

# BPL\_YEAST\_AIR\_Fedbatch - demo

This notebook demonstrate yeast fedbatch cultivation. We look at impact of changes in the glucose feeding. We also take a look at tuning of the DO-control system. Both liquid- and gasphase are included in the model. The culture growth and metabolism are formulated in relation to the respiratory capacity [1] and the model is expanded to describe also the gas phase as well as the culture heat production [2]. The model was derived mainly from continuous culture data but proved to capture dynamic aspects well of ethanol production and consumption [3].

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-p` 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.

Enjoy!

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

Windows - run FMU pre-compiled JModelica 2.14

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'] = [36/2.54, 30/2.54]
```

## About the process model

We can get information about the process, liquid- and gas-phase by the command `describe()`. 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 values used during the simulation.

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

Saccharomyces cerevisiae - default parameters for strain H1022

Reactor broth substances included in the model

|               |       |   |   |   |                    |          |
|---------------|-------|---|---|---|--------------------|----------|
| Cells         | index | = | 1 | - | molecular weight = | 24.6 Da  |
| Glucose       | index | = | 2 | - | molecular weight = | 180.0 Da |
| Ethanol       | index | = | 3 | - | molecular weight = | 46.0 Da  |
| Dissolved O2  | index | = | 4 | - | molecular weight = | 32.0 Da  |
| Dissolved CO2 | index | = | 5 | - | molecular weight = | 44.0 Da  |

Reactor gasphase substances included in the model

|         |       |   |   |   |                    |         |
|---------|-------|---|---|---|--------------------|---------|
| N2 etc  | index | = | 1 | - | molecular weight = | 28.0 Da |
| O2      | index | = | 2 | - | molecular weight = | 32.0 Da |
| CO2     | index | = | 3 | - | molecular weight = | 44.0 Da |
| Ethanol | index | = | 4 | - | molecular weight = | 46.0 Da |

The model of the process has parameters both for culture, gas\_liquid\_transfer, as well as feeding procedure. The parameters that are available for changes you find by the command `disp()` and you get a long list and you change by them by command `par()`. The model has even more parameters in the background but not made available for interaction.

## First simulations - adjusting start of substrate feeding

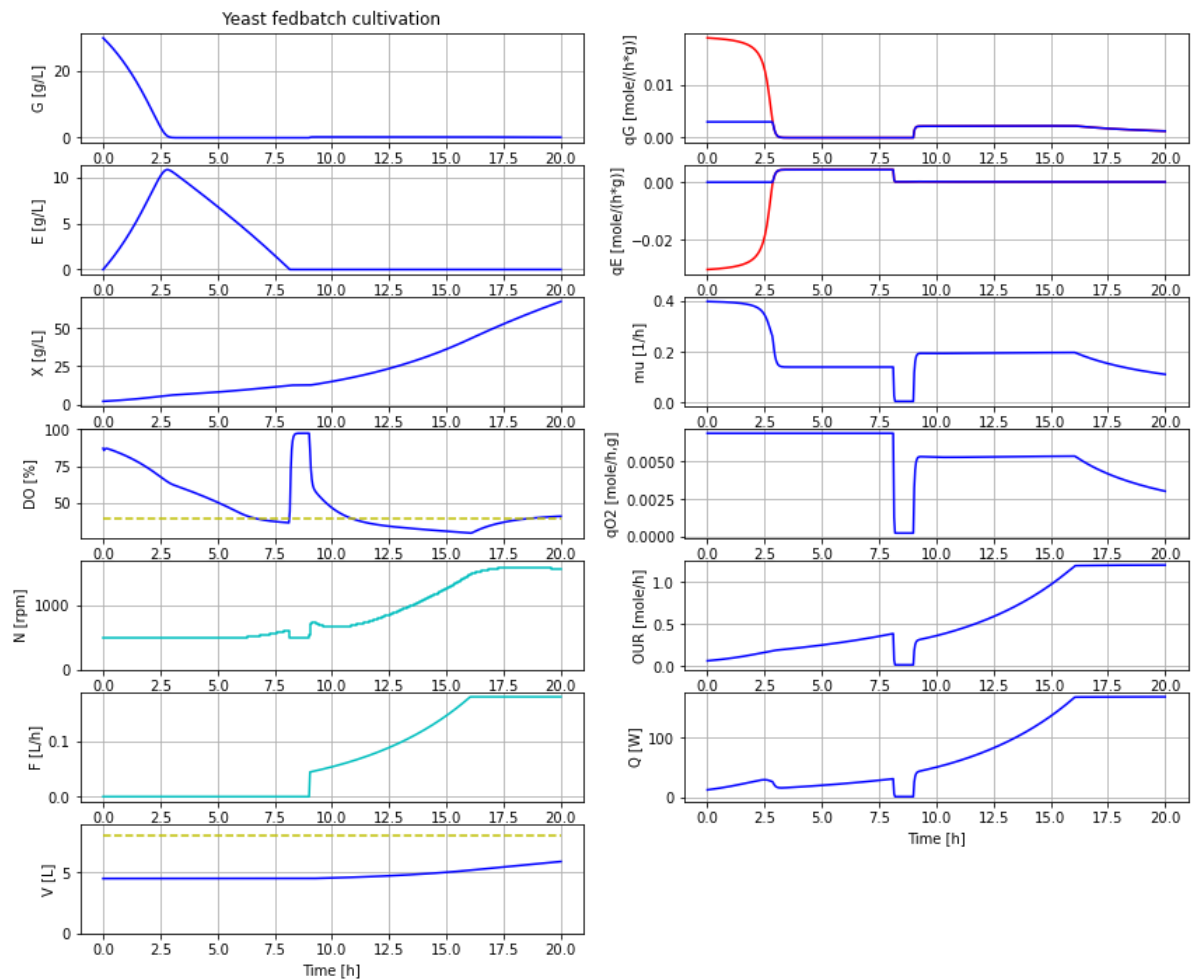
```
In [4]: # Culture parameters and others at default values
        par(q02lim=0.0069)

