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Effect of Fermentation Time on Xanthan Gum Production from Sugar Beet Molasses

Marzieh Moosavi- Nasab, Safoora Pashangeh, and Maryam Rafsanjani

Abstract**—**Xanthan gum is a microbial polysaccharide of great commercial significance. The purpose of this study was to select the optimum fermentation time for xanthan gum production by *Xanthomonas campestris* (NRRL-B-1459) using 10% sugar beet molasses as a carbon source. The pre-heating of sugar beet molasses and the supplementation of the medium were investigated in order to improve xanthan gum production. Maximum xanthan gum production in fermentation media (9.02 g/l) was observed after 4 days shaking incubation at 25°C and 240 rpm agitation speed. A solution of 10% sucrose was used as a control medium. Results indicated that the optimum period for xanthan gum production in this condition was 4 days.

Keywords**—**Biomass, Molasses, Xanthan gum, *Xanthomonas campestris*

I. Introduction

THE areas of interest for microbial exo-polysaccharides (EPS), or biopolymers are extensive and including: food industry, agro-chemistry, crude oil recovery, medical and pharmaceutical, chemical and cosmetic industries [8]. Xanthan gum is a water-soluble heteropolysaccharide produced by fermentation using the gram-negative bacterium *Xanthomonas campestris* and some bacteria of the genus *Xanthomonas*.

It was discovered in the 1950s at the Northern Regional Research Laboratories (NRRL) of the United States Department of Agriculture [6]. The polysaccharide B-1459, or xanthan gum, produced by the bacterium *Xanthomonas campestris* NRRL B-1459 was extensively studied because of its properties that would allow it to be an suitable alternative for other known natural and synthetic water-soluble gums. [2]. It was not until 1969 that the FDA issued the final approval for the use of xanthan gum in food products. The demand for xanthan gum produced by *Xanthomonas campestris ssp*. has increased steadily every year and is estimated to grow continuously at an annual rate of 5–10%.

M. Moosavi-Nasab is with the Department of Food Science and Technology, College of Agriculture, Shiraz University, Shiraz, Fars 7144165186 I.R.Iran (corresponding author to provide phone: +98-711-6138227; fax: +98-711-2287029; e-mail: marzieh.moosavi-nasab@mail.mcgill.ca).

S. Pashangeh is a graduate student at the Department of Food Science and Technology, Shiraz University, Shiraz, Fars 7144165186 I.R.Iran (e-mail: safoorap_1985@yahoo.com).

M. Rafsanjani is a graduate student at the Department of Food Science and Technology, Shiraz University, Shiraz, Fars 7144165186 I.R.Iran (e-mail: mrafsanjani@gmail.com).

Commercial production of xanthan gum uses glucose as the substrate, and generally batch production is used instead of continuous production due to the batch process having been proven to work successfully.

Xanthan gum is used in pharmaceutical formulations, cosmetics and agricultural products. It is used in textile printing, ceramic glazes, slurry explosive formulations and rust removers. It is also used in drilling fluids and in enhanced oil recovery processes [2]-[7]. Commercially available xanthan is relatively expensive due to glucose or sucrose being used as the sole carbon source and the very stringent purity standards of the food and drug administration for foods. For food-grade xanthan, up to 50% of the production costs are related to down stream purification steps, many of which would not be necessary for non-food applications. Another cost reduction could be achieved by using less expensive substrates, such as waste agricultural products [5].

Molasses is a co-product of sugar production, both from sugar beet as well as from sugar cane, and is defined as the runoff syrup from the final stage of crystallization, from which further crystallization of sugar is uneconomical. Despite their similarities, beet and cane molasses exhibit significant differences with regards to nitrogenous compounds, fermentable sugars, ash and vitamin content. Sugar beet molasses, therefore, is a solution of sugar, organic and inorganic matter in water with a dry substance of 74-77% (w/w). Total sugars (mainly sucrose) constitute approximately 47-48% (w/w) of molasses, ash 9-14% (w/w) and total nitrogen containing compounds (mainly betaine and glutamic acid) 8-12% (w/w). Sugar beet molasses is widely used as a substrate in fermentations since it constitutes a valuable source of growth substances such as pantothenic acid, inositol, and trace elements and, to a lesser extent, biotin [4].

In the present study, the optimum fermentation period for xanthan gum production from sugar beet molasses considering medium composition and molasses pre-treatment was determined.

