

▼ BPL_CHO_Fedbatch script with PyFMI ver 2.9.8

The key library PyFMI v2.9.8 is installed.

After the installation a small application BPL_CHO_Fedbatch is loaded and run. You can continue with this example if you like.

```
lslsb_release -a # Actual VM Ubuntu version used by Google

No LSB modules are available.
Distributor ID: Ubuntu
Description:   Ubuntu 20.04.5 LTS
Release:       20.04
Codename:      focal

%env PYTHONPATH=

env: PYTHONPATH=

!wget https://repo.anaconda.com/miniconda/Miniconda3-py38_22.11.1-1-Linux-x86_64.sh
!chmod +x Miniconda3-py38_22.11.1-1-Linux-x86_64.sh
!bash ./Miniconda3-py38_22.11.1-1-Linux-x86_64.sh -b -f -p /usr/local
import sys
sys.path.append('/usr/local/lib/python3.8/site-packages/')

--2023-03-07 09:26:15--  https://repo.anaconda.com/miniconda/Miniconda3-py38_22.11.1-1-Linux-x86_64.sh
Resolving repo.anaconda.com (repo.anaconda.com)... 104.16.131.3, 104.16.130.3, 2606:4700::6810:8303, ...
Connecting to repo.anaconda.com (repo.anaconda.com)|104.16.131.3|:443... connected.
HTTP request sent, awaiting response... 200 OK
Length: 64630241 (62M) [application/x-sh]
Saving to: 'Miniconda3-py38_22.11.1-1-Linux-x86_64.sh'

Miniconda3-py38_22. 100%[=====] 61.64M  177MB/s   in 0.3s

2023-03-07 09:26:15 (177 MB/s) - 'Miniconda3-py38_22.11.1-1-Linux-x86_64.sh' saved [64630241/64630241]

PREFIX=/usr/local
Unpacking payload ...

Installing base environment...

Downloading and Extracting Packages

Downloading and Extracting Packages

Preparing transaction: done
Executing transaction: done
installation finished.

!conda update -n base -c defaults conda --yes
```

```
- numpy -> libgcc-ng[version='>=11.2.0'] -> __glibc[version='>=2.17']
- openssl -> libgcc-ng[version='>=7.5.0'] -> __glibc[version='>=2.17']
- python=3.8 -> libgcc-ng[version='>=11.2.0'] -> __glibc[version='>=2.17']
- scipy -> libgcc-ng[version='>=11.2.0'] -> __glibc[version='>=2.17']
- suitesparse -> libgcc-ng[version='>=11.2.0'] -> __glibc[version='>=2.17']
```

Your installed version is: 2.31

Collecting package metadata (current_repodata.json): done

Solving environment: -

The environment is inconsistent, please check the package plan carefully

The following packages are causing the inconsistency:

```
- conda-forge/linux-64::libopenblas==0.3.21=threads_h78a6416_3
- conda-forge/linux-64::sundials==6.4.1=h89a52a3_0
- conda-forge/linux-64::libblas==3.9.0=16_linux64_openblas
- conda-forge/linux-64::fmlib==2.4.1=h27087fc_0
- conda-forge/linux-64::assimulo==3.3=py38h71f17ff_1
- conda-forge/linux-64::libcblas==3.9.0=16_linux64_openblas
- conda-forge/linux-64::suitesparse==5.10.1=hd8046ac_0
- conda-forge/linux-64::scipy==1.10.1=py38h10c12cc_0
- conda-forge/linux-64::liblapack==3.9.0=16_linux64_openblas
- conda-forge/linux-64::pyfmi==2.9.8=py38h26c90d9_1
- conda-forge/linux-64::numpy==1.24.2=py38h10c12cc_failed with repodata from current_repodata.json, will retry with ne
```

Solving environment: failed with repodata from current_repodata.json, will retry with next repodata source.

Collecting package metadata (repodata.json): done

Solving environment: |

```
!conda --version
```

```
!python --version
```

```
conda 22.11.1
Python 3.8.15
```

```
!conda install -c conda-forge pyfmi --yes # Install the key package
```

```
- conda-forge/linux-64::libblas==3.9.0=16_linux64_openblas
- conda-forge/linux-64::fmlib==2.4.1=h27087fc_0
- conda-forge/linux-64::assimulo==3.3=py38h71f17ff_1
- conda-forge/linux-64::libcblas==3.9.0=16_linux64_openblas
- conda-forge/linux-64::suitesparse==5.10.1=hd8046ac_0
- conda-forge/linux-64::scipy==1.10.1=py38h10c12cc_0
- conda-forge/linux-64::liblapack==3.9.0=16_linux64_openblas
- conda-forge/linux-64::pyfmi==2.9.8=py38h26c90d9_1
- conda-forge/linux-64::numpy==1.24.2=py38h10c12cc_done
```