        # Process initial conditions
        init(V_0=4.5, VG_0=4.5*30, VX_0=4.5*2, VE_0=4.5*0)

        # Feed profile
        par(t_start=9, F_start=0.044, mu_feed=0.20, F_max=0.18)

        # DO-control parameters
        par(samplePeriod=1/60, K=10, Ti=0.5, I_0=500)

        # Simulate and plot
        newplot(title='Yeast fedbatch cultivation', plotType='Overview')
        simu(20)
```



Now we can get value of broth volume as well as the headspace and values are the last ones in the simulation

```
In [5]: describe('bioreactor.V')
```

```
Reactor broth volume : 5.892 [ L ]
```

```
In [6]: describe('bioreactor.V_gasphase')
```

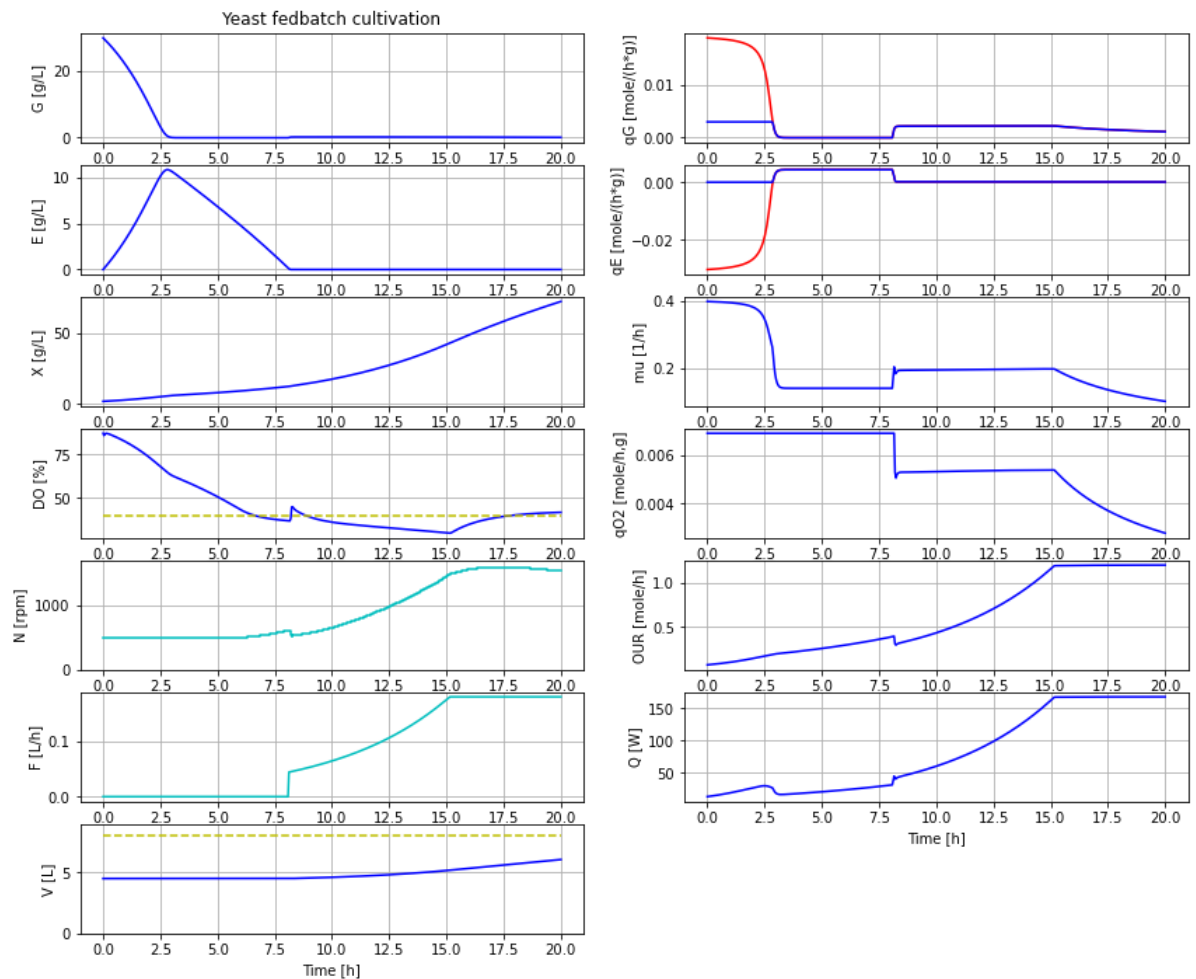
```
Volume of the gas phase : 2.108 [ L ]
```

```
In [7]: # Take a Look at the parameters available to adjust the dosage scheme
disp('dosage', decimals=4)
```

```
mu_feed : 0.2
F_0 : 0.0
t_start : 9.0
F_start : 0.044
F_max : 0.18
```

```
In [8]: # Let us start the feeding just after the batch phase has ended and keep other par
par(t_start=8.1)

