055100220721 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')
```

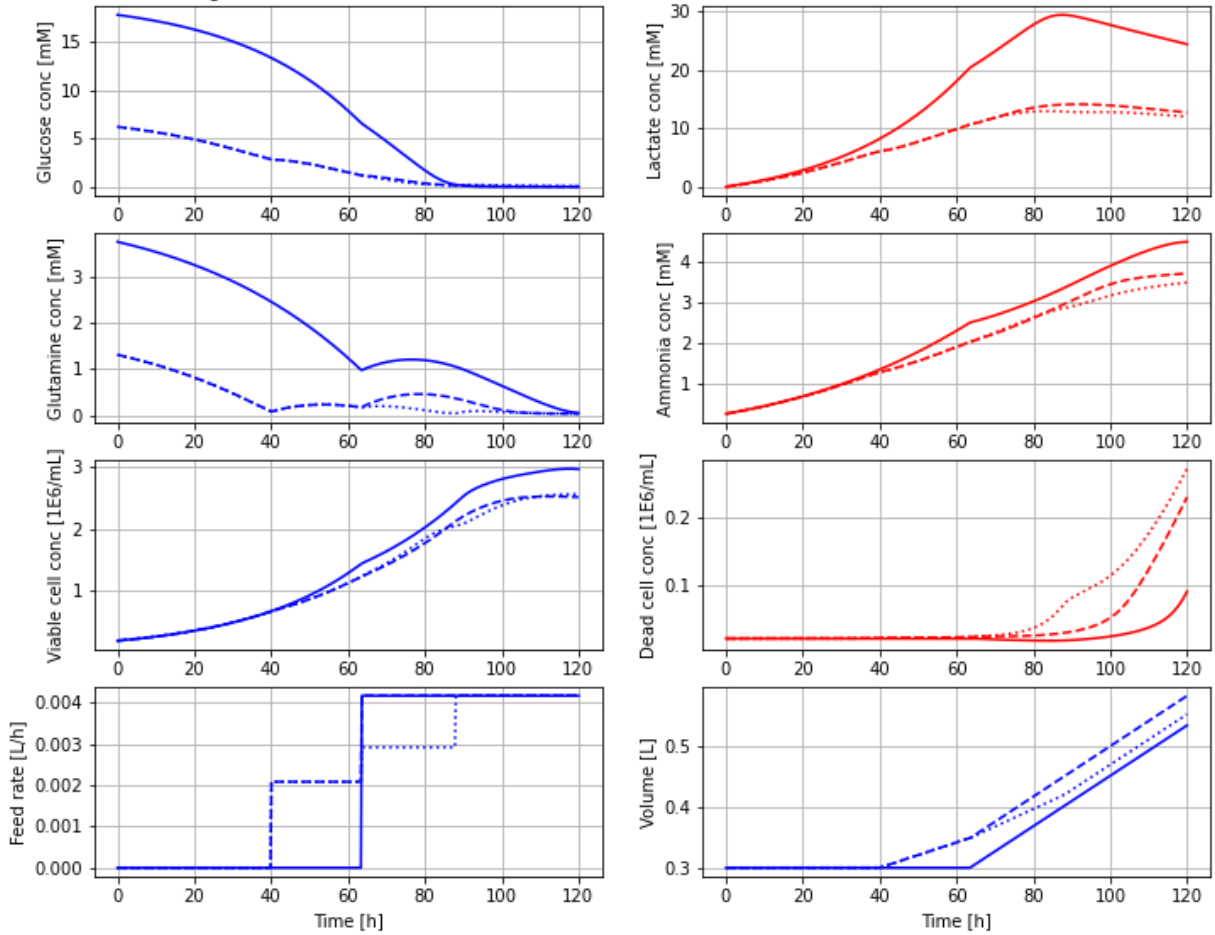
```
Flow rate : 0.004 [ L/h ]
```

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: 0.048440259000926744 seconds.
Simulation interval    : 0.0 - 119.99999999999999 seconds.
Elapsed simulation time: 0.031460684000194306 seconds.
Simulation interval    : 0.0 - 119.99999999999999 seconds.
Elapsed simulation time: 0.044258991998503916 seconds.
```

