Fensterle, Lucassen

# Anaerobic fermentation

## 1. General objective The aim is to

plan, carry out and analyze a fermentation with yeast and to analyze the ethanol and CO2 production in real time. The experiments are carried out with different glucose concentrations. The overall goal is the (kinetic) analysis of the fermentation process, so at the point at which the glucose is completely used up, glucose is “spiked” again.

## 2. Requirements You should

have read this quick guide.

It is expected that you will independently develop a plan for the experiment using these instructions (sampling times, sample volume, media composition...). Please take into account the document on microbiological methods.

## 3. Preparation of the culture media (day 1)

Liquid media must be produced for fermentation and analysis, as well as agar plates for the dilution series. The liquid and solid media are prepared on the first day of the laboratory.

The exact instructions for this can be found in the tables at the end of this script!

## 4. Fermenter (Day 2)

### 4.1. Preparing the fermenters

BlueSens fermenters with 2 (optionally 4) large screw threads (GL 45) are used for fermentation. Attention: (blue) caps or gas sensors attached to the large screw threads must be fitted with sealing rings, otherwise the vessels are not gas-tight!

Each fermentation is carried out with a total volume of 500 mL:

| 400 mL YPD medium + X mL glucose + Y mL water = 500 mL |
|---|

#### 4.1.1. Set initial glucose concentration (day 2)

Each group uses a different concentration. The required volume of glucose solution (X) must be calculated beforehand. The following amounts (g glucose!) are required:

0.3 g, 0.6 g, 1.2 g, 2.5 g, 5.0 g, 10 g (for group 1, 2, 3, 4, 5, 6 respectively)

The required amounts of glucose solution must be calculated in advance and aseptically pumped into the reactor vessel.

#### 4.1.2. Adjust reactor volume (day 2)

After the glucose has been added to the YPD medium, the volume should be adjusted to 500 mL with sterile demineralized water.

#### 4.1.3. Inoculation

The fermentation is inoculated with 20 mL of Saccharomyces cerevisiae suspension. The suspension is prepared by suspending a block of fresh baker's yeast in 200 mL of YPD medium.

Before inoculation, a group should determine the total cell count.

### 4.2. Sampling

At each specified time (see table at the end of this script), 5 mL of the fermentation broth should be drawn and analyzed accordingly.

## 5. Required contents of the protocol

You can find a protocol template on Moodle, please stick to the form given.

The following points must be included:

Preliminary remark: the calculations must be carried out separately for the areas before and after glucose addition (groups 1-5). The maximum rates should be compared accordingly.

1.) Short introduction (1-2 sentences)
2.) Determination of the conversion factor OD600 ÿ biomass (please always use the correct units!)

2.) Graphical representation of the amount formed in moles (not formation rate!) of CO2 and EtOH based on the sensor data vs. time; Graphical representation of the measured total and live cell numbers and
Biomass vs. time

2.) Calculate the maximum molar formation rates of CO2 and EtOH (average the formation rates in a suitable range).

3.) Calculation of the maximum growth rate based on the biomass 4.)
Calculation of the molar yield of CO2, EtOH (mol / mol glucose) and biomass (g / mol glucose)

5.) Calculation and comparison of the “lead time” for both areas.

5.) Optional: Set up the stoichiometric sum equation based on the one formed
Total masses.

The following assumptions apply to the evaluation:

- the existing glucose is completely consumed as soon as the CO2 concentration remains constant • CO2 is considered an ideal gas, the conditions are standard conditions, so the following applies:
  Vm = 22.46 L/mol.
- Oxygen consumption is neglected, concentration changes of CO2 are based
  exclusively on CO2 formation
- Please note that the raw data represents percentage values (so must be corrected with 10-2
  become!)
- Both the gas volume and the liquid volume become independent of sampling
  considered a constant 500 mL
- The density of the culture broth is considered to be constant 1 g/cm³ • For ethanol,
  the concentration dependence of the partial molar volume is neglected, the density of pure ethanol is 0.789 g/mL (thus the
  approximate weight of the pure ethanol contained in 100 mL of 0.5% ethanol solution Ethanol: 100 mL*0.5%*0.789 g/mL =
  0.39 g). • Molar masses: H = 1 g/mol, C = 12 g/mol, O = 16 g/mol • For the groups in which EtOH formation was not recorded, the amount formed should be taken from the