# Simulate and plot
newplot(title='Yeast fedbatch cultivation', plotType='Overview')
simu(20)
```



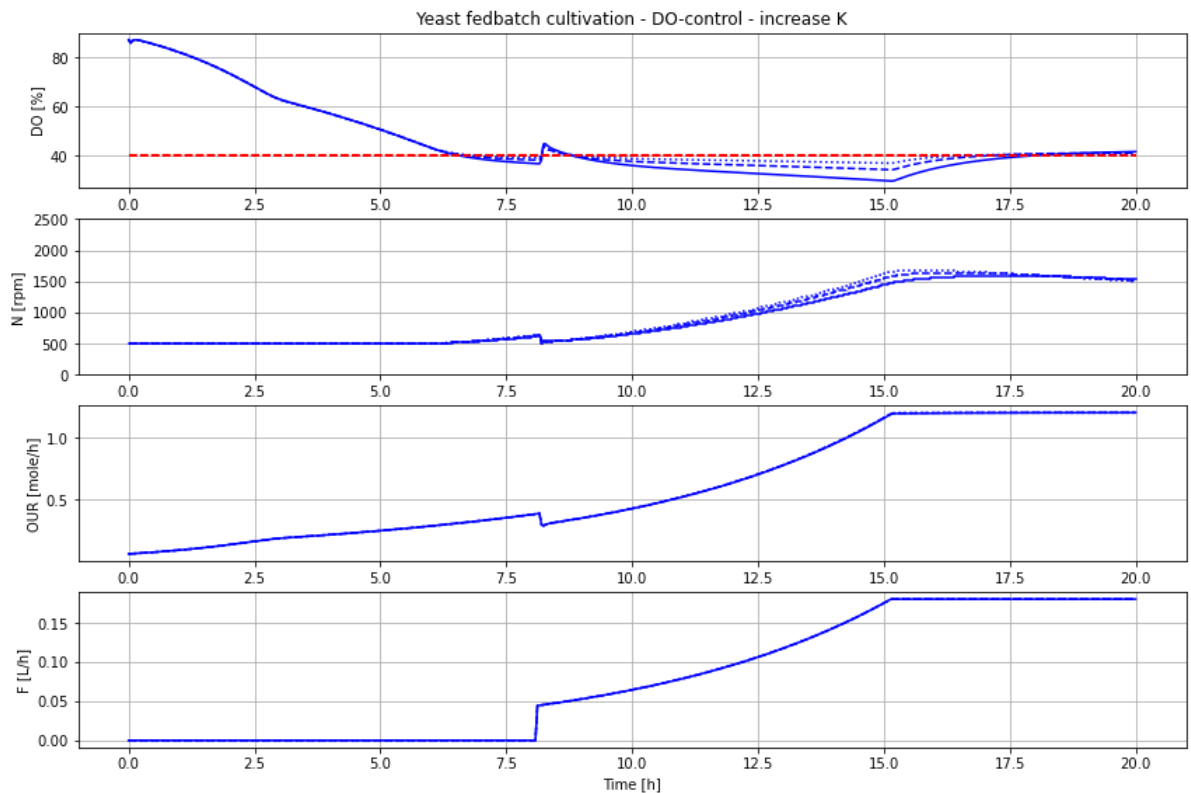
The increase of DO to about 50 % at end of batch phase should be possible to detect easily. This simulation is more realistic and we use these settings from now on.

## DO-control - tuning of PI-regulator parameters

Let us focus on the DO-control system and choose a more limited plotType. We study the impact of PI control parameters and see if we can decrease the control error without loosing stability.

```
In [9]: # Let us take a closer look at the DO-control system and try to make control error
newplot(title='Yeast fedbatch cultivation - DO-control - increase K', plotType='For
for value in [10, 20, 40]: par(K=value); simu(20)