Figure 5 - Fedbatch cultivation




# 4 Simulation of optimal feed profile for cell growth

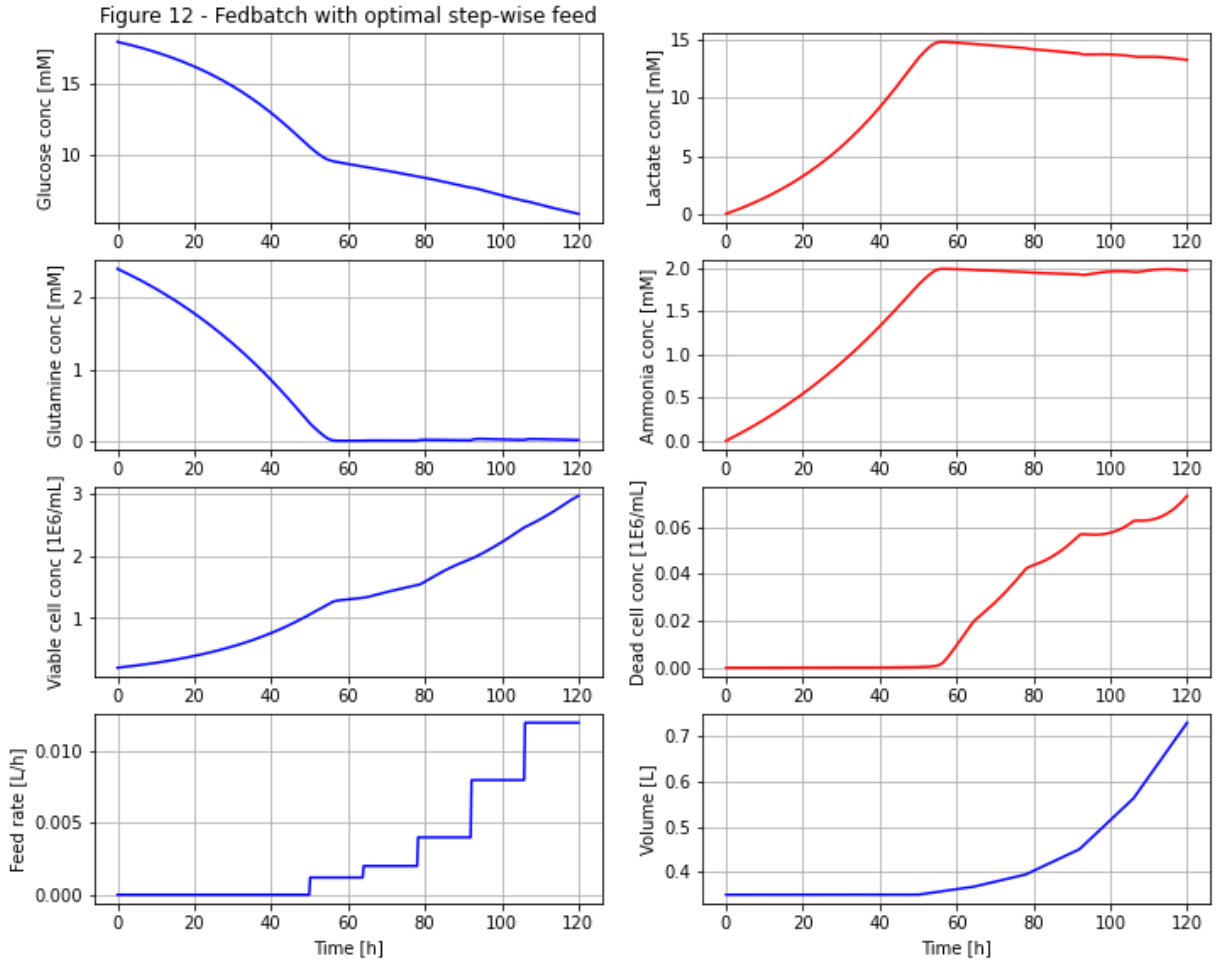
At the end of the original paper [1], section 5, the derived model is used to find an optimal feeding profile for high final cell concentration. The optimzation is first done using simulation with first a step-wise increase in the feed rate and after that with an epxonential structure of the feed rate. 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: 0.05743271800020011 seconds.
```




# 5 Simulation of different feed profiles to increase recombinant protein production

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

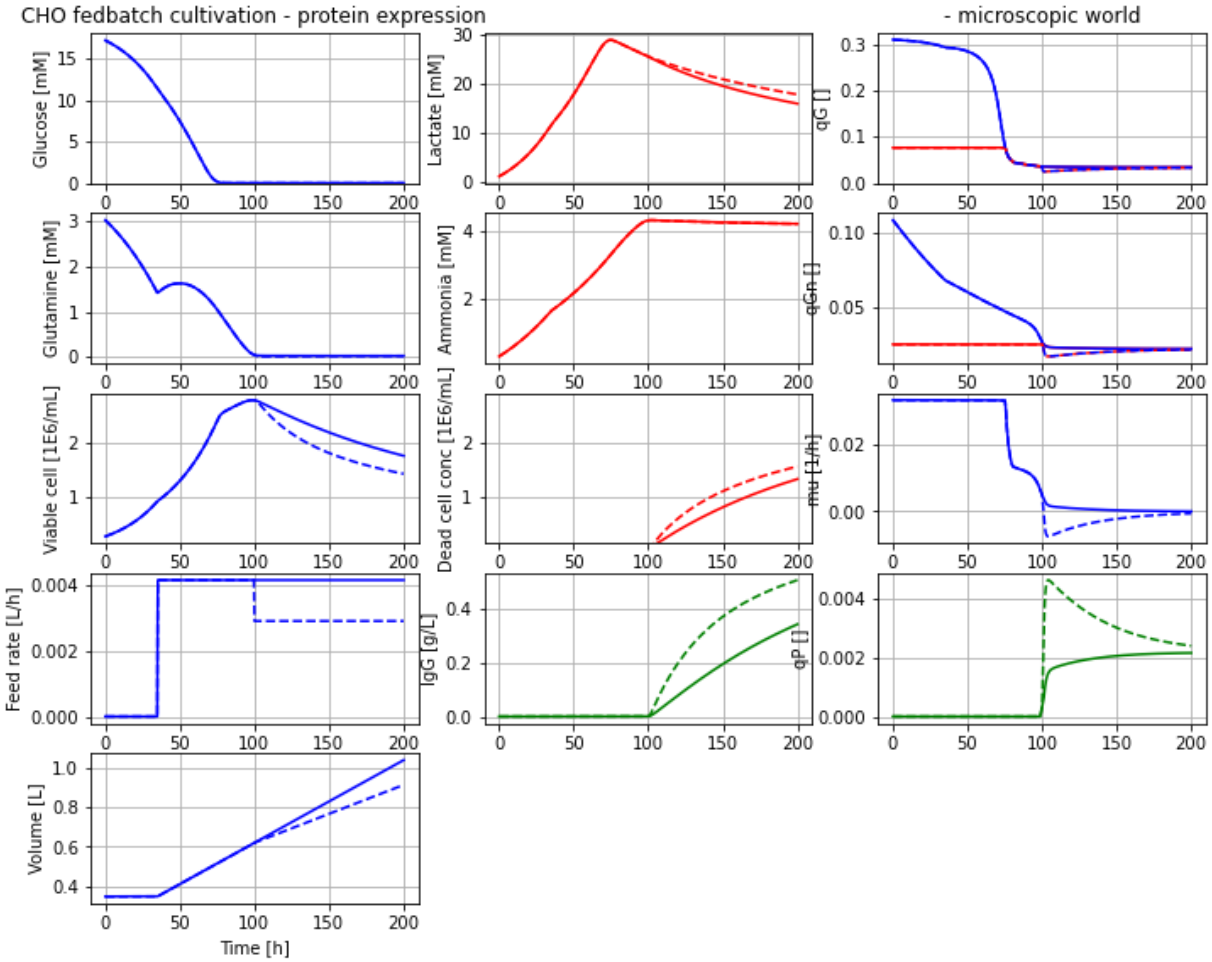
$$q_P = \alpha \cdot \mu + \beta$$

Here we choose a negative value of growth-associated protein production production $\alpha$ while keeping the non-growth associated 4\beta$ 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 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: 0.03388082200035569 seconds.
Simulation interval    : 0.0 - 200.00000000000003 seconds.
Elapsed simulation time: 0.03476670699819806 seconds.
```




The simulation results show that actually a decease 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 Bioproces 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', 'dosageschem
e', '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 [ ]:
```

```