Fermentation of molasses to ethanol with yeast

(Item No.: P1313600)

Curricular Relevance

Area of Expertise: Biology → **Education Level:** University → **Topic:** Biotechnology → **Subtopic:** Biotechnology → **Experiment:** Fermentation of molasses to ethanol with yeast

Difficulty	Preparation Time	Execution Time	Recommended Group Size
Difficult	30 Minutes	3 Hours	1 Student

Additional Requirements:

- Molasses
- Nutrient salts
- Yeast
- Oleic acid

Experiment Variations:

Keywords:

Fermentation, Ethanol, Bioreactor, Yeast

Principle and equipment

Principle

Notes

As a result of the need to save energy and the increased consciousness of environmental problems, biotechnological production methods are on the advance. The list of products produced using bacteria, yeasts or cell cultures grows almost daily. The majority of these products are produced in bioreactors (fermenters), in which the mixing, gassing, pH value, temperature, oxygen concentration etc. can be exactly regulated.

The spectrum of bioreactors ranges from 1 litre laboratory models up to large containers of 1,000 m3 capacity. Such commercially available bioreactors are mostly too elaborate for teaching purposes, however. Components that can all be sterilised, a complicated automatic control or the realisation of strictly anaerobic conditions (without oxygen), can be done without when experimental examples are appropriately chosen. If required, 3 ml of formalin (4% solution) can be added to the molasses to act against bacterial contamination.

The bubble reactor is designed as a very simple bioreactor, that is nevertheless completely sufficient for student experiments. It consists of a long glass tube which can be thermostatted, and in which an insert extends right up to the top. An opening at the bottom serves for the passage of gas into the bioreactor, two openings at the top allow nutrient solution to be added or final product to be removed. Two side nipples allow connection for the ciculation of water from a thermostat.

In this experiment, molasses (a waste product from the production of sugar) is fermented to ethanol in a so-called batch process. This example is a discontinuous fermentation, i.e. fermentation is stopped after a certain time and the fermentation product harvested. Subsequently, the bioreactor is re-filled with a new filling („batch“). Continuous fermentation has recently asserted itself, as it requires far less working time per kg of final product. In this type of process, there is continuous feeding of starting substance and retrieval of final product. This can also be accomplished with the bioreactor. A flask above the bioreactor holds the mash to be fermented, a bottle below the bioreactor collects the fermented mash.
The following are additionally required for continuous fermentation:

Apparatus carrier with fixing magnets	45525.00	1
Aspirator bottle, 1000 ml	34175.00	1
Rubber stopper, PN 19, 1 hole 7 mm	39255.01	1
Rubber stopper, 26/32, 1 hole 7 mm	39258.01	1

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Equipment

Position No.	Material	Order No.	Quantity
1	Precision Balance, Sartorius ENTRIS822-1S, 820 g / 0,01 g	49295-99	1
2	Immersion thermostat Alpha A, 230 V	08493-93	1
3	Heating mantle f. roundbottom flask, 250ml	49542-93	1
4	Bubble bioreactor	65999-00	1
5	Frame for complete experiments	45500-00	1
6	Power regulator	32288-93	1
7	Bath for thermostat, makrolon	08487-02	1
8	Liebig Condenser, with head, GL18/8	35795-15	1
9	Panel for complete experimental setups	45510-00	2
10	Apparatus carrier w. fix. magnet	45525-00	1
11	External circulation set f. thermostat Alpha A	08493-02	1
12	Stutzer attachment GL25/12	35791-15	1
13	Calciumbishydrogenphosphate,500g	30056-50	1
14	Apparatus holder, variable	45526-00	1
15	Aquarium pump, 150 l/h, 230 V AC	64566-93	1
16	Pycnometer, calibrated, 25 ml	03023-00	1
17	Clamping holder, turnable, 18-25 mm	45521-00	1
18	Flask,round,1-neck,250ml,GL25/13	35812-15	1
19	Oleic acid pure 500 ml	31685-50	1
20	Round bottom flask, 100ml, GL 25/12	35841-15	1
21	Rear-cover for compl.-exp. panel	45501-00	1
22	Clamping holder,18-25mm	45520-00	4
23	Hook on fixing magnet	02151-03	1
24	pH test sticks 4.0-7.0,100 sticks	30301-03	1
25	Clamp for heating mantle	49557-01	1
26	Glass tube,right-angled, 10 pcs.	36701-52	1
27	Tube Coupling, d = 8 mm	47521-00	2
28	Spring plugs, 50 off	45530-00	1
29	Pipettor	36592-00	1
30	Glass tubes,straight with tip, 10	36701-63	1
31	Ammonium sulphate 250 g	30027-25	1
32	Magnesium sulphate 500 g	30136-50	1
33	Sodium hydroxide sol.,10%, 500ml	31630-50	1
34	Sulphuric acid, 10%, tech.gr., 1000 ml	31828-70	1
35	G-clamp	02014-00	2
36	Dropping pipette with bulb, 10pcs	47131-01	1
37	Bottle,nar.mouth,1000ml,clear,p.s	41104-01	1
38	Fixing bands,universal,100 pcs.	45535-00	1
39	Cotton wool, white 200 g	31944-10	1
40	Erlenmeyer flask,narrow n.,100 ml	36118-00	1
41	Erlenmeyer flask,narrow n.,250 ml	36124-00	2
42	Graduated cylinder 100 ml	36629-00	1
43	Pinchcock, width 15 mm	43631-15	1
44	Funnel, glass, top dia. 80 mm	34459-00	1
45	Spoon, special steel	33398-00	1
46	Silicone tubing i.d. 7mm	39296-00	1
47	Graduated pipette 10 ml	36600-00	1
48	Silicone tubing, ID 4 mm	47529-00	1
49	Rubber tubing, i.d. 6 mm	39282-00	6
50	Tubing connector, ID 6-10mm	47516-01	2
51	Hose clip, diam. 8-16 mm, 1 pc.	40996-02	13
52	Hose clip f.12-20 diameter tube	40995-00	1