# Reset K to the original value
par(K=10)
```



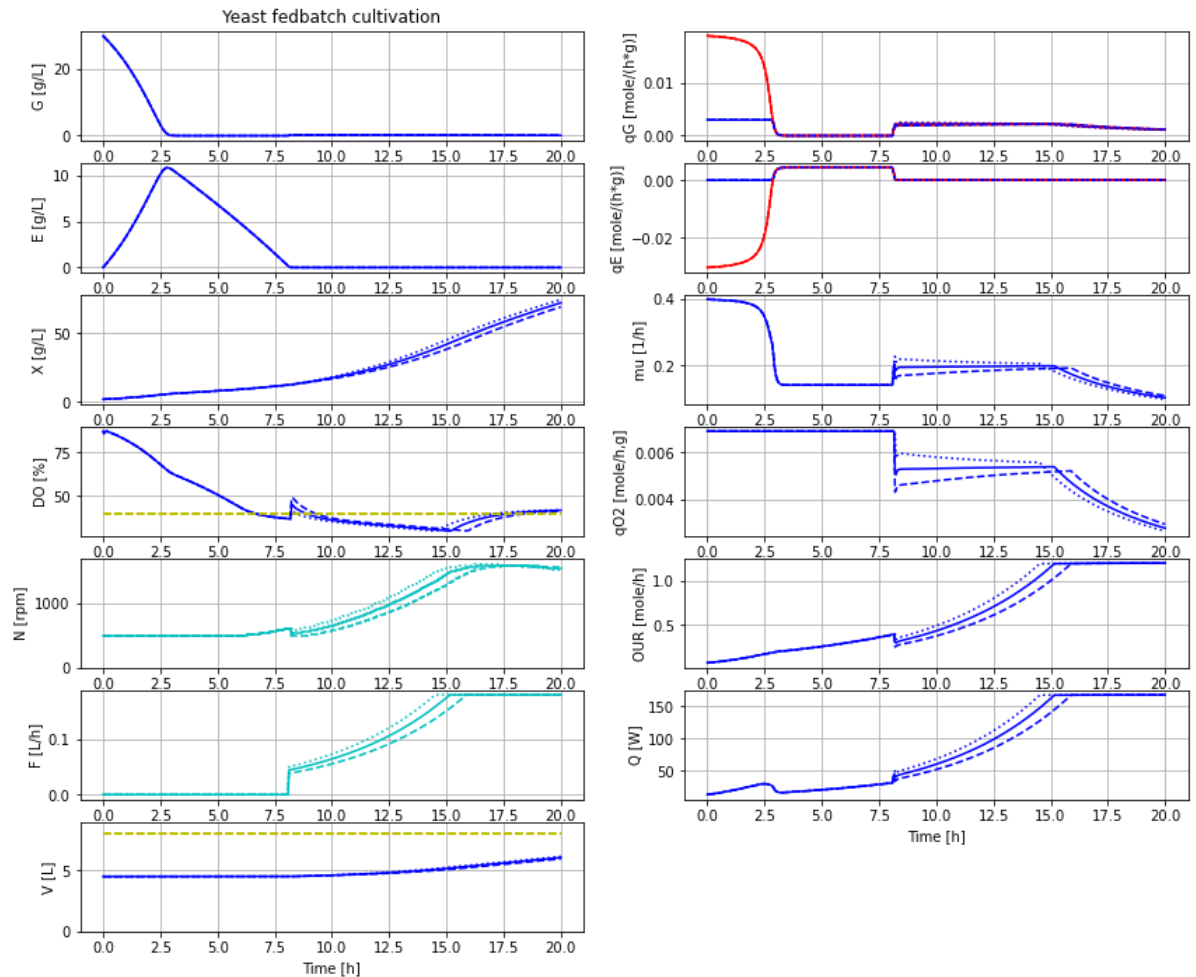
We see that by a higher control gain  $K$  the DO-control error get smaller and the stability of the control system is maintained.

**Exercise** I leave for you to study the impact variation of the  $T_i$ -parameter. Just make a new cell below. Then copy and paste the cell above and change parameter to  $T_i$ .

## Sensitivity to changes in feed-profile

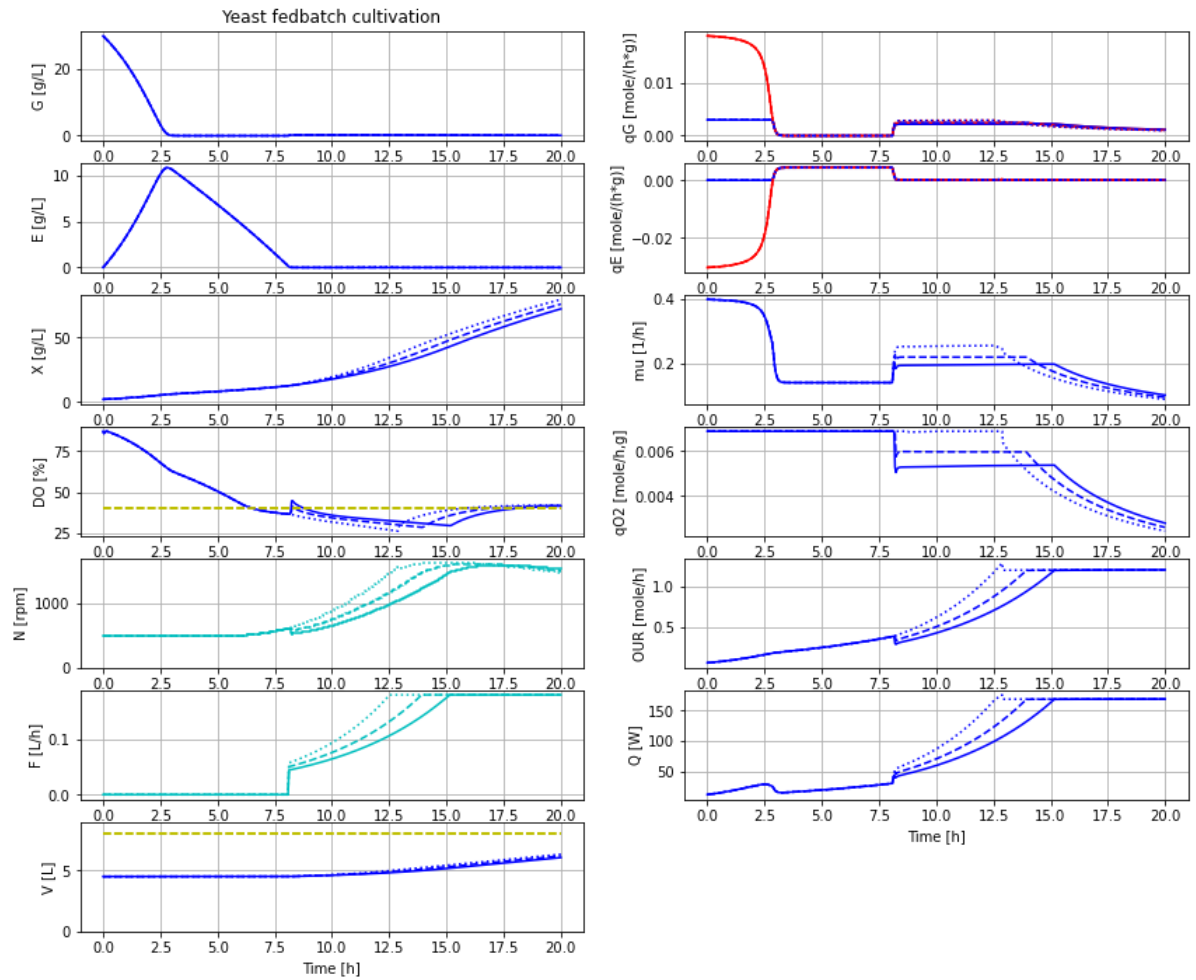
Now, let us focus on investigating impact of changes in the feed-profile. The goal is to increase the produced cell mass without accumulation of by-product ethanol. Simulation can bring some insight into how behaviour of the different variables change when by-product is formed. This insight can help to interpret experimental results.

