InternshipLM-Biotechnology

Laboratory course in food science

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Aerobic culture of yeast in a small fermenter

1. General objective The aim is to
plan, carry out and analyze an aerobic cultivation of yeast in the fermenter and to analyze product formation rates, substrate consumption rates and growth rates. The experiments are carried out with different glucose concentrations.

2. Requirements You should
have read this quick guide to the experiment.

It is expected that you will independently develop a plan for the experiment using these instructions (sampling times, sample volume, media composition...).

Consider the microbiological methods document.

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

The following points must be included:

1.) Graphical representation of the measured total, live cell numbers and biomass vs. time

2.) Calculation of the maximum division rate and doubling time based on the number of living cells.

4. Planning

4.1.Day 1

- Agar plates have already been prepared during the anaerobic fermentation experiment
- Preparation of the solutions •
  Preparation and sterilization of the bioreactors and accessories

4.2.Day 2

- Pump glucose, water and inoculum into the reactor medium in a sterile
  manner. • Sampling t=0.
- Duration of culture as specified by the
  supervisor • Sampling and analysis at regular intervals according to the table.

o OD 600 and total cell count (t=0; then every half hour)

o Live cell count (t=0; then hourly) and dry matter (t=0 and last sampling)

o Ethanol (first sampling after 2 hours then every hour)

o Glucose (t=0 then every half hour until value = 0)

4.3.Test setup for sterilization (Day 1)

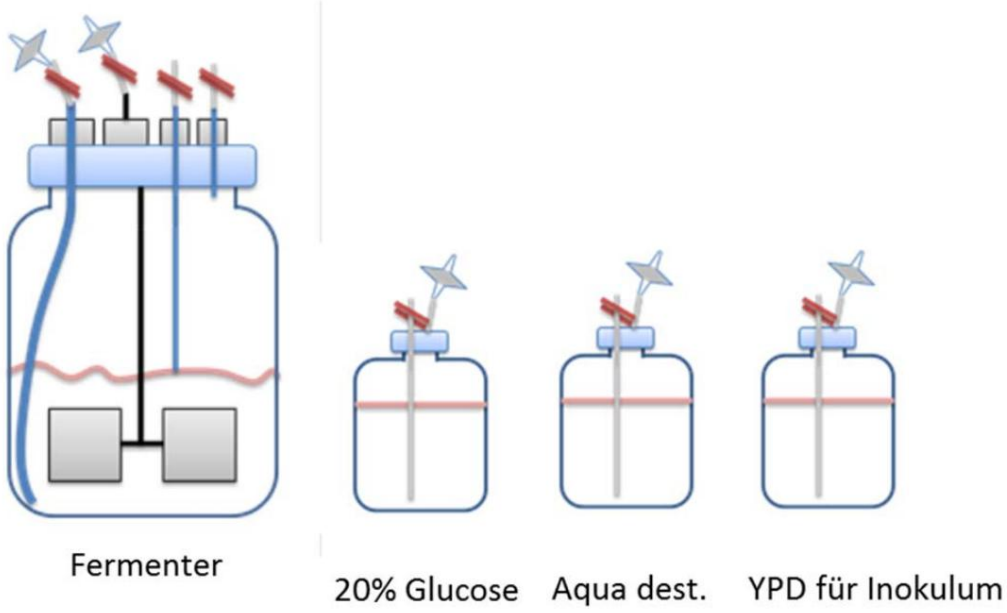

Figure 1: Structure of the fermenter for sterilization. The hose feed can optionally be connected to the fermenter be. All open hose ends must be wrapped with aluminum foil.