Safety information

Sodium hydroxide is highly corrosive to skin, eyes and mucous membranes. Dust from it irritates respiratory organs. Chemical burns destroy tissue and cause great pain. Dilute sulphuric acid is highly irritative to skin and eyes. Fine spray irritates the respiratory organs, whereby the mucous membranes of the upper respiratory organs are particularly affected. Gaseous hydrogen can be produced on contact with metals (danger of explosion!).

Calcium bis(dihydrogen phosphate) is irritative to skin, eyes and mucous membranes. Dusts irritate the respiratory organs. Do not inhale vapours or dusts. Avoid contact of the chemicals with skin and eyes.Wear suitable protective clothing, protective gloves and protective goggles when working with them! Observe the detailed information on safety measures in the appendix.

Set-up and procedure

Set-up

First prepare a suspension from molasses, nutrient salts, yeast and oleic acid (antifrothing agent).

- 83,3 g Molasses
- 0,3 g Nutrient salt mixture
- 3,0 g Fresh yeast
- 2 drops Oleic acid

The nutrient salt mixture consists of:

- 50 g $Ca(H_2PO_4)_2 \cdot H_2O$
- 20 g $(NH_4)_2H_2O$
- 5 g $MgSO_4$ dissolved in 75 ml of tap water

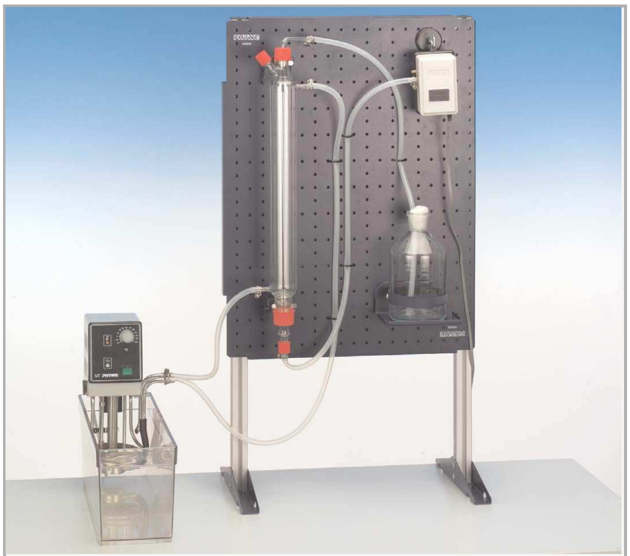

Abb. 1

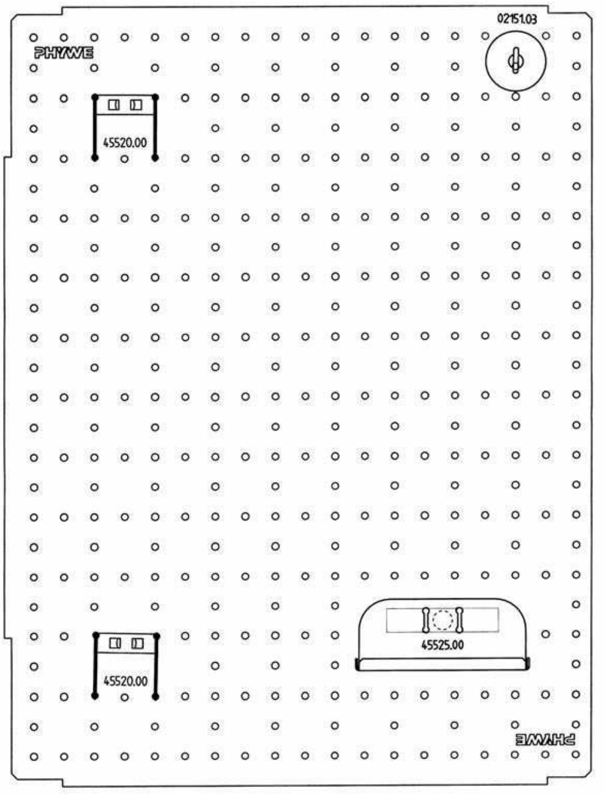

Abb. 3

The suspension that is to be fermented (called the „mash“), should have a pH of 5 to 6. Adjust pH values that are outside of this range with sodium hydroxide solution or sulphuric acid.

Position the clamping holders on the panel for complete experiments as shown in Fig. 2. The equipment is to be subsequently assembled and fixed to the clamping holders as shown in Fig. 1. To start with, insert the inner tube (d = 18 mm) through the sealing ring of the GL 32 screw cap until it cannot be inserted further, then tighten the screw cap to fix it in position. Fix the bioreactor to the panel with two clamping holders, as shown in Figures 1 and 2. Secure the clamping holders at the back of the panel with spring plugs.

For aeration of the bioreactor, fix a glass tube with drawn - out tip in the bottom end, so that the tip end is just above the side holes in the inner tube. Use silicone tubing to connect the glass tube to an aquarium pump. As the connecting tube of the aquarium pump is of too small a diameter, increase this diameter by fitting a short piece of 4 mm tubing over it. Position the pump higher up than the bioreactor, so that, when the pump is switched off, suspension cannot flow into it and dirty it.

To maintain a constant temperature in the bioreactor, connect the two hose nipples on the outer glass jacket to a circulating thermostat. When the production process can take place at room temperature (30°C), however, then external thermostatting is not required.