==> WARNING: A newer version of conda exists. <==

```
current version: 22.11.1
```

```
latest version: 23.1.0
```

Please update conda by running

```
$ conda update -n base -c defaults conda
```

Or to minimize the number of packages updated during conda update use

```
conda install conda=23.1.0
```

Package Plan

```
environment location: /usr/local
```

```
added / updated specs:
```

```
- pyfmi
```

The following packages will be REMOVED:

```
libgomp-11.2.0-h1234567_1
```

The following packages will be UPDATED:

```
ca-certificates  pkgs/main::ca-certificates-2022.10.11~ --> conda-forge::ca-certificates-2022.12.7-ha878542_0
conda            pkgs/main::conda-22.11.1-py38h06a4308~ --> conda-forge::conda-23.1.0-py38h578d9bd_0
libgcc-ng       pkgs/main::libgcc-ng-11.2.0-h1234567_1 --> conda-forge::libgcc-ng-12.2.0-h65d4601_19
libstdcxx-ng    pkgs/main::libstdcxx-ng-11.2.0-h12345~ --> conda-forge::libstdcxx-ng-12.2.0-h46fd767_19
openssl         pkgs/main::openssl-1.1.1s-h7f8727e_0 --> conda-forge::openssl-1.1.1t-h0b41bf4_0
```

```
Downloading and Extracting Packages
```

```
Preparing transaction: done
Verifying transaction: done
Executing transaction: done
```

▼ BPL_CHO_Fedbatch setup

Now specific installation and the run simulations. Start with connecting to Github. Then upload the two files:

- FMU - BPL_CHO_Fedbatch_linux_om_me.fmu
- Setup-file - BPL_CHO_Fedbatch_explore

```
%%bash
git clone https://github.com/janpeter19/BPL_CHO_Fedbatch

Cloning into 'BPL_CHO_Fedbatch'...

%cd BPL_CHO_Fedbatch

/content/BPL_CHO_Fedbatch/BPL_CHO_Fedbatch
```

▼ BPL_CHO_Fedbatch - demo

Author: Jan Peter Axelsson

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 in section 5 expanded with the classical empirical Luedeking-Piret model recombinant protein production, see chapter 5 in [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 here 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!

```
run -i BPL_CHO_Fedbatch_explore_me.py

Linux - run FMU pre-compiled OpenModelica 1.21.0

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 simula
- describe() - describe culture, broth, parameters, variables with values

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()
<Figure size 708.661x566.929 with 0 Axes>

%matplotlib inline
plt.rcParams['figure.figsize'] = [25/2.54, 20/2.54]
```

▼ 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.

```
describe('culture'); print(); #describe('liquidphase') # Pump schedule parameter
```

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

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

▼ 2 Simulation reproducing the original paper

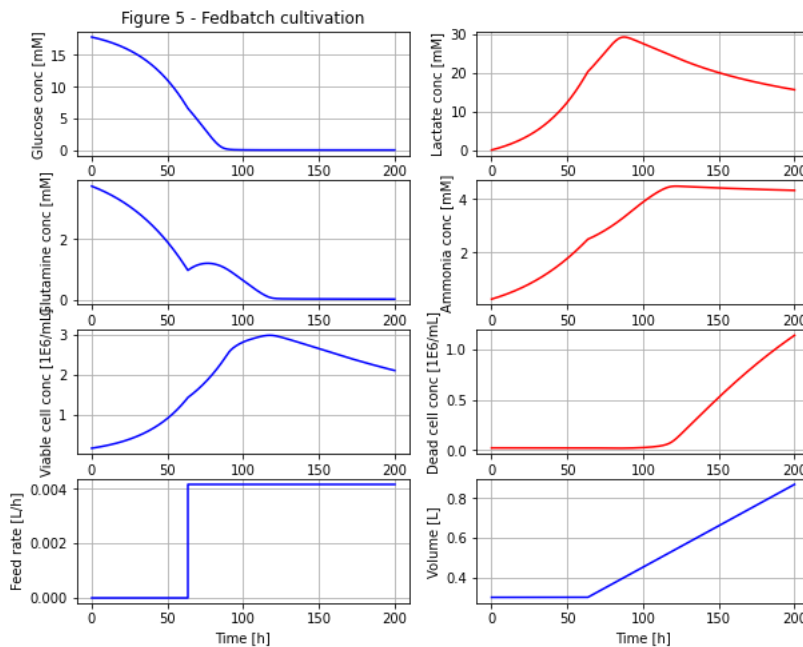
The simulation below reproduce diagrams in Figure 5 in the original paper. There are several simulation in the paper showing how well the model describe different experiments and here I just choose one of them.

```
# 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 1,2,and 3

# 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)
```



Comment: The simulation results looks very similar to the published diagram Figure 5 in [1]. The model pass this quality check.