4.4.Test setup (day 2)

The aerobic cultivation of *S. cerevisiae* takes place in 1000 mL glass fermenters (setup, sterilization - see Figure 1 and **error! Reference source could not** be found . Figure 2):

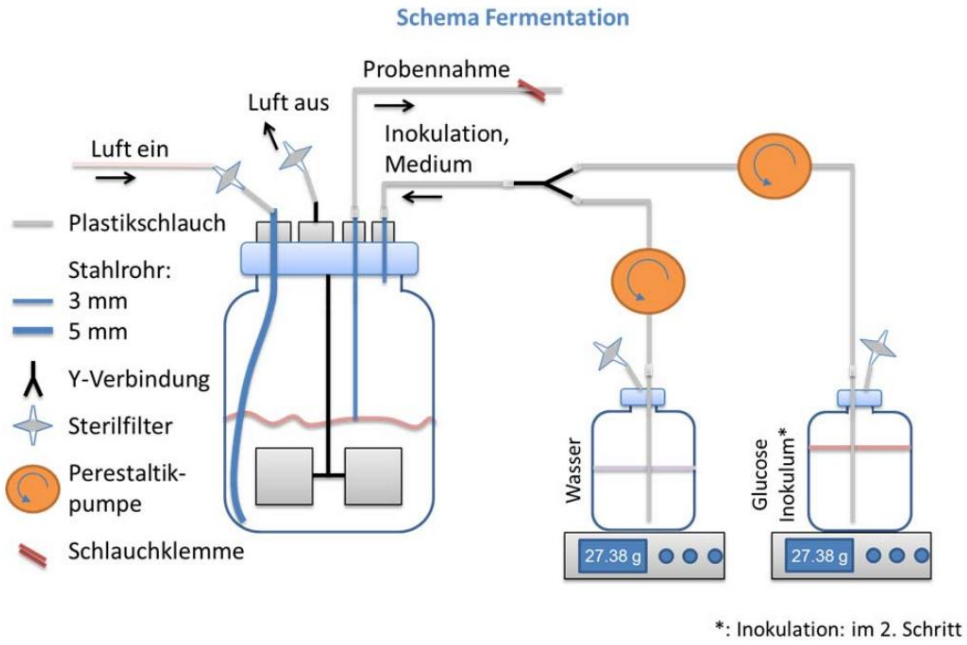

Figure 2: Structure of the fermenter for fermentation

4.5.Preparing for fermentation

Before fermentation begins, water and glucose are aseptically added to the YPD medium. The addition is done by pumping.

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

400 mL YPD medium + X mL glucose + Y mL water = 500 mL total volume

4.5.1.Setting the glucose concentrations in the reactor 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)

4.6.Inoculation

The medium is inoculated with cells from an overnight preculture. The target concentration in the bioreactor is (unless otherwise announced by the supervisor) 5 x 106 cells / mL.

4.7. sampling

At each given point in time, just as much fermentation broth should be drawn as is necessary for the analysis at that point in time.

- Sampling and analysis at regular intervals according to the table. o OD 600 and total cell count (t=0; then every half hour) o Live cell count (t=0; then every hour) and dry matter (t=0 and last sampling) o Ethanol (first sampling after 2h then every hour) o Glucose (t=0 then half hourly until value = 0)

5. Aerobic fermentation

The goal is:

- Determination of the biomass and total cell formation rates and the respective maximum Formation rates •

Determination of product formation rates • Calculation of the molar yield for biomass (end point determination)

Work instructions for the first day of the laboratory (Table 1ÿ6)

