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Homo-/hetero-fermentative lactic acid fermentation

1. General objective The aim is to

plan, carry out and balance a homo- or heterofermentative lactic acid fermentation with Lactococcus lactis or Leuconostoc mesenteroides. The parameters lactate, CO2, EtOH, acetate, total gas, cell number, biomass and OD are determined at the beginning and at the end of the fermentation and a balance is created. The experiments are carried out with three different carbon sources.

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 refer to the document on microbiological methods.

3. Preparation of the culture media (day 1)

Liquid media must be produced for fermentation and analysis. 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 fermentersBlueSens

fermenters with 2 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 MRS medium + X mL sugar + Y mL water = 500 mL

4.1.1.Setting the initial concentration of C
sourcesEach group uses a different concentration, the following amounts of glucose, sucrose or xylose should be set:

tribe	Lactococcus lactis			Leuconostoc mesenteroides		
Group (=fermenter)	1	2	3	4	5	6
Glucose (g) per fermenter 10 10						
Sucrose (g) per fermenter		10			10	
Xylose (g) per fermenter		10				10

The required volumes (in mL), based on a 20% stock solution, must be calculated in advance be pumped aseptically to the reactor vessel.

4.1.2.Adjust the reactor volumeAfter
the sugar solution has been added to the medium, the volume should be 500 mL.

4.1.3.Inoculation
The fermentation is carried out with 50 mL of a Lactococcus lactis or Leuconostoc mesenteroides preculture inoculates. The total cell number should be determined beforehand for the inoculum.

4.2. Sample name
At each specified time (see table at the end of this script) 5 mL of the
Fermentation broth is drawn.

5. Required contents of the protocol
Please use the protocol template on Moodle and stick to the given form.

The protocol must contain the following:

1.) Short introduction (1-2 sentences)
2.) Calculation of the molar yield of CO2, EtOH, lactate, acetate (mol / mol glucose/xylose/sucrose) and biomass (g/mol glucose/xylose/sucrose)
3.) Optional: Set up the stoichiometric sum equation based on the one formed Total masses.

6. Experimental procedure

Work instructions for the first day of the laboratory

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

MRS fermentation medium		Date:	
		Editor (name) abbreviation	
• Tryptone (1% m/v) • Meat extract (1% m/v)			
• Yeast extract (0.5% m/v) • Buffer solution pH 6.5 (v/v			
10%) • C sources are variable (will only posted on the			
2nd day)			
	Calculated quantity (g) for 500 mL	Actually weighed (g)	
composition			abbreviation
Tryptone			
Meat extract			
Yeast extract			
Buffer solution Demin.			
Water	Prepare 350 g of medium directly		
in the fermenter (1000 mL reactor vessel). To fill in the culture media and water, please use the powder funnel use.			
Screwing on the lids. Attach hose clamps and hose			
ends and connections with aluminum foil wrap around.			
Sterilize in an autoclave at 121ÿC for 20 min.			

Table 2: C source (one approach for two groups together; a total of 3 different sugars)

C source (see 4.1.1) A 20% solution should be prepared!		Date:	
		Editor (name) abbreviation	
composition	Calculated (g) for 500mL	Actually weighed (g)	abbreviation
CÿSource: Attach screw cap with			
sampling tube and sterile filter. Sterilize the bottle in an autoclave at 121ÿC for 20 min.			

Table 2: Sterile water (should only be carried out by one group)

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

Table 3: Medium for OD measurement and preculture (one approach for each C source)

MRS medium		**Date:**	
(as a blank solution for OD measurements and pre-culture)		**Editor (name) abbreviation**	
Concentrations see Table 1			
composition	**Calculated Quantity (g) for 500mL**	**Actually weighed (g)**	**abbreviation**
Tryptone			
Meat extract			
Yeast extract			
Buffer solution Demin.			
Sterilize the water bottle	400g	-	
in an autoclave at 121ýC for 20 min.			
Sterile addition of the sugar solution in the desired concentration.			

Table 4: PBS - Isotonic diluent (should only be carried out by one group)

PBS	Date:	
	Editor (name) abbreviation	
composition	Amount required for 1000 mL	abbreviation
Phosphate Buffered Saline (PBS) Ready-made tablets		
Demineralized water Phosphate Buffered Saline (PBS) ready-		
made tablets are prepared according to the Manufacturer's instructions dissolved in demineralized water.		
Sterilize the bottle in an autoclave at 121ÿC for 20 min.		

Table 6: Preparation of the pre-culture (should be carried out by each group)

pre-culture	Date:	
	Editor (name) abbreviation	
Pipette 50 mL of medium into a 100 mL sterile Erlenmeyer flask		
Inoculate the medium with 250 µL Bacterial suspension		
Incubation at 30°C (shaking) for 12-24 hours		

Work instructions for the second day of the laboratory

Table 7: Determine total cell count and OD of preculture (should be performed by each group)

pre-culture					
The following parameters of the inoculum should be measured at the time of transfer to the fermenter become:					
Transfer time:		Cell count (cells/mL)		OD600	
Date transfer:					

Table 8: Fermenter set-up (should be carried out by each group)

Fermentation (preparation)				
		Date:		
		Editor (name) abbreviation		
Setting the C source of the MRS medium				
Aseptically connect the storage container of the sugar solution and water to the fermenter. First this Bring in the calculated amount by pumping. Then pump in the remaining amount of water Volume in the reactor of 500 mL is reached.				
composition	Calculated volume	Calculated Pumping time	Difference abbreviation	
Sugar _____				
demineralized water				
After adding (pumping) the two solutions, the hose is clamped and the metal one Hose coupling wrapped with sterile aluminum foil. The reactor is now placed in a 30ÿC water bath and stirred for at least 30 minutes tempered.				

Table 9: Carrying out the fermentation

Fermentation (implementation)	Date:	
	Editor (name) abbreviation	
Inoculation of the fermenter		
50 mL of the preculture are aseptically pipetted into the preheated fermenter (sterile workbench!). The time of inoculation is referred to here as t(0).		
The reactor is placed in a 37ÿC water bath and the EtOH and CO2 sensors connected.		
sampling		
A sample must be taken at time t(0) and after 6 h. For this purpose, the overall, Live cell count, dry mass and OD can be determined. 1 mL of the sample is centrifuged (10000g/5min/10°C), the supernatant was transferred to a new vessel and frozen at -20°C Lactate and acetate analysis should be carried out on the 3rd day of the laboratory.		