Escherichia coli batch Fermentation

Introduction
Escherichia coli is a rod-shaped bacteria belonging to the family of the Enterobacteria aceae. Due to its rapid generation time (< 30 min) this microorganism is commonly used as an expression system for heterologous proteins.

1. Equipment and Materials Used
- BIOSTAT® Aplus 5L, MO-System
- Balance (e. g. Sartorius CP-Model)
- pH-Meter
- Photometer
- Shaker (e. g. Certomat, Sartorius)
- Cuvettes
- Heated magnetic stirrer plate
- 5 Petri dishes
- Inoculation loop
- 2× 500 mL Schott flasks
- 2× 500 mL Schott flasks with stainless steel head plate
- 1× 1000 mL Schott flasks
- 3× 10 mL Measuring pipettes
- 2× 1000 mL Inoculation flasks
- 1× 1000 mL Erlenmeyer flask
- 1× 100 mL Amber bottle
- Lactose test kit
- Drying chamber, microwave or moisture analyzer
- Centrifuge tubes
- Incubator
- E.coli DSM 498

2. Overview of setting-up procedures

a) Timetable
Day 1: Preparation of agar plates
Day 2: Inoculation of agar plates
Day 2: Preparation and sterilization of preculture I
Day 3: Inoculation of preculture I
Day 3: Preparation and sterilization of preculture II
Day 4: Inoculation of preculture II
Day 5: Preparation of main culture medium and bioreactor assembly

b) Bioreactor
- Calibration and installation of pH electrode
- Installation of pO_2 probe
- Preparation and sterilization of corrective agents
- Sterilization of the culture vessel including the main culture media
- Calibration pO_2 probe at cultivation temperature and mixing speed
- Manual fi lling of the tubes
- Sterile connection of peripheral equipment

c) Agarplates
Prepare 100 mL of nutrient solution, pH = 7.0 in a 500 mL Schott flask as follows using:

Peptone	0.5 g
Meat extract	0.3 g
Agar	1.5 g

Dissolve the components in warm distilled water (50°C) and adjust the pH to 7.0. After autoclaving distribut the nutrient broth equally on 5 Petri dishes. Inoculate the dry agar plates with Escherichia coli according to 13-line procedure and incubate for 24 hours at 37°C.

d) Preculture I
Prepare 250 mL nutrient solution, pH = 7.0 in a 1000 mL Erlenmeyer flask as follows using:

Peptone from casein	1.25 g
Peptone from meat	0.75 g

Dissolve the components in distilled water and adjust the pH to 7.0. Aufter autoclaving and cooling down transfer Escherichia coli from the agar plates to the flask. Incubate for 25 hrs at 37°C and 100 U/min in an incubator shaker (e. g. Certomat, Sartorius).

e) Stock solution trace elements
Prepare 1 L trace elements solution using 1 g of the following Specpure chemicals in a 1 L Schott flask:
$FeSO_4 * 7\ H_2O$
$CoSO_4 * 7\ H_2O$
$ZnCl_2$
$CuSO_4 * 5\ H_2O$
$Na_2MoO_4 * 5\ H_2O$
H_3BO_3
$NiCl_2 * 6\ H_2O$
H_3BO_3
$NiCl_2 * 6\ H_2O$
$MnSO_4 * H_2O$
$Al_2(SO_4)_3 * 18\ H_2O$

Use 5 mL of this stock solution per litre nutrient broth for both in preculture II and the main culture.

f) Preculture II
Prepare 500 mL nutrient solution, pH = 6.9 and distribute equally on both 1 L inoculation flasks, using:

Di-Ammonium hydrogen citrate	0.25 g
Ammonium sulphate	6.0 g
KH_2PO_4	0.8 g
$Na_2HPO_4.2H_2O$	3.3 g
NaCl	1.0 g
Yeast extrract	0.5 g
$MgSO_4.7\ H_2O$	0.15 g
Lactose	3.5 g
Trace elements	2.5 mL

Note
Lactose is only soluble up to 30% w | v.

Dissolve 40 g lactose in 133.4 mL warm (50°C) distilled water (this is equivalent to a 30% solution). Autoclave the lactose solution separately for 20 min at 121°C. Dissolve the salts and yeast extract in 244 mL distilled water and adjust the pH to 6.9.

Autoclave the salt solution separately.

Transfer 5.8 mL lactose per flasks and incubate for 24 hrs at 37°C and 100 U/min in an incubator shaker (e. g. Certomat, Sartorius.).

g) Main culture
Medium composition should be the same as for preculture II. The amount of nutrients should be calculated to a working volume of 5.0 L and dissolved in 4.5 L distilled water. After adjusting the pH to 6.9 transfer the nutrient solution into the culture vessel and sterilize at 121°C for 20 minutes. Add the rest of the already prepared lactose solution under sterile conditions into the bioreactor. Inoculate using both flasks of preculture II.

h) Corrective agents

Base:	10 M NaOH
Antifoam:	1% (w | w) silicon oil (e. g. Serva)

i) Fermentation conditions

Culture volume:	5 L
Aeration:	from 0.125 vvm
Stirrer:	from 200 U/min
Temperature:	37°C
pO_2:	40°C, controlled
pH value:	6.9 controlled

3. Analytical procedure

Measurement of biomass
A number of different methods can be used for determining biomass. These include:
- BM determination using a moisture analyzer (e. g. Sartorius MA 45)
- BM determination in a drying chamber
- BM determination in a microwave

Measurement of Optical Density
Optical density (OD can be determined using a spectrophotometer at a wavelength of 600 m. Samples should be diluted in such a way that the measured extinction is between 0.2 and 0.4. Measurements should made in cuvettes with a layer thickness of 1 com. OD can be calculatede according to the following formula:

$$\mathbf{OD_{600mm} = E * F\ [-]}$$

With E = measured extinction
F = dilution factor

Measurement of lactose concentration
Lactose measurements can be made using Test kit No. 176303 for Lactose (Boehringer Mannheim).

Determination of ß-Galactosidase activity
Since ß-Galactosidase is an intracellular formed enzyme, the E.coli bacteria must be broken down prior to activity measurement. This is carried out chemically using toluene. Per mL bacteria suspension 2 drops toluene are added and the resulting solution incubated for 15 minutes under ambient conditions. The test solution for activity determination comprises:

KH_2PO_4	0.165 g
$Na_2HPO_4.2\ H_2O$	1.158 g
$MgCl_2.6H_2O$	0.010 g
2-Mercaptoethanole	0.367 g
ONPG	0.069 g

Dissolve these components in 100 mL distilled water and adjust the pH to 7.6. Store the solution at 4°C in a dark bottle (amber bottle).

The test analysis is carried out as followed:

Test solution	2.00 mL
Disintegrated sample	0.02 - 0.1 mL

Sample should be selected in such a way that the measured extinction change △ E/min is between 0.02 – 0.2 E/min. Extinction change is determined at 30°C using a spectrophotometer equipped with a temperature controlled cuvette at a wavelength of 405 nm.

ß-Galactosidase activity can be calculated according to the following equation:

$$\text{Activity}_{\text{ß-Gal}} = \frac{\text{Vcomplete} \times \text{F X} \triangle\text{E/min}}{V_{sample} \times 3.1}\ [\text{U/mL}]$$

With F = dilution factor
V = volume

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