```
In [10]: # Let us check the sensitivity to changes in the feed profile design
newplot(title='Yeast fedbatch cultivation', plotType='Overview')
for value in [0.044, 0.038, 0.050]: par(F_start=value); simu(20)
```

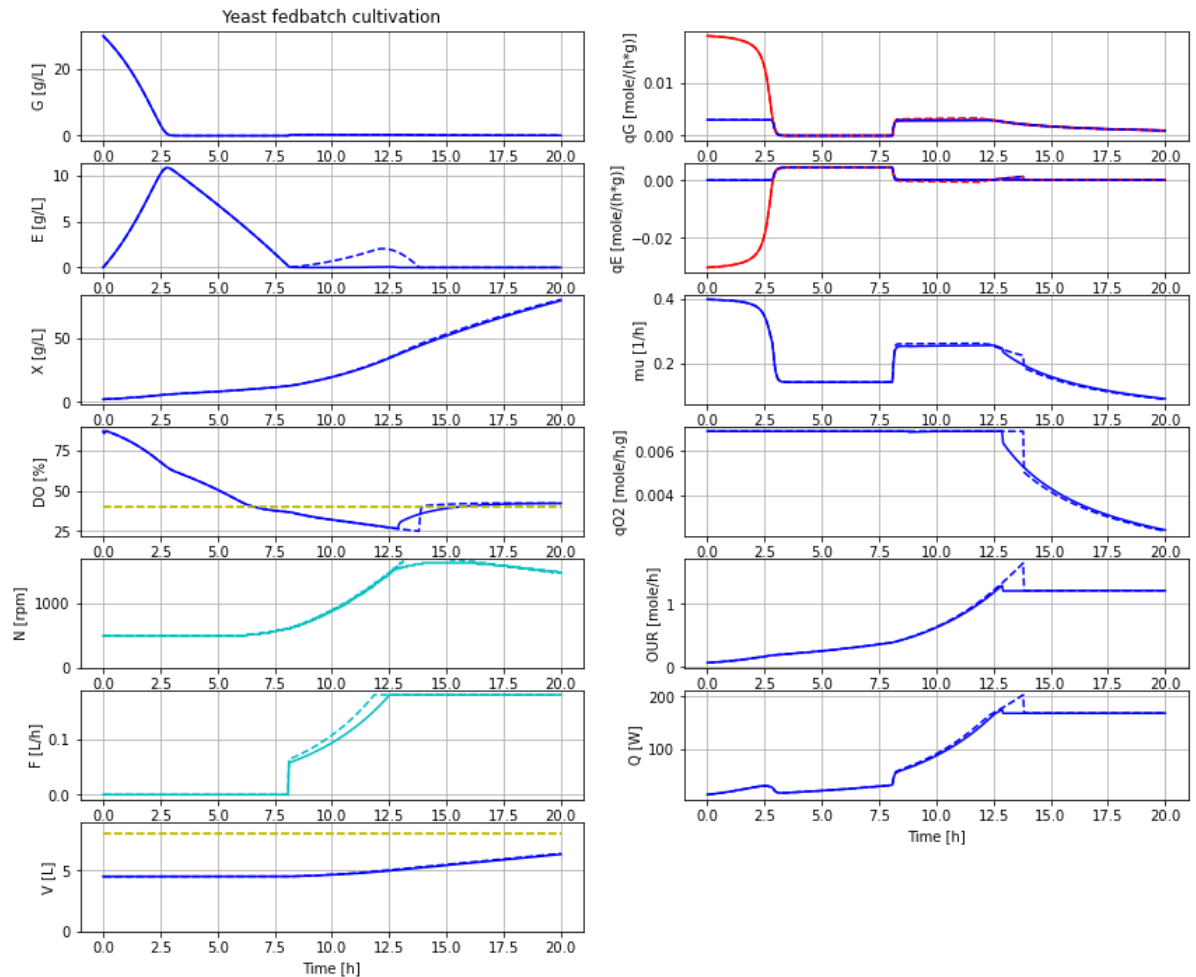


The variation in  $F_{\text{start}}$  has an impact and we see that the actual growth rate during fedbatch phase do converge to the set growth rate of the feed, but it takes more than 5 hours.

```
In [11]: # Let us investigate a feedprofile that is closer to the maximal capacity
newplot(title='Yeast fedbatch cultivation', plotType='Overview')
par(F_start=0.044, mu_feed=0.20); simu(20)
par(F_start=0.050, mu_feed=0.22); simu(20)
par(F_start=0.057, mu_feed=0.26); simu(20)
```