▼ 3 Simulation of different start-up feeding strategies

```
# 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)

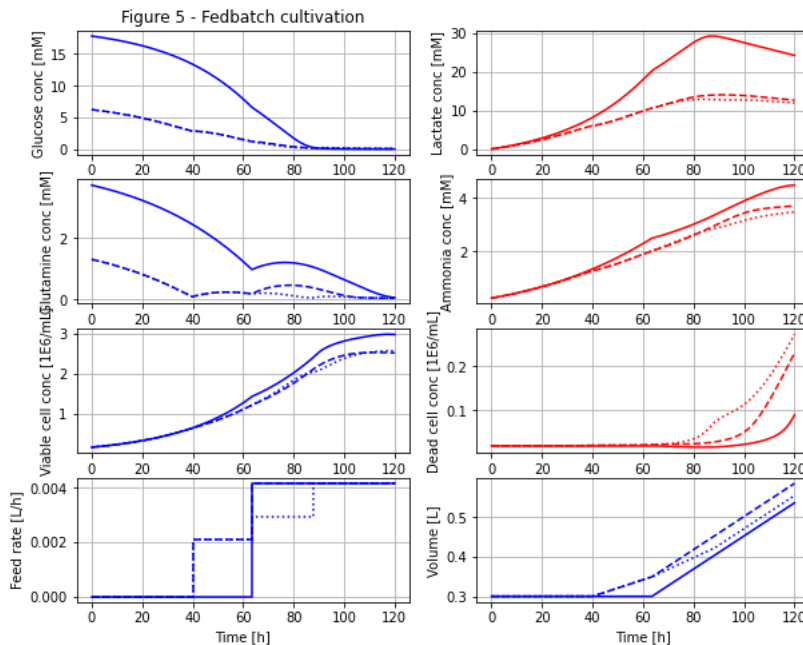
newplot(title='Figure 5 - Fedbatch cultivation')
simu(120)

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, t4=300, F4=Feed)
simu(120)

# Reset time table to avoid problems below
par(t1=1001, t2=1002, t3=1003, t4=1004, t5=1005, t6=1006)

```



Comment: We see that starting the feed a day earlier at lower rate and then increase decreases lactate formation to half, while the cell concentration is just slightly lower. With a more careful design of the feedprofile the ammonia formation can be decreased more than shown here.

4 Simulation of optimal feed profile for cell growth

At the end of the original paper section 5 in [1], the derived model is used to find an optimal feeding profile for high final cell concentration. It is stated that protein productivity is assumed to be mainly positively growth associated and therefore optimization of cell concentration is very similar to optimization of protein product. The optimization of feed profile is done with different structures of the feed profile. All of them have a start-time and all of them has a fixed amount of substrate and concentrations in the media are also the same.

- The first optimization is for a feed profile similar to the experimental, i.e. after start the feed rate remains constant throughout the cultivation. Thus the start time and the actual feed rate are optimized. The result was that the start time was about the same as experimentally but the feed rate was 50% higher, see Figure 7 and Figure 10 in [1].
- The second optimization is for a feed profile with not just one increase but three steps of increase of feed rate. The result is a somewhat higher final cell concentration, see Figure 11.
- The third optimization is for a feed profile with five steps of increase of feed rate. The result is a slightly higher final cell concentration than for three steps, see Figure 12.
- The fourth optimization is for a feed profile with continuous exponential increase of the feed rate. The result is a bit higher final concentration than the previous with five steps, see Figure 13 but not shown in the figure below.

