Fermentation Procedures using a new „pre-packaged" BIOSTAT®A plus system
Saccharomyces Cerevisiae - Aerobic Baker´s Yeast Fermentation

1. Introduction

Yeast belongs to the class of Protoascomyceten and more specifically to the physiological class of Saccaromyces cerevisiae. Saccaromyces cerevisiae is 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 metabolised to ethanol. This metabolic pathway can be reduced by introduction of a regulated feed procedure adapted to the specific growth rate of the yeast.

2. Equipment and Materials Used

- BIOSTAT®A plus 2L, 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 flask 500mL 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
- Baker´s yeast

3. 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 pO_2 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 pO_2 probe at cultivation mixing speed
- Sterilie connection of peripheral equipment

c.) Medium

2 Litres of nutrient medium are prepared as follows:

$(NH_4)_2SO_4$	2,0 g/L
K_2HPO_4 * 3 H_2O	2,0 g/L
$MgSO_4$ * 7 H2O	0,5 g/L
KCl	2,0 g/L
Yeast extract	0,1 g/L
Glucose * H_2O	11 g/L
H_2SO_4	5,0 mol/L

Add salts to a 2L 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 1M 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 inoculation 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_2SO_4
Base	1 mol/L NaOH

f.) Culture Conditions

Culture volume	2 L
Temperature	30° C
pO_2	40%, controlled
pH value	4,5 controlled
Stirrer	from 250 rpm
Aeration	from 0.5 vvm

Sartorius BBI Systems GmbH
Schwarzenberger Weg 73–79
34212 Melsungen
Germany

Phone +49 (0) 5661.71.3400
Fax +49 (0) 5661.71.3702
E-Mail info@sartorius-bbi-systems.com
www.sartorius-bbi-systems.com

sartorius

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4. Analytical Procedure

: Measurement of Optical Density

Optical density (OD) is determined using a spectrophotometer at a wavelength of 600 mm. 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:

$$OD600_{nm} = E * F \quad [-]$$

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 chamger
- 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 specifity, and reproducibility.

Sartorius BBI Systems GmbH
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Germany

Phone +49 (0) 5661.71.3400
Fax +49 (0) 5661.71.3702
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www.sartorius-bbi-systems.com