```
In [12]: # And let us see what happens if the feedprofile exceed the culture capacity
newplot(title='Yeast fedbatch cultivation', plotType='Overview')
par(F_start=0.057, mu_feed=0.26); simu(20)
par(F_start=0.063, mu_feed=0.28); simu(20)
par(F_start=0.044, mu_feed=0.20)
```



Note that with the feedprofile that exceed culture respiratory capacity, ethanol is accumulated during time 8-12.5 hours. When the feedprofile then is constant from time 12.5 hours and on, then the accumulated ethanol is consumed over about an hour. This leads to a higher oxygen demand and heat production during this time. The specific cell growth rate is also slightly higher during this period.

**Exercise** You can investigate the impact of changing the maximal feedrate  $F_{\max}$ . Make sure that the DO level do not get too low.

## Make your own diagrams

There are a couple of pre-defined `plotType` for the application that you make by the command `newplot()`. The command result in a list "diagrams" that describe the commands that make the plot when you call `simu()` or you just want to look at the last simulation again with a changed `plotType` using `show()`.

You can also in Jupyter notebook directly define the list "diagrams" and then that will be used for subsequent calls of `simu()` or `show()`. When you have made a diagram that you want to reuse many times you can bring it into the python-setup file and edit the `newplot()` command and add a new `plotType`.

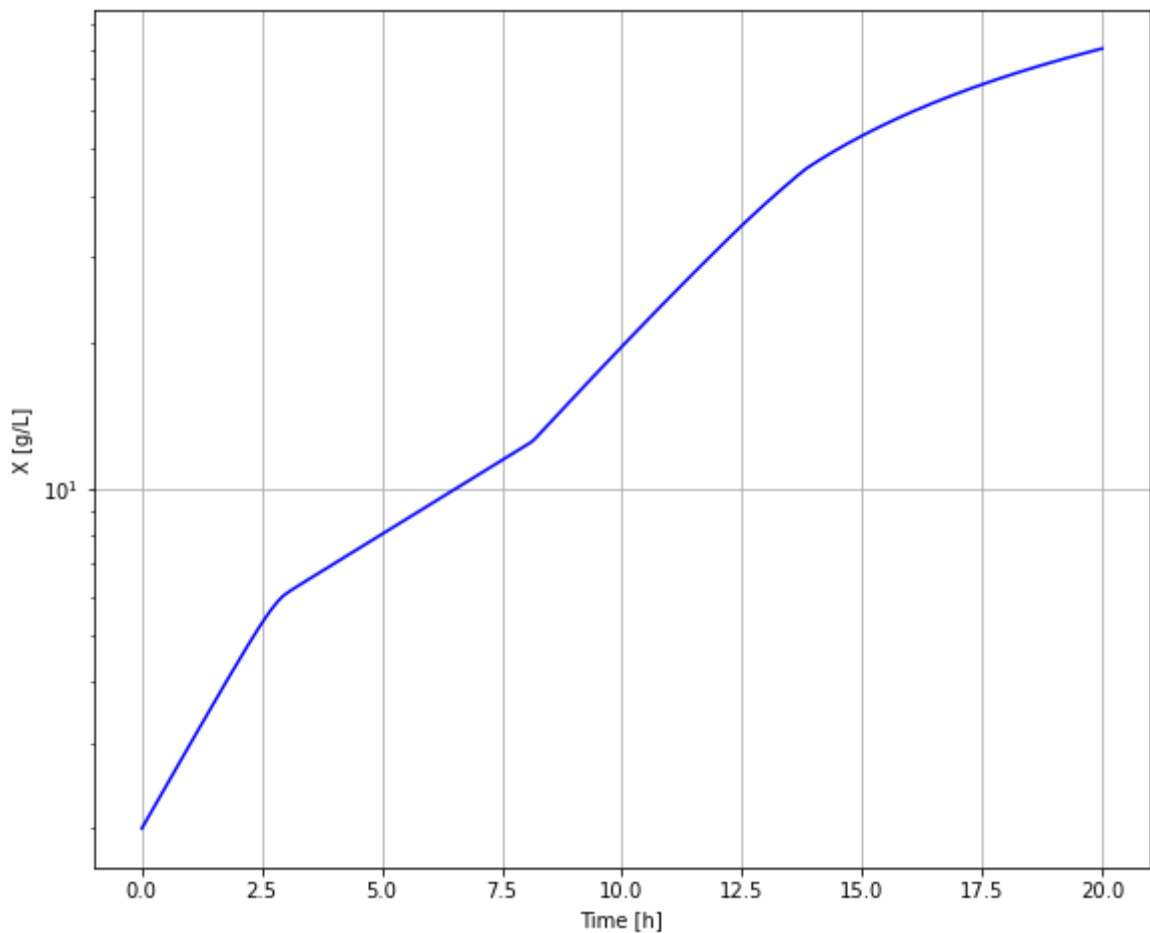
Below a few simple examples that show how to do a diagram directly in the notebook