Use an apparatus carrier with fixing magnets to fix the narrow- necked glass bottle to the panel, and secure it with the Velcro band. Connect a right-angled glass tube in the central upper opening of the bioreactor to the glass bottle that is to act as a safety bottle with a length of silicone tubing.

Use cotton wool to plug the bottle opening around the tubing. For a batch process, close the upper side opening of the bioreactor with a screw cap. For a continuous process, the mash must flow in here. Use a 1 litre decanting bottle to hold the supply of mash. Mount the bottle on an apparatus carrier fixed to the upper edge of the panel, and fix a venting tube in the upper opening of it to ensure that the mash flows evenly.

Procedure

Fill the bioreactor up to about 1 cm from the upper end of the inner tube with mash. Switch on the aquarium pump. Air bubbles rise up and, in doing so, effect an upwards movement of the suspension in the inner tube. When the suspension has reached the top of the tube, it sinks back down in the outer tube to the holes in the inner tube, which are intended for this purpose. The air bubbles so cause a permanent mixing of the suspension, and obviate the need for a stirring motor. Use a hose clip to adjust the air flow, so that aerobic conditions are given in the inner tube (propagation of yeast). This requires an air flow at the inlet of 5 to 10 ml per minute.

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Observation and evaluation

After 24 hours, distil the fermented mash. To do this, fit the holders to the second panel and assemble the apparatus as shown in Fig. 3. The set-up and procedure are extensively the same as described in experiment KV 1.4 „Distillation“. As molasses have a strong tendency to foam, however, it is necessary here to insert a Stutzer attachment between the distillation flask and the distillation bridge, to prevent foam from rising up and into the condenser, and so contaminating the distillate. Stop distillation when the temperature of the distillate vapour begins to rise above 78-80°C.

Subsequent to distillation, weigh the distillate and determine its alcohol content from its density (pyknometer). As example, from the Table (see KV 1.4), a distillate of density 0.9887 g/ml contains 65% ethanol, i.e. 65 g of ethanol per litre. At the beginning of the fermentation process, one litre of molasses contained 150 g of sugar (value given by the sugar factory). On complete fermentation, therefore, and according to the equation for the reaction:

$$C_{12}H_{22}O_{11} \, + \, H_2O \rightarrow 4\, C_2H_5OH \, + \, 4\, CO_2$$

80.7 g of ethanol must be produced. The yield would then be $\frac{65 g/L}{80{,}7 g/L}$ ·100 % = 80,5 %