Fermentation and photometry schedule

Step 2 and 3: Fermentation of glucose to ethanol by yeast

1.4 Filtration of the hydrolysate

Filtrate the hydrolysate after the hydrolysis with a Nutsche filter.

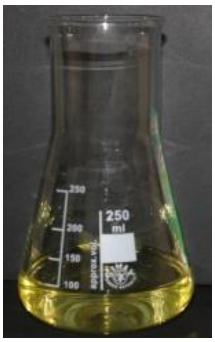
2. Fermentation

2.1 Preparation of the nutrient medium (2-fold concentrated)

Look at teachers schedule.

2.2 Preparation of the subculture

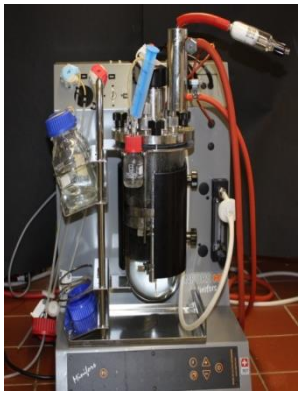
1. Add 20 g fresh yeast to the 50 ml 2-fold concentrated nutrient medium and the 50 ml distilled H_2O.
2. Resuspend the fresh yeast.



2.3 Fermentation in a bioreactor

1. Fill the fermenter with
   - 800 ml nutrient medium (2-fold concentrated) and
   - 800 ml filtrated hydrolysate.

2. Start the fermentation with the following parameters:
   - anaerobic
   - 30 °C
   - 200 rpm
   - pH 4,5



Put 100 ml of the subculture into the fermenter after the parameters are set!

3. Photometric measurements

3.1 Sample extraction

The samples are extracted at the very beginning (t_0) and during the fermentation after 15 (t_1), 45 (t_2), 90 (t_3) and 150 (t_4) minutes.

1. Wash the sample extractor pipe before each sample extraction and extract 5 ml fermentation medium.
2. Discard the 5 ml.
3. Extract 5 ml fermentation medium one more time.
4. Pipette 1 ml of the medium for the glucose- and ethanol determination (3.2.1) and another 1 ml for the optical density (OD)-measurement (3.2.2) in two tubes.
5. Centrifuge the tube for glucose- and ethanol determination for one minute at 14000 rpm.
6. After centrifugation pipette 500 µl of the supernatant into a new tube.
7. Freeze this tube at -20 °C until the photometric determination of glucose or ethanol.

3.2 Photometric determination

3.2.1 Determination of ethanol and glucose

Pay attention during the determination of ethanol to:

1. A lid has to be on the cuvette after each pipetting step and during the measuring!
2. Determine the concentration of glucose and ethanol according to the accompanying pipette schemes.

3.2.2 Determination of the optic density

1. Determine the blank value with a 1 ml 1-fold nutrient medium at 600 nm.
2. Extract 125 µl of the 1 ml sample and add it to the 875 µl 1-fold nutrient medium.
3. Measure the OD of the sample at 600 nm and write the measured values in the attached worksheet.