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

YPD fermentation medium		**Date:**	
• Tryptone (2% w/v) • Yeast extract (1% w/v) • Glucose concentration (variable) (will only be posted on the 2nd day) **Please note:** The amounts of tryptone + yeast Calculate extract to 500 mL (The medium volume is only 400 mL).		**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). **To fill in the nutrient media and water, please use the** **Use powder funnel.**			
Screwing on the lid (adjusting the stirring rod and the test tube).			
Attach hoses and sterile filters. Wrap all sterile filters and hose ends with aluminum foil.			
Disconnect hoses. Autoclave tape on the fermenter Stick it on and label the fermenter on it.			
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	1000mL	Actually weighed (g)	abbreviation
glucose			
Adjust glucose to 1000 mL with demineralized water. Screw cap with sampling tube and			
sterile filter attach. Disconnect hoses. All sterile filters and Wrap the ends of the hose with aluminum foil.			
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. Disconnect hoses. All sterile filters and wrap the hose ends with aluminum foil.		
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 (100%) **(as a blank solution for OD measurements)**		**Date:** **Editor (name) abbreviation**	
composition	**Calculated** **Quantity (g) for 1000mL**	**Actually weighed (g) Abbreviation**	
Tryptone (2% w/v)			
Yeast extract (1% w/v)			
Demineralized Water			
Dissolve all substances in 80 mL of demineralized water and Adjust volume to 100 mL.			
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	**Amount required for 1000 mL**	**abbreviation**
Buffered Saline (PBS) pre-filled tablets		
Demineralized water Phosphate Buffered Saline		
(PBS) ready-made tablets are according to 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 the cultivation of S. cerevisiae (should only be carried out by one group)

YPD medium 100% (for inoculum) • 2% (w/v) tryptone • 1% (w/v) yeast extract		Date:	
		Editor (name) abbreviation	
Composition Calculated quantity	(mg) for 500 mL	Actually weighed (g)	abbreviation
Adjust tryptone yeast extract medium			
to 500 mL with demineralized water			
and add 250 mL each to two 500 mL Erlenmeyer flasks. Please close Erlenmeyer flasks with metal caps.			
Sterilize both flasks in an autoclave at 121ýC for 20 min.			

Work instructions for the second day of the laboratory (Table 7 to 9)

Table 7: Preparation of the *S. cerevisiae* starter culture (should only be carried out by one group the day before)

<table>
<tr><td colspan="6">S. cerevisiae starter</td></tr>
<tr><td colspan="6">culture Working lots (yeast cells frozen at -80ÿC) are used as inocula. Aseptically add 200 ÿl of the inocula using a shake flask. All inocula must be inoculated the day before at 30ÿC (~300 rpm) and grown overnight the day before.</td></tr>
<tr><td colspan="6">The following parameters of the inoculum should be measured at the time of transfer:</td></tr>
<tr><td>time Transfer:</td><td></td><td rowspan="2">Cell number (cells/mL)</td><td rowspan="2"></td><td rowspan="2">OD600</td><td rowspan="2"></td></tr>
<tr><td>Date transfer:</td><td></td></tr>
</table>

After successful incubation, **aseptically** transfer 250 mL of yeast suspension into the sterilized inoculum bottle (see Fig. 1) !

Table 8: 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			
Storage 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	Needed Quantity (mL)	Pumping time (sec)	abbreviation
Glucose (___% w/v)			
demineralized Water			
After adding the two solutions, the hose is clamped and the metal hose coupling wrapped with sterile aluminum foil.			

Table 9: Carrying out the fermentation

Fermentation (implementation)	Date:	
	Editor (name) abbreviation	

Inoculation of the fermenter

The target cell number in the fermenter (unless otherwise communicated) is 5x106 cells/mL. The appropriate amount of yeast starter culture is added to the fermenter pumped aseptically (please loosen the clamp on the connecting hose). After addition the starter culture, the connecting hose is disconnected again. The reactor is now placed in the 30ÿC water bath and gassed with air (1 vvm). The The reactor should be connected quickly, the same applies to the Sampling t0.

Composition	Quantity (mL)	Pumping time (sec)	Abbreviation	
Starter culture				

sampling

t	sampling (hr:min)	Ethanol	glucose	dry Dimensions	OD 600	Alive cell number	In total cell number
0			X	X	X	X	X
0.5			X		X		X
1			X		X	X	X
1.5			X		X		X
2		X	X		X	X	X
2.5 3h			X		X		X
		X	X		X	X	X
End		X	X	X	X	X	X