Fermentation protocol using BIOFERM bioreactor

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. This is done using a fermentor.

2. Equipment and Materials Used

- BIOFERM 7L Bioreactor Assembly
- Balance
- pH Meter
- Photometer
- Magnetic stirrer plate
- Miscellaneous lab glassware like:
    - 1 graduated flask 5000 ml
    - 1 graduated flask 1000 ml
    - 1 graduated cylinder 250 ml
    - 1 graduated cylinder 100 ml
    - 1 beaker 500 ml
    - 1 beaker 250 ml
    - 1 beaker 100 ml
    - 2 graduated pipettes 10 ml
    - 1 graduated pipettes 1 ml
- Ethanol test kit
- Glucose analyzer or glucose test kit
- Drying chamber (Oven)
- Baker´s yeast
- Autoclave

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3. Setting up procedure

a.) Timetable

Step 1: Preparation main culture medium and bioreactor assembly
Step 2: Inoculation bioreactor / fermenter

b.) Bioreactor

- Calibration and installation of the pH-electrode
- Installation of the pO_2 probe
- Preparation and sterilization of base, acid manual filling of the tubes
- Sterilization of the culture vessel including the medium
- Calibration of the pO_2 probe at cultivation mixing speed
- Sterile connection of peripheral equipment

c.) Medium

4 litres of nutrient medium are prepared as follows:

$(NH_4)_2SO_4$	2.0 g/L
$K_2HPO_4.3H_2O$	2.0 g/L
$MgSO_4.7H_2O$	0.5 g/L
KCl	2.0 g/L
Yeast extract	0.1 g/L
Glucose in H_2O	11 g/L
H_2SO_4	5.0 mol/L

Add salts to a 5L flask and dissolve in 3 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 4.0 L with distilled water.
Transfer the salt solution into the prepared culture vessel and autoclave at 121°C for 20 minutes.
Dissolve 44 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 bakers yeast with 40 ml sterile nutrient medium.

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e.) Corrective Agents
Antifoam 1.0% (w/w)
Acid 0.1% (w/w) H_2SO_4
Base 1 mol/L NaOH

f.) Culture Conditions
Culture volume 4 L
Temperature 30 ° C
pO_2 40%
pH value 4,5 controlled
Stirrer 250 rpm

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:

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

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 test kit for glucose (Roche Diagnostics) according to the respective manufacturer protocol.

: Measurement of Ethanol Concentration
Ethanol concentration can be determined using an ethanol kit. 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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