Fermentation Procedures using a new pre-packaged BIOSTAT®Aplus system
Xanthomonas campestris - Consumer Product Additives Fermentation

Introduction

Bacteria of the type Xanthomonas belong to the family of plant pathogenous, yellow-pigmented pseudomonades. Strains of Xanthomonas campestris secrete enzymatic partially degredable exopolysaccarides (Xanthan).
Xanthan has become a widely available commercial product used in food, chemical and oil production industries. For these applications the viscosity characteristics of Xanthan solutions over a wide pH and temperature range as well as the influence of salt contents is of prime importance.

1. Equipment and Materials Used

- BIOSTAT®A plus 5 L MO-system
- Inoculation needle
- 10 Petri dishes
- Inoculating loop
- 2 x 250 mL Schott or graduated flask
- 1 x 50 mL Erlenmeyer flasks
- 14 x 100 mL Erlenmeyer flasks
- 7 x 500 mL shaking flasks
- Centrifuge bottles
- Moisture analyzer
- Drying chamber
- Viscometer
- Glucose analyzer or glucose kit
- Whirl-Mix
- Centrifuge
- Magnetic stirrer plate
- Balance
- Xanthomonas campestris DSM 1706

2. Overview of setting up procedures

a.) Time table

Day 1: Preparation agar plates
Day 2: Inoculation agar plates
Day 2: Preparation and sterilization of preculture I
Day 3: Inoculation preculture I
Day 3: Preparation and sterilization of preculture II
Day 4: Inoculation preculture II
Day 5: Preparation main culture medium and bioreactor assembly
Day 6: Inoculation bioreactor / fermenter

- Preparation agar plates (Day 1)

Note:

Glucose and nutrient solutions have to be autoclaved separately (Mailard reaction!)

b.) Assembling of fermentor

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

c.) Growth in petri dishes

200 mL of nutrient solution, pH = 7,0 prepared as follows:

Glucose monohydrate	20 g/L
Yeast extract	10 g/L
CaCO3	10 g/L
Agar	25 g/L

- Dissolve 4 g glucose in 50 mL distilled water
- Dissolve 2 g yeast extract, 2 g CaCO3 and 5 g agar in 150 mL distilled water, prewarmed to 80°C.
- Glucose solution and nutrient solution have to be autoclaved separately
- After autoclaving and sterile mixing of both solution distribute on the 10 petri dishes
- Inoculate the dryer agar plates with Xanthomonas campestris according to 13-line procedure and incubate for 24 hours at 27°C.

d. Preculture I

Prepare 7 x 20 mL nutrient solution, pH = 7,0 as follows:

Glucose monohydrate	10	g/L
Yeast extract	5	g/L
Peptone	5	g/L

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- Dissolve 1.4 g Glucose in 14 mL distilled water
- Dissolve 0.7 g Yeast extract and 0.9 Peptone from Casein in 126 mL distilled water, adjust pH to 7.0
- Distribute 18 mL of the solution to seven 100 mL-Erlenmeyer flask
- Glucose and nutrient solution have to be autoclaved separately
- Mix glucose and nutrient; 2 mL glucose per flask
- Transfer 4 full inoculation loops os Xanthomonas campestris from the agar plates to every flask
- Incubate for approximately 36 h at 27°C and 100 U/min in a thermo-shaker (e.g. CERTOMAT® Sartorius)

e.) Preculture II

Prepare 7 x 200 mL nutrient solution, pH = 7,0 as follows:

Glucose monohydrate	55	g/L
Citric acid monohydrate	2.3	g/L
KH_2PO_4	5.0	g/L
KH_4Cl	2.0	g/L
Na_2CO_3	0.5	g/L
Na_2SO_4	0.114	g/L
$MgCl_2$ x 6 H_2O	0.163	g/L
$FeCl_3$	0.0014	g/L
$ZnCl_2$	0.0067	g/L
$CaCl_2$ x 2H_2O	0.112	g/L
H3BO3	0.006	g/L

- Dissolve 7 x 11 g glucose in 7 x 50 mL distilled water
- Add salts for 1.4 and dissolve in 910 mL distilled water, adjust pH to 7.0
- Add 130 mL salt solution to 7 shaking flasks
- Glucose solution and nutrient solution have to be autoclaved separately
- After cooling down add 5 mL glucose and 20 mL preculture I to each flask

Incubate for approximately 148 h at 27°C and 100 U/min in a thermo-shaker (e.g. CERTOMAT®, Sartorius).

f.) Main culture:

Medium composition is the same as for preculture II. The amount of glucose and salts should be calculated to a working volume of 4.0 L. After adjusting the pH to 7.0 transfer the salt solution into the culture vessel and sterilize at 121°C for 20 minutes.
Add glucose solution sterile to the bioreactor.
Inoculum consists of 1,400 mL preculture II.
Increasing the inoculation volume will result in a reduced fermentation time.

g.) Corrective agents

Acid	2 N H_3 PO_4
Base	20% (w/w) KOH
Antifoam	1% (w/w) silicon oil (Serva)

h.) Culture conditions

Culture volume	4 L
Aeration	from 0,125 vvm
Stirrer	from 100 U/min
Temperature	27°C
pO2	40% controlled
pH value	7,0 controlled

3. Analytical Procedures

: Biomass production

There are different methods for biomass determination;
- BM determination using a moisture analyzer
- BM determination in a drying chamger
- BM determination in a microwave

: Determination of Xanthan concentration

Precipiate the polysaccaride Xanthan using methanol, wash, dry, and weigh. Pipette 5 mL of the supernatant into a dried and weighed centrifuge tube. Add 15 mL methanol drop by drop and mix thoroughly. Centrifuge for 30 min at 15°C and 1700-x g. Re-mix the pellet with 1% KCL and repeat the precipitation step.
Discard the supernatant and dry the pellet for 24 h at 80°C in a drying chamber. After cooling in an Exicator the tubes should be weighed again. Xanthan concentration can be calculated using the following equation:

$$c_{Xanthan}\ [g/L] = (weight_{full}\ [g] - weight_{empty}\ [g])*DF*1000\ [mL]/5\ [mL]$$

with DF = Dilution factor

: Viscosity

This can be determined directly using a direct reading viscometer (i.e. FANN Direct Reading Viscosimeter model 35 A)

: Glucose

Glucose measurements can be made using:
- Glucose analyzer (e.g. YSI-Model) or
- Test kit Nr. 71 6251 for glucose (Roche Diagnostics)

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