



Saccharomyces Cerevisiae

Aerobic Baker's Yeast Fermentation



Introduction

Yeast belongs to the class of Protozoa-comyceten and more specifically to the physiological class of *Saccharomyces Cerevisiae*. *Saccharomyces Cerevisiae* is a Crabtree positive yeast, which is sensitive to high substrate concentrations that results in the reduction of the oxygen uptake rate. As a result glucose is metabolized to ethanol. This metabolic pathway can be reduced by introduction of a regulated feed procedure adapted to the specific growth rate of the yeast.

1. Equipment and Materials Used

- BIOSTAT® Aplus 2 L, MO-Assembly
- Balance (Sartorius CP series)
- pH-Meter
- Photometer
- Magnetic stirrer plate
- 1 graduated flasks 2000 mL
- 1 graduated cylinder 50 mL
- 1 beaker 250 mL
- 1 beaker 50 mL
- 1 Schott flask 250 mL
- 3 Schott flasks 500 mL with stainless steel head plate
- 2 graduated pipettes 10 mL
- 1 graduated pipettes 1 mL
- Ethanol test kit
- Glucose analyzer or glucose test kit
- Drying chamber, Microwave or Moisture Analyzer
- Baker's yeast

2. Overview setting up procedure

a) Timetable

- Day 1: Preparation main culture medium and bioreactor assembly
- Day 2: Inoculation bioreactor | Fermenter

b) Bioreactor | Fermenter

- Calibration and installation of the pH-electrode
- Installation of the pO₂ probe
- Calibration of the pumps
- Preparation and sterilization of base, acid and antifoam; manual filling of the tubes
- Sterilization of the culture vessel including the medium
- Calibration of the pO₂ probe at cultivation mixing speed
- Sterilize connection of peripheral equipment

c) Medium

2 Litres of nutrient medium are prepared as follows:

(NH ₄) ₂ SO ₄	2.0 g/L
K ₂ HPO ₄ * 3 H ₂ O	2.0 g/L
MgSO ₄ * 7 H ₂ O	0.5 g/L
KCl	2.0 g/L
Yeast extract	0.1 g/L
Glucose * H ₂ O	11 g/L
H ₂ SO ₄	5.0 mol/L

Add salts to a 2 L flask and dissolve in 1.5 L distilled water. Add 10 mL sulphuric acid (1 mol/L). Adjust the pH value to pH = 4.5 with 1 M NaOH, add 1 mL antifoam agent and adjust to 1.9 L with distilled water. Transfer the salt solution into the prepared culture vessel and autoclave at 121 °C for 20 minutes.

Dissolve 22 g glucose in 100 mL distilled water and autoclave in a separate flask. Transfer the sterile glucose solution into the bioreactor vessel.

d) Inoculum

For inoculum mix 15 g baker's yeast with 40 mL sterile growth medium.

e) Corrective Agents

Antifoam	1.0% (w w) PEG
Acid	0.1% (w w) H ₂ SO ₄
Base	1 mol/L NaOH

f) Culture Conditions

Culture volume	2 L
Temperature	30 °C
pO ₂	40 °C, controlled
pH value	4.5, controlled
Stirrer	from 250 rpm
Aeration	from 0.5 vvm

3. Analytical Procedure

Measurement of Optical Density

Optical density (OD) is determined using a spectrophotometer at a wavelength of 600 nm. Samples should be diluted in such a way that the measured extinction is between 0.2 and 0.4. Measurements are made in cuvettes with a layer thickness of 1 cm. OD is calculated according to the following formula.

$$OD_{600\text{nm}} = E * F [-]$$

With E = measured extinction
F = dilution factor

Measurement of Biomass Production

There are different methods for biomass detection available:

- BM determination using a moisture analyzer
- BM determination in a drying chamber
- BM determination using a microwave

Measurement of Glucose Concentration

Glucose measurements can be made using - Glucose analyzer (e.g. YSI-Model) or - Test kit No. 71 6251 for glucose (Roche Diagnostics) according to the respective manufacturer protocol.

Measurement of Ethanol Concentration

Ethanol concentration can be determined using an ethanol kit (i.e. 176290 Roche Diagnostics). This photometric method of ethanol determination using enzyme alcohol dehydrogenase (ADH) is simple to use and characterised by high specificity, and reproducibility.

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