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® Aplus 5 L MO-system
- Inoculation needle
- 10 Petri dishes
- Inoculating loop
- 2 × 250 mL Schott or graduated flasks
- 1 × 50 mL Erlenmeyer flask
- 14 × 100 mL Erlenmeyer flasks
- 7 × 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) Timetable
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 of preculture II
Day 5: Preparation main culture medium and bioreactor assembly
Day 6: Inoculation bioreactor|fermenter

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

b) Assembling of fermenter
- Calibration and installation pH-electrode
- Installation pO_2 probe
- Calibration of the pumps
- Preparation and sterilization of acid & base, manual fi lling of the tubes
- Sterilization of the culture vessel including the main culture medium
- Calibration pO_2 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
$CaCO_3$	10 g/L
Agar	25 g/L

- Dissolve 4 g glucose in 50 mL distilled water
- Dissolve 2 g yeast extract, 2 g $CaCO_3$ 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 dry agar plates with Xanthomonas campestris according to 13-line procedure and incubate for 24 hours at 27°C.

d) Preculture I
Prepare 7 × 20 mL nutrient solution, pH = 7.0 as follows:

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

- Dissolve 1.4 g Glucose in 14 mL distilled water
- Dissolve 0.7 g Yeast extract and 0.7 g Peptone from Casein in 126 mL distilled water, adjust pH to 7.0
- Distribute 18 mL of the solution to seven 100 mL Erlenmeyer flasks
- Glucose and nutrient solution have to be autoclaved separately
- Mix glucose and nutrient; 2 mL glucose per flask
- Transfer 4 full inoculation loops of 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 × 200 mL nutrient solution, pH = 7,m0 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
$MgCl_2 \times 6\ H_2O$	0.163 g/L
$FeCl_3$	0.0014 g/L
$ZnCl_2$	0.0067 g/L
$CaCl_2 \times 2H_2O$	0.012 g/L
H_3BO_3	0.006 g/L

- Dissolve 7 × 11 glucose in 7 × 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 50 mL glucose and 20 mL preculture I to each flask

Incubate for approximately 48 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. Add glucose solution into culture vessel and sterilize at 121°C for 20 minutes. After adjusting the pH to 7.0 autoclave the salt solution and transfer 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
pO_2	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 chamber
– BM determination in a microwave

Determination of Xanthan concentration
Precipitate 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 × g. Re-mix the pellet with 5 mL 1% KCl and repeat the precipitation step. Discard the supernatant and dry the pellet for 25 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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