  CO2 formation can be extrapolated. To do this, use the following equation: o Mol EtOH = 0.92 *
  Mol CO2 The molecular formula of
- yeast is: CH1.83O0.56N0.17

6. Anaerobic fermentation

Work instructions for the first day of the laboratory (Table 1-7)

Table 1: YPD fermentation medium (to be carried out by each group)

| YPD fermentation medium<br>• Tryptone (2% w/v) • Yeast extract (1% w/v) • Glucose concentration<br>(variable)<br>(will only be posted on the 2nd day) | | Date: | |
|---|---|---|---|
| | | Editor (name) abbreviation | |
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |
| Composition Calculated | Quantity (g) for 500mL | Actually weighed (g) | abbreviation |
| Tryptone | | | |
| Yeast extract | | | |
| demineralized Water | 400g | | |
| Prepare medium directly in the fermenter (1000 mL reactor vessel).<br>To fill in the nutrient media and water, please use the<br>Use powder funnel. | | | |
| Screwing on the lids. Attach hose clamps | | | |
| and hose ends and connections<br>wrap with aluminum foil. | | | |
| Sterilize the fermenter in an autoclave at 121ÿC for 20 min. | | | |

Table 2: Glucose solution (should only be carried out by one group)

| Glucose solution • Glucose (20% w/v) | | Date: | |
|---|---|---|---|
| | | Editor (name) abbreviation | |
| | | | |
| Composition Calculated (g) for | 500mL | Actually weighed (g) | abbreviation |
| glucose | | | |
| Adjust glucose to 1000 mL with demineralized water. Screw cap with sampling tube and | | | |
| sterile filter attach. | | | |
| Sterilize the bottle in an autoclave at 121ÿC for 20 min. | | | |

Table 3: Sterile water (should only be carried out by one group)

| water (sterile) | Date: | |
|---|---|---|
| | Editor (name) abbreviation | |
| | | |
| 2x 1000 mL demineralized water in a 1L Duran each Add bottles. Screw cap with sampling tube and attach sterile filter. | | |
| Sterilize both bottles in an autoclave at 121ÿC for 20 min. | | |

Table 4: YPD medium for OD measurement (should be carried out by a group)

| YPD Medium<br>(as a blank solution for OD measurements) | | Date: | |
|---|---|---|---|
| | | Editor (name) abbreviation | |
| | | | |
| composition | Calculated<br>Quantity (g) for<br>1000mL | Actually weighed in<br>(G) | abbreviation |
| Tryptone (2% w/v) Yeast Extract (1% w/v) | | | |
| Demineralized | | | |
| Water | | | |
| Sterilize the bottle in an autoclave at 121ÿC for 20 min. | | | |

Table 5: PBS - Isotonic diluent (should only be carried out by one group)

| PBS media | | Date: | |
|---|---|---|---|
| | | Editor (name) abbreviation | |
| | | | |
| Composition Phosphate Buffered | Amount required for 1000 mL | | abbreviation |
| Saline<br>(PBS) pre-filled tablets | | | |
| Demineralized water Phosphate Buffered Saline | | | |
| (PBS) ready-made tablets are according to<br>dissolved in demineralized water according to the manufacturer's instructions. | | | |
| Sterilize the bottle in an autoclave at 121ÿC for 20 min. | | | |

Table 6: YPD medium for ***S. cerevisiae*** **slurry (to be performed by one group only)**

| **YPD medium (for inoculum) • 2% (w/v) tryptone • 1% (w/v) yeast extract** | | **Date:** | |
|---|---|---|---|
| | | **Editor (name) abbreviation** | |
| | | | |
| **Composition Calculated quantity** | **(mg) for 500 mL** | **Actually weighed (mg)** | **abbreviation** |
| Tryptone | | | |
| Yeast extract | | | |
| Adjust medium to 500 mL with demineralized water and 250 mL each into a 1L Duran bottle with sampling give tube. | | | |
| Sterilize both bottles in an autoclave at 121ÿC for 20 min. | | | |