```
In [13]: # First decrease the diagram size
plt.rcParams['figure.figsize'] = [24/2.54, 20/2.54]
```



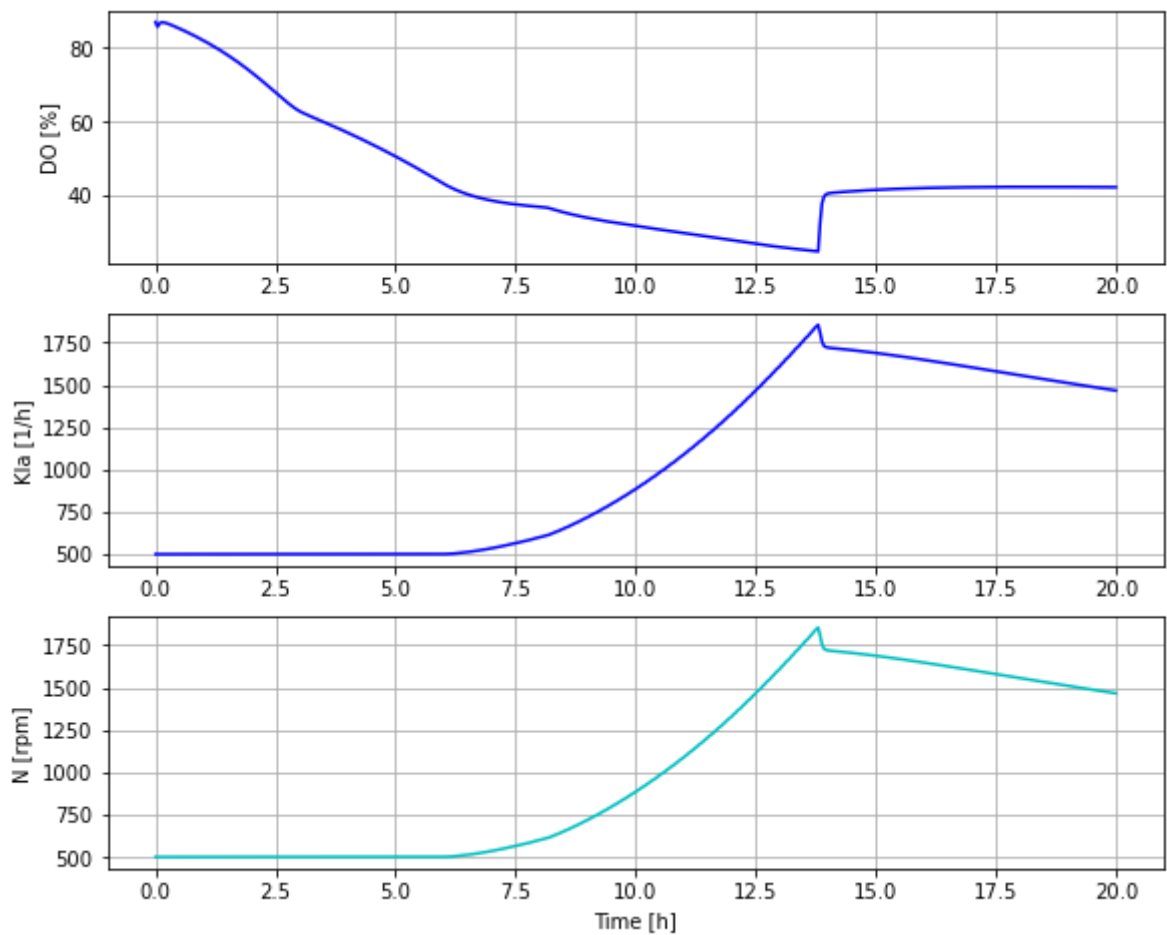
```
In [14]: # Improvise and make your own diagram - cell concentration in a Logarithmic plot
plt.figure()
ax1 = plt.subplot(1,1,1)
ax1.set_ylabel('X [g/L]')
ax1.set_xlabel('Time [h]')
ax1.grid()

setLines()
diagrams.clear()
diagrams.append("ax1.semilogy(sim_res['time'], sim_res['bioreactor.c[1]'], color='b'
show()
```



```
In [15]: # - study the variation of Kla together with DO and N during cultivation
plt.figure()
ax1 = plt.subplot(3,1,1); ax2 = plt.subplot(3,1,2); ax3 = plt.subplot(3,1,3)
ax1.set_ylabel('DO [%]'); ax1.grid()
ax2.set_ylabel('Kla [1/h]'); ax2.grid()
ax3.set_ylabel('N [rpm]'); ax3.grid()
ax3.set_xlabel('Time [h]')

setLines()
diagrams.clear()
diagrams.append("ax1.plot(sim_res['time'], sim_res['DOsensor.out'], color='b', line
diagrams.append("ax2.plot(sim_res['time'], sim_res['bioreactor.gas_liquid_transfer.
diagrams.append("ax3.plot(sim_res['time'], sim_res['bioreactor.N'], color='c', line
show()
```



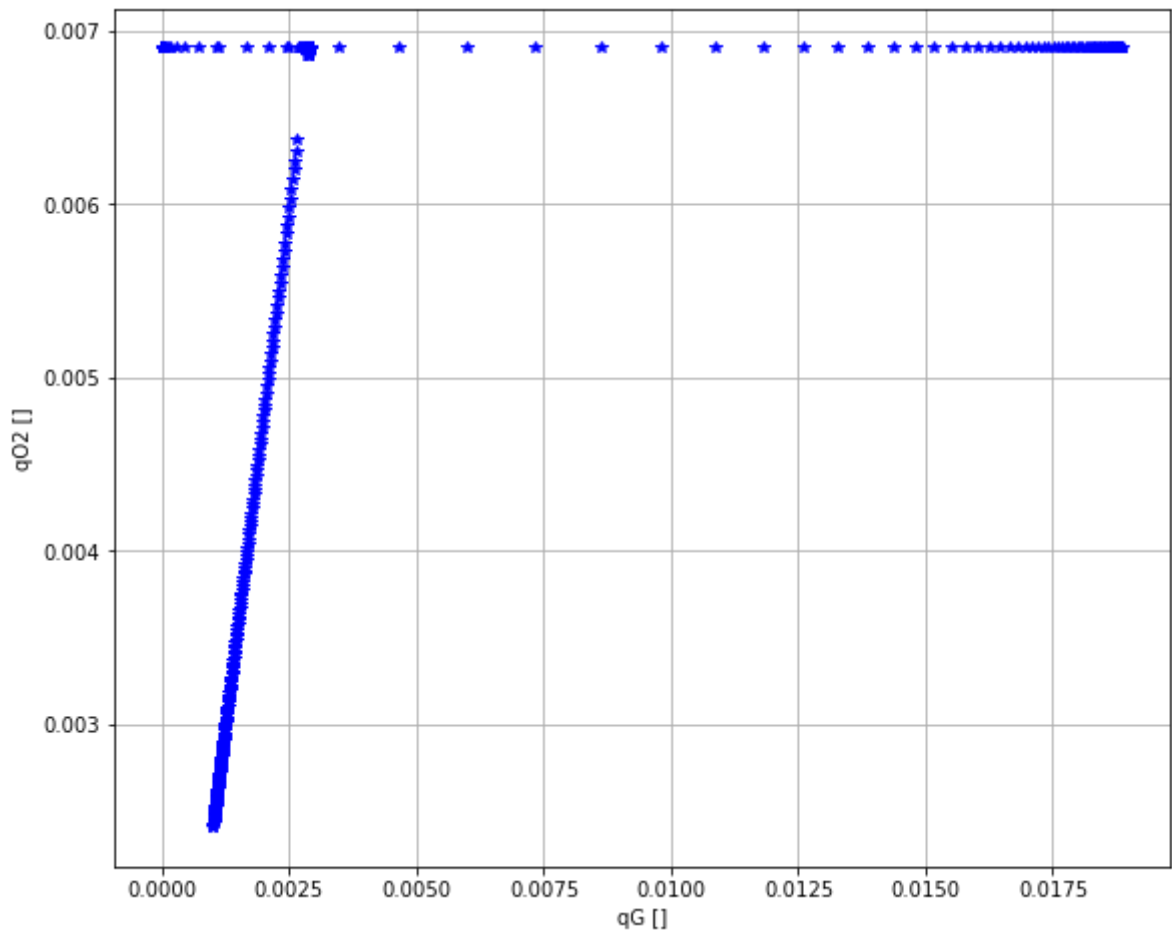
The relation is  $KLa = \alpha_{O2} \cdot N$  and we see the value of the parameter should be around 1.0, and we check below