Below we just show the results of the original experimental cultivation, compared with results from three and five steps. It is possible to do the optimization in Python with the FMU, but we save that for a future notebook.

```

# Culture parameters taken from Table 5 identified parameters for cultures 1,2,and 3

# 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 n=1 - experimental and lower feed rate
par(G_in=15, Gn_in=4.0)
par(t0=0, F0=0, t1=49, F1=0.00417)
par(t2=1002, t3=1003, t4=1004, t5=1005)

# Simulation
newplot(title='Figure 12 - Fedbatch with optimal step-wise feed')
simu(125)

# Feeding n=1
par(G_in=15, Gn_in=4.0)
par(t0=0, F0=0, t1=52, F1=0.00625)
par(t2=1002, t3=1003, t4=1004, t5=1005, t6=1006)

# Simulation
simu(125)

# Feeding n=3
par(G_in=15, Gn_in=4.0)
par(t0=0, F0=0, t1=52, F1=0.002, t2=74, F2=0.0045, t3=98.0, F3=0.010)
par(t4=99.0, F4=0.010, t5=106, F5=0.010, t6=150, F6=0.010)

# Simulation
simu(125)

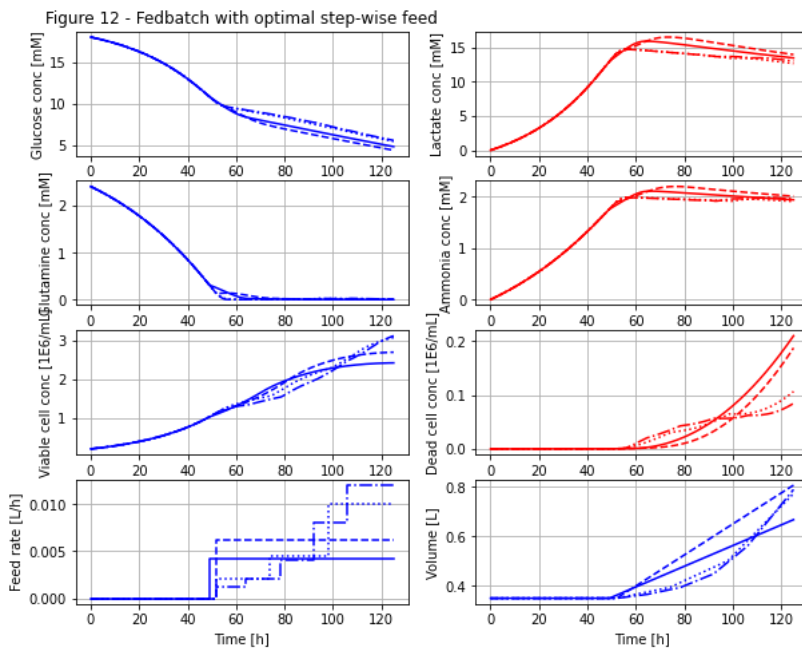
# Feeding n=5
par(G_in=15, Gn_in=4.0)
par(t0=0, F0=0, t1=52, F1=0.0012, t2=64, F2=0.0020, t3=78.0, F3=0.0040)
par(t4=92.0, F4=0.0080, t5=106, F5=0.012, t6=150, F6=0.012)

# Simulation
simu(125)

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

```

Error - the following requirements do not hold:
 parDict['t4'] < parDict['t5']



Comment: We see that that already the better tuned constant feed rate (dashed) compared to the experimental (solid) gives higher final cell concentration.

Breaking up the constant feed rate in three (dotted) and five (dash-dotted) steps with a more gradual increase of the feed rate gives even higher final cell concentration. The difference between $n=3$ and $n=5$ is small. The change to continuous exponential feed is even smaller and not shown here.

The results shown here are similar to what is presented in Table 7 in [1] but our simulation are slightly longer and here are small differences in the final cell concentration too. The qualitative result is the same though. The difference we see to the result in the original paper is most likely due to the fact that we here use the full model with 17 parameters while in the paper they have reduced the model to 15 parameters for the optimization work.

▼ 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 this choice 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.

```
# 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)

Error - the following requirements do not hold:
parDict['t4'] < parDict['t5']
Error - the following requirements do not hold:
parDict['t4'] < parDict['t5']
Error - the following requirements do not hold:
parDict['t4'] < parDict['t5']
Error - the following requirements do not hold:
parDict['t4'] < parDict['t5']
Error - the following requirements do not hold:
parDict['t4'] < parDict['t5']
Error - the following requirements do not hold:
parDict['t4'] < parDict['t5']
```

Comment: 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 actually 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 optimal 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 protein productivity is actually 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 increasing feed rate. Simulation confirms this idea. The results gives some possible background to why the constant feed rate was used experimentally 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 Bioprocess 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

```
# List of components in the process setup and also a couple of other things like liquidphase and gasphase etc
describe('parts')
```

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

```
describe('MSL')
```

```
MSL: 3.2.3 - used components: RealInput, RealOutput, CombiTimeTable, Types
```

```
system_info()
```

```
System information
-OS: Linux
-Python: 3.8.10
-Scipy: not installed in the notebook
-PyFMI: 2.9.8
-FMU by: OpenModelica Compiler OpenModelica 1.21.0~dev-185-g9d983b8
-FMI: 2.0
-Type: FMUModelME2
-Name: BPL_CHO.Fedbatch
-Generated: 2023-01-20T10:09:49Z
-MSL: 3.2.3
-Description: Bioprocess Library version 2.1.1-beta
-Interaction: FMU-explore version 0.9.6
```

✓ 28s completed at 10:26