**Table 7: YPD Agar (to be performed by each group)**

| **YPD agar • 2% (w/v) tryptone • 1% (w/v) yeast extract • 1.5% agar • Glucose 4%** | | **Date:** | |
|---|---|---|---|
| | | **Editor (name) abbreviation** | |
| | | | |
| | | | |
| **Composition Calculated quantity** | **(g) for 1000 mL** | **Actually weighed (g)** | **abbreviation** |
| Tryptone | | | |
| Agar | | | |
| glucose | | | |
| Yeast extract | | | |
| Adjust medium to 1000 mL with demineralized water and add a stir fry. | | | |
| Sterilize the bottle in an autoclave at 121ÿC for 20 min. | | | |

## Work instructions for the second day of the laboratory (Table 8 to 10)

Table 8: Preparation of the S. cerevisiae starter culture (should only be carried out by one group)

| S. cerevisiae starter culture | | | | | |
|---|---|---|---|---|---|
| Approximately 20 g/250 mL of fresh baker's yeast was added to the YPD medium. Please Clean the spatula with alcohol before use. The Duran bottle comes in one Incubator tempered at 30ÿC and shaken. | | | | | |
| The following parameters of the inoculum should be measured at the time of transfer become: | | | | | |
| time Transfer: | | Cell count (cells/mL) | | OD600 | |
| Date transfer: | | | | | |

Table 9: Fermenter set-up (should be carried out by each group)

| Fermentation (preparation) | Date: | |
|---|---|---|
| | Editor (name) abbreviation | |
| | | |

| Adjustment of the glucose concentration of the YPD medium | | | | |
|---|---|---|---|---|
| Store container of glucose solution (20%) and water aseptically with the Connect fermenter. First the calculated amount of glucose by pumping bring in. Then pump in the remaining amount of water to create a volume Reactor of 500 mL is reached. | | | | |
| composition | Calculated volume | Calculated Pumping time | Difference abbreviation | |
| Glucose (___% w/v) demineralized | | | | |
| Water | | | | |
| After adding (pumping) the two solutions, the hose is clamped and the metal hose coupling is wrapped with sterile aluminum foil. The reactor is now placed in a 35ÿC water bath and at least 30 mins while stirring. | | | | |

Table 10: Carrying out the fermentation

| Fermentation (implementation) | Date: | |
|---|---|---|
| | Editor (name) abbreviation | |
| | | |

Inoculation of the fermenter

20 g (assumption: 1 g ~ 1 mL) of the yeast starter culture are added to the fermenter pumped aseptically (please loosen the clamp on the connecting hose). For this purpose The preheated reactor is inoculated with yeast cells by pumping. Currently- The point of inoculation is referred to here as t(0). After adding the starter culture the connecting hose is disconnected again.

| Composition Required | Quantity (g) | Bottle previously (G) | Bottle afterwards r (G) | difference (G) | abbreviation |
|---|---|---|---|---|---|
| Starter culture | 20mL | | | | |

The reactor is placed in a 35ÿC water bath.

sampling

A sample must be taken at time t(0). For this purpose, the overall, Live cell count, dry matter, OD and CFU can be determined.

After every 30 minutes, another sample (5 mL) should be taken. OD and total cell count are measured every half hour, and the live cell count is measured determined every 1.5 hours. For the first and last sample, the Biomass can be determined.

| T (min) sampling (hr:min) | Dry-biomass | In total-cell number | Live-cell number | O.D |
|---|---|---|---|---|
| 0 Please register | x | x | x | x |
| 30 Please register | | x | | x |
| 60 Please register | | x | | x |
| 90…. End | | x | x | x |
| Please register | x | x | x | x |