II. Material And Methods

2-1. Microorganism and Media

X. campestris NRRL B-1459 was obtained from Persian Type Culture Collection. The strain were maintained on glucose yeast extract agar (20 g/l glucose, 10 g/l Yeast extract, 20 g/l $CaCO_3$ (light precipitate), and 17 g/l Agar). Cultures were transferred at two week intervals. Plates were incubated at 26°C.

The same medium without agar were used for stock culture in test tube and incubated at 28°C. The pH of media was adjusted to between 6.5 and 7.5 [1].

After 2 days, the stock culture was transferred to seed culture containing 200 ml distilled water, 5g/l yeast extract, 25g/l sucrose, 10g/l trypton and 10g/l NaCl. The pH of media was adjusted to between 6.5 and 7.5 [1]. *X. campestris* was incubated overnight in an orbital shaking incubator (240 rpm) at 28°C [9].

2.2. Substrate Preparation

The total soluble solids of sugar beet molasses were adjusted to 10% with distilled water. Diluted molasses was preheated in 90°C water bath for 10 minutes and centrifuged two times in 8000 rpm for 20 min. Addition of 6 g/l NH_4NO_3, 4 g/l KH2PO4, 0.2g/l Mg_2SO_4, 5 g/l NaCl was performed before adjusting pH at 7 and sterilization of fermentation media [4]-[9]. The same treatments were used for 10% sucrose fermentation media. Fermentation was carried out in 100 ml Erlenmeyer flasks with 50 ml of medium.

2.3. Inoculum Preparation

Incubating seed culture, defined as the time allowed for cell growth. The initial absorbance at 650 nm (A650) of the medium was adjusted to approximately 0.6 and flasks were inoculated by 10 ml of the inoculum culture. Cultures were grown in triplicates. Flasks incubated in a rotary shaking incubator (25°C and 240 rpm) for 5 days and gum separation was carried out with 24 h intervals.

2.4. Determination of pH Variation

The changes in pH values of fermentation broth were measured before separation of the gum in each interval.

2.5. FT-IR Spectra

FTIR spectra of xanthan gum was recorded in the range of 400–4000 cm^{-1} on a Perkin Elmer Paragon 500 FTIR spectrophotometer using KBr pellets.

2.6. Xanthan Gum Production

For the sucrose fermentation medium, the cell mass were separated by centrifugation process. The amount of xanthan gum produced was determined by precipitating the whole fermentation broth with three volumes of 95% ethanol [1]. The weight of the dried mass obtained was the amount of xanthan gum.

For the fortificated molasses media, the amount of xanthan gum produced was determined by precipitating the whole fermentation broth with three volume of 95% ethanol. The precipitate contained xanthan gum plus insoluble molasses solids. Since xanthan gum is water soluble, washing the precipitate will remove the xanthan gum.

2.7. Scanning Electron Microscopy

The SEM of gold-coated samples was obtained using JSM - 6390LV scanning electron microscope (Jeol Ltd, Japan) at a magnification of 200-1000 (Resolution-HV 3.0 nm).

2.8. Statistical Analysis

Statistical analysis of test results was performed by SPSS (16 for windows) using Duncan and factorial test and also the regression between xanthan gum production and pH and biomass was done.

III. Results And Discussion

3.1. pH Determination

The initial pH that was selected in this study was 7.0 (Fig. 1) [1]. Addition of phosphate salts to the sterilized molasses solutions affected greatly their pH values as buffer solution. In all cultures that initial pH was above 5.9, the biomass production was higher than when pH reached under 5.9. After 20 h, the pH of the cultures dropped to approximately 6.0 whereas after 96 h it rose to approximately 6.6. Fig. 1(a).

As it shown in Fig. 1(a), the pH above 6.4 is the optimum pH for production of xanthan gum. It seems gum production increases with increasing pH.

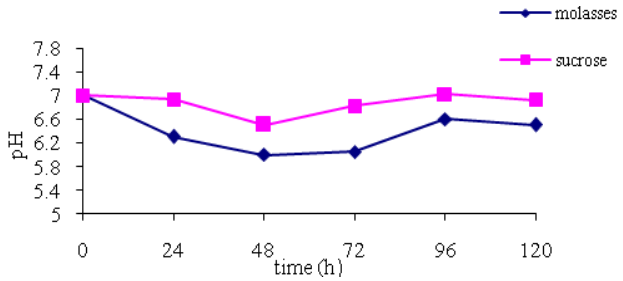


Fig. 1(a). pH variation during fermentation in sucrose and molasses media.

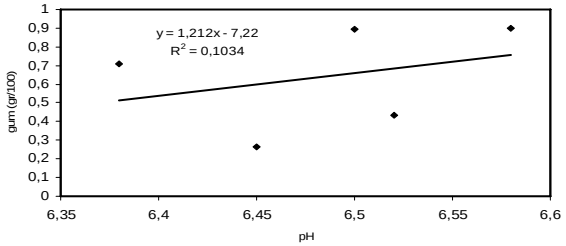


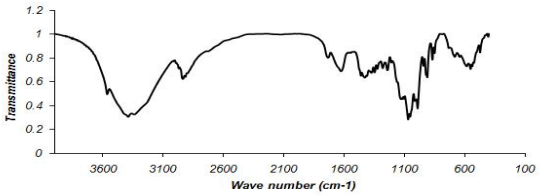
Fig. 1(b). Correlation between gum production yield and pH

In molasses, variation in pH is more noticeable because of its components. Thus, the role of KH2PO4 as buffering agent is a key factor for holding the pH up. This result showed that there is a positive and significant correlation between the pH above 6.6 and increasing the gum production. Fig. 1(b).

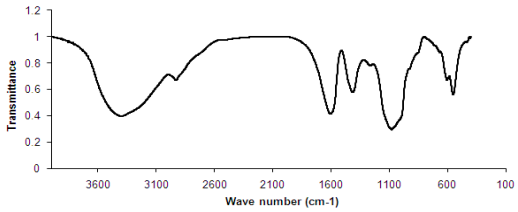
3.2. FT-IR Spectra

The FT-IR spectra of the pure xanthan, a broad absorption peak at 3450cm^{-1} indicate the hydrogen bonded OH groups. Two peaks, one at 615cm^{-1} and the other at 1476 cm^{-1}, are attributed to −COO groups [10].

a)



b)



c)

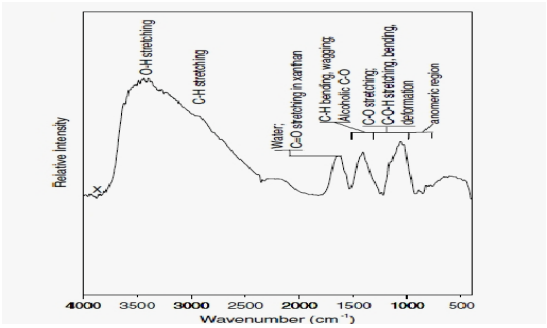


Fig .2. FTIR spectra of xanthan gum in sucrose (a) in molasses (b) and standard of xanthan gum (c)

Additional characteristic absorption bands of xanthan gum appeared at 417cm^{-1} and 1023cm^{-1} due to C–H bending and O–H bending vibrations, respectively. The peaks at 1568 cm^{-1} (C-O asymmetric stretching) and 1406 cm^{-1} (C-O symmetric stretching) are due to the carboxylate anion (Gils et al, 2009). Fig. 2 showes the FTIR spectra of the produced xanthan gum in (a) 10% molasses; (b) in sucrose and (c) is the standard FTIR spectra for xanthan gum [3]-[10]. (a) and (b) showed the value of transmittance and in (c) curve relative intensity is used.

3.3. Xanthan Gum Production

Xanthan gum production using two fermentation media is summarized in Table 1. The xanthan gum yield from molasses media ranged from 0.264 to 0.902 g/100 ml. while from sucrose was 0.290 to 1.066 g/100 ml, The yields of xanthan gum from molasses in this study are similar to sucrose and regarding the low sugar concentration of used molasses, it is noticeable and economical.

TABLE I
XG PRODUCTION (G/100ML)

Time (Day)	Sucrose (g/100ml)	Molasses (g/100ml)
1	0.290	0.264
2	0.524	0.434
3	0.616	0.708
4	1.066	0.896
5	1	0.902

Increasing in biomass weight was observed until 72h in both media (Fig.3a) but maximum xanthan gum production was obtained after 96 h (Fig.3b). It means the best time for adaptation the microorganism for gum producing is 96h after incubation although the maximum number of microorganism was observed after 72h after incubation. The maximum gum production by this microorganism in 10% molasses is too similar to 10% sucrose media. Fig. 3 (c) shows the significant correlation between the biomass weight and increasing the amount of xanthan gums.

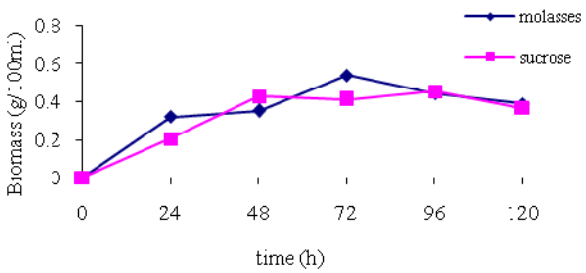


Fig. 3 (a). Bio cell mass from cultures inoculated and grown on molasses and sucrose.

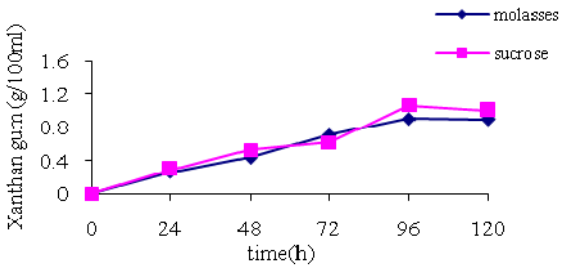


Fig. 3 (b) Xanthan gum produced by cultures inoculated and grown on molasses and sucrose.

The results obtained in the present study permit us to conclude that, the yields of xanthan gum production from molasses considering the low sugar concentration are similar to sucrose and consequently industrial by-products such as molasses can be used as a substrate to produce microbial gums such as xanthan.

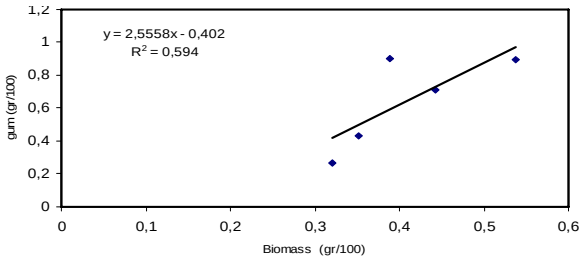


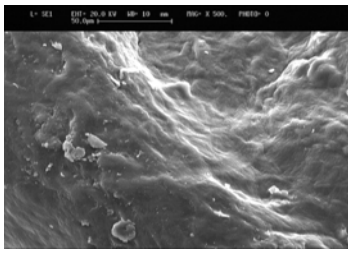
Fig. 3 (c). Correlation between gum production and the weight of biomass

3.4. Scanning Electron Microscopy

Figure 4 shows SEM images of xanthan gum produced by *X. campestris*. In molasses fermentation media.

Fig. 4. These micrographs demonstrate the homogeneity of xanthan gum produced by X. campestris. The novel properties of xanthan gum might be responsible for salad dressings and sauces, stabilizing the colloidal oil and solid components against creaming by acting as an emulsifier. Also used in frozen foods and beverages, xanthan gum creates the pleasant texture in many ice creams. Toothpaste often contains xanthan gum, where it serves as a binder to keep the product uniform.

a)



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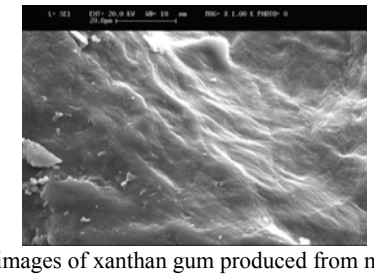

Fig. 4 SEM images of xanthan gum produced from molasses at a magnification of × 500 (a) and ×1000 (b)

Statistical Analysis

The amount of yields from each sampling time was tested by Duncan. The obtained results showed four statistical significant groups at the level of 0.05 that were shown in Table 2. These results demonstrated that the yield production of xanthan for 120 h fermentation significantly increased during 96h after fermentation and then non-significantly decreased. Factorial test (time × treatments (molasses and sucrose media) × repetition) results (Table III) represented the significant difference for sampling times alone at the level of 0.001 while the treatments and time × treatment interaction did not show significant difference even at the level of 0.05.

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TABLE II
RESULTS OF DUNCAN ANALYSIS FOR SAMPLING TIME

Time (hour)	Weight (g/ml)
24	0.27700^{a}±0.66636
48	0.47900^{b}±0.64156
72	0.66200^{c}±0.79995
96	0.98400^{d}±0.126469
120	0.94800^{d}±0.126206

TABLE III
ANOVA RESULTS BY FACTORIAL TEST

Source	df	Mean Square	F	Sig
Time	4	0.550	72.427	0.000^{**}
Treatment	1	0.026	3.367	$0.081^{n.s}$
Time*Treatment	4	0.014	1.870	$0.155^{n.s}$
Error	20	0.008		
Corrected Total	29			

n.s: Non-Significant
**: Significant difference at level of 0.001