```
In [16]: disp('bioreactor.gas_liquid_transfer.alpha_O2')
```

```
alpha_O2 : 1.0
```

```
In [17]: # - study the relation qO2 vs qG(G)
plt.figure()
ax1 = plt.subplot(1,1,1)
ax1.set_ylabel('qO2 [ ]')
ax1.set_xlabel('qG [ ]')
ax1.grid()

setLines()
diagrams.clear()
diagrams.append("ax1.plot(sim_res['bioreactor.culture.qGm'], sim_res['bioreactor.cu
par(F_start=0.057, mu_feed=0.26)
simu(20)
```



During the cultivation we have a number of data points for  $qG$  and  $qO_2$  at the same time, during different conditions. What we see in the diagram is that  $qO_2$  increase with  $qG$  until  $qG$  reach a level of just above 0.0025 and then  $qO_2$  saturates for higher  $qG$ . This what expect to see.

We also see that for lower  $qG$  we have also  $qO_2$  values at saturation level. This points correspond to a situation where ethanol is consumed with the remaining respiratory capacity. Glucose is consumed by priority.

## Summary

- We have first seen an overview diagram of a typical yeast fedbatch cultivation where the feed started about an hour after the batch phase was finished. A new simulation was made where the feed started directly after detection of lack of substrate.
- We also took a look at the DO-control system and saw that we could decrease the control error by increasing the PI-controller gain. Stability of the control system remained.
- Then we tested variations in the feed dosage scheme and investigated the possibilities to increase the production.
- We also saw what happens if the feed dosage exceed the culture respiratory capacity and what to look for during the experimental work.
- Finally we saw some examples of how to improvise new diagrams.

## References

[1] Sonnleitner, B. and O. Käppeli "Growth of *Sacharomyces cerevisiae* is controlled by its limited respiratory capacity: formulation and verification of a hypothesis", Biotech. Bioeng., 1986.

[2] von Stockar, U., Gustafsson, L., Larsson, C., Marison, I., Tissot, P. and Gnaiger E. "Thermodynamic considerations in constructing energy balances for cellular growth", Biochimica et Biophysics Acta, vol 1183, p 221-240, 1993.

[3] Axelsson, J. P. "Experimental techniques and data analysis to determine baker's yeast ethanol dynamics", Anal. Chim. Acta, vol 213, p 151-163, 1988.

## Appendix

In [18]: `disp('culture')`

```
qGmax : 0.02
Ks : 0.01
qO2lim : 0.007
```

In [19]: `describe('mu')`

```
Cell specific growth rate variable : 0.088 [ 1/h ]
```

In [20]: `# List of components in the process setup and also a couple of other things like li`  
`describe('parts')`

```
['airFlow_setpoint', 'airtube', 'atmosphere', 'bioreactor', 'bioreactor.culture',
'bioreactor.gas_liquid_transfer', 'compressor', 'DO_setpoint', 'dosagescheme', 'DO
sensor', 'feedtank', 'gasphase', 'liquidphase', 'MSL', 'N_high', 'N_low', 'PIreg',
'pump']
```

In [21]: `describe('MSL')`

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

In [22]: `system_info()`

```
System information
-OS: Windows
-Python: 3.9.2
-Scipy: not installed in the notebook
-PyFMI: 2.9.8
-FMU by: JModelica.org
-FMI: 2.0
-Type: FMUModelCS2
-Name: BPL_YEAST_AIR.Fedbatch_DOcontrol
-Generated: 2022-10-10T09:27:00
-MSL: 3.2.2 build 3
-Description: Bioprocess Library version 2.1.0
-Interaction: FMU-explore ver 0.9.5
```

In [ ]: