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Aeration Rate Effect in Xanthan Production by *Xanthomonas campestris* ATCC 13951 on Synthetic and Sugar Cane Medium

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ABSTRACT

Xanthan gum is a heteropolysaccharide produced by *Xanthomonas campestris* ATCC 13951 (TISTR 1100). This polysaccharide has been extensively used in many industries such as food, adhesive, ceramic, cosmetics, leather, pharmaceutical and petrochemical. The aeration rate effects at 0, 5, 10 and 15 ppm on xanthan production were evaluated in a batch fermentation. Both of synthetic and sugar cane medium were compared in order to determine the optimal condition for xanthan production. Results indicated that the 15 ppm aeration rate was suitable for both substrates, whereas the xanthan yield was higher in sugar cane medium than synthetic medium. The experiment showed that the maximum xanthan production was 117.3 g/L with 6.8 g/L maximum biomass concentration. When accounting for the growth fermentation kinetics, xanthan gum was considered as a growth associated product. Results showed that the growth rate in synthetic medium increased 3 and 5 times as the aeration rate was increased by 2 and 3 times. The increment in the growth was resulting in the greater rate of xanthan production. Data revealed that an increasing of aeration rate from 2 and 3 times was influenced the rate of xanthan production by 3 and 3.5 times. In the sugar cane medium, the growth rate multiplied from 2 to 4 times as 2 and 3 times increasing in aeration rate. To the same amount of this aeration rate, the greater rates of xanthan production were obtained by 3 and 3.5 times.

Keywords: Xanthomonas campestris, xanthan gum, growth kinetics, aeration, sugar cane

INTRODUCTION

Xanthan gum is produced commercially by fermentation on commercial grade glucose or starch, following degradation by a combined acid and enzyme treatment. This polymer is majorly used for food applications as thickener and viscosifier. Reports currently suggested that the gum could be produced in fermentations on whey, chestnut flour, coconut water, sugar beet molasses and olive mill wastewaters (Liakopoulou-Kyriakides *et al*., 1997; Liakopoulou-Kyriakides *et al*., 1999; Kongruang and Kongtun, 2005; Kalogiannis *et al*., 2003; Lopez *et al*., 2001). Sugar cane is a solution of sugar, organic and inorganic matter in water with a dry substance of 30% (w/w). Total sugars (mainly sucrose) constitute approximately 11% (w/w) of solution, ash 11% (w/w) and total nitrogen containing compounds (mainly amion acids and ammonia) 1% (w/w). Therefore, sugar cane solution has a high potential application as an alternative suitable industrial substrate

for xanthan gum fermentations. Many factors such as the type of growth medium, growth conditions, the aeration rates, agitation rates, the fermentation temperature and the microogransism strain influenced xanthan production. Other than these parameters, reserachers on xanthan fermentation have been faced problems on poor bulk mixing and low oxygen transfer rates (García-Ochoa *et al*., 2000; García-Ochoa *et al*., 1997; Funahashi *et al*., 1987) in high viscous broth during the fermentation process resulting in a low xanthan yield. Moreover, the cost of downstream processing determines whether the manufacture of the gum is commerical viable or not, a high production concentration is therefore essential. This present work aims at evaluating the alternative growth medium, a sugar cane medium, for xanthan production while accounting for the aeration rates in order to increase the production yield and choose a cost effective approach.

METHODOLOGY

Microorganism and Inoculum Preparation

Xanthomonas campestris TISTR 1100 was obtained from Thailand Institute of Scientific and Technological Reserach, Bangkok, Thailand. This strain was used throughout this experiment. Inoculum preparation was transferred from the stock solution to YM agar slants and incubated for 2 days at 28°C. Following this period, single colonies were transferred into the broth media according to Pons, 1989, which contained the following: yeast extract (3 g/L), malt extract (3 g/L), peptone (5 g/L), glucose (10 g/L); the pH was adjusted to 7.0 by adding NaOH. Inoculum media was sterilized in 100 mL erlenmeyer flasks at 121°C for 20 min. Following sterilization, the medium pH was 7. Cultures were grown in duplicates and sterile additives were added under aseptic conditions after autoclaving. The cultures were then incubated at 28°C in an orbital shaking incubator with agitation of 250 rpm for 24 h. until they reached an optimal density of 0.8 at 600 nm. These were used as inoculum for the batch fermentation.

Media

The synthetic medium was prepared according to Pons, 1989. The composition was as the following: 0.1% w/v yeast extract, 0.1% w/v malt extract, 0.17% w/v peptone, 2.5% w/v glucose, 0.5% w/v K_2HPO_4, 0.03% w/v $MgSO_4$, 0.05% w/v $CaCl_2$. For the sugar cane medium, the fresh sugar cane was collected in plastic container and stored at 4 °C. Before preparation of the culture media, sugar cane solution was filtered through a cotton layer and adjusted the pH to 7.0 with 1 N NaOH and 1 N HCl. This neutralized solution was used as the culture media after authoclaving.

Fermentations

Xanthan production was tested on cultures in 300 mL erlenmeyer flasks of two different substrates, a synthetic medium and a sugar cane medium. These media were inoculated with a 10% (v/v) of inoculum. Each media was applied the aeration rates at 0, 5, 10 and 15 ppm with incubation at 28 °C on a rotary shaker at 250 rpm for 35 h. Aliquots of approximately 12 mL were withdrawn aseptically at regular 5 h

intervals. The samples were analysed for pH, viscosity, and biomass and xanthan production. All runs were done in triplicated with no inoculated media used as a control. The averaged values are presented in this work.

Analytical procedures

The apparent viscosity was measured in the fermentation broth. For viscosity determination a Brookfield viscometer DV-I (Massachusetts, USA) with spindle number 27 at 100 rpm was used. Biomass was calculated by dry cell-weight estimation. The culture was taken 5 mL and added 1% KCl to reduce the viscosity. The cell were collected after centrifugation at 10,000 rpm for 30 min. at 4 °C. The supernatant was collected for the determination of residue sugar and xanthan content. The biomass residue was then washed with 1 mL of conc. HCl and distilled water to remove traces of xanthan before passed through the 0.2 μm cellulose nitrate membrane. Finally, cell were dried in an oven at 60 °C for 48 h and weighed.

Determination of xanthan gum concentration

The collected exopolysaccharide was recovered from the cell-free supernatant by precipitation with two volumes of cool isopropanol. The solution was then centrifuged at 10,000 rpm at 4 °C for 15 min. The supernatant was saved for total sugar determination. The precipitates were further rinsed with isopropanol and passed through 0.45 μm cellulose nitrate membrane. The residues were dried in an oven at 60 °C for 48 h and weighed.

Determination of residual sugar concentration

The saved supernatant from the xanthan gum determination process was measured the sugar content by the colorimetric assay by using dinitrosalicylic acid method.

RESULTS AND DISCUSSION

The quantitative analysis of *Xanthomonas campestris* TISTR 1100 growth kinetics on xanthan production were monitored in a batch fermentor without a pH control. Experiments were carried out under the optimum temperature for biomass and xanthan production at 28 °C with agitation of 250 rpm. The influences of the aeration rates on xanthan production by the comparison between synthetic medium and sugar came medium were shown in Figures 1 and 2. The similar trends of increasing viscosity over the course of fermentation were observed as the increasing of aeration rates from 0 to 15 ppm. According to aeration rates, table 1 summarizes the kinetic parameters of the maximum values of biomass and xanthan concentration, substrate utilization, specific growth rates and xanthan production during the growth of *X. campestris* in the synthetic medium while Table 2 reported those of sugar cane medium. Results indicated that the biological proformance of the culture was dependent on the aeration rates. The maximum xanthan production was obtained when the culture was maintained 15 ppm of aeration rate in both synthetic and sugar cane medium, yielding the xanthan concentration of 108 and 177.30 g/L.

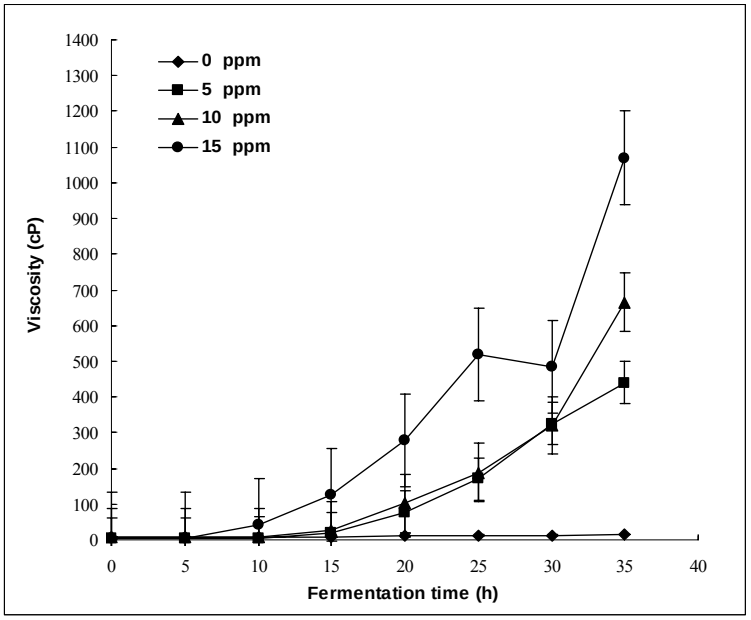

Figure 1 Comparison of aeration rates on xanthan production time courses on synthetic medium, including the aeration rates at 0, 5, 10, and 15 ppm.

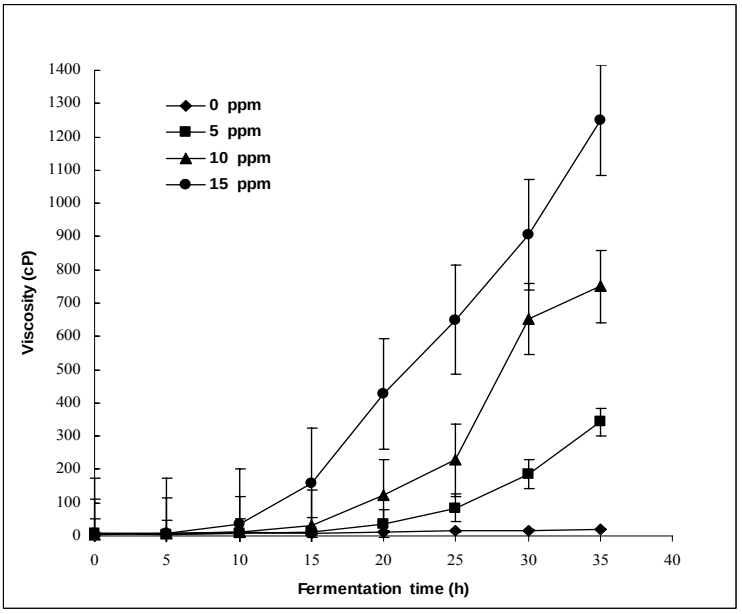

Figure 2 Comparison of aeration rates on xanthan production time courses on sugar cane medium, including the aeration rates at 0, 5, 10, and 15 ppm.

Table 1 Maximum values of biomass and xanthan concentration, substrate utilization, specific growth rates and xanthan production during growth of *Xanthomonas campestris* TISTR 1100 in the synthetic medium with the aeration rate of 0, 5, 10 and 15 ppm at 28 °C.

Aeration Rate (ppm)	Biomass Conc. (g/L)	Xanthan Conc. (g/L)	Substrate Utilization (g/L)	Growth Kinetic Parameters											
				$^{a}r_x$ (g/L/h)		$^{b}\mu$ (g/g/h)		$^{c}r_s$ (g/L/h)		$^{d}q_s$ (g/g/h)		$^{e}r_p$ (g/L/h)		$^{f}q_p$ (g/g/h)	
				Max.	Period (h)	Max.	Period (h)	Max.	Period (h)	Max.	Period (h)	Max.	Period (h)	Max.	Period (h)
0	3.2	27.8	23.45	0.12	35	0.04	35	0.12	15	0.09	15	1.28	35	0.09	15
5	4.1	47.6	12.01	0.16	35	0.04	35	0.96	35	0.23	35	3.22	35	0.23	35
10	4.5	93.4	8.88	0.24	35	0.06	35	1.05	35	0.23	35	7.28	35	0.23	35
15	5.5	108	3.63	0.32	35	0.08	35	1.88	10	1.04	10	8.14	35	1.04	10

$^{a}r_x$ = Growth rate (g/L/h); $^{b}\mu$ = Specific growth rate (h^{-1}) ; $^{c}r_p$ = Rate of xanthan production (g/L/h)
$^{d}q_p$ = Specific rate of xanthan production (g/g/h); $^{e}r_s$ = Rate of substrate utilization (g/L/h); $^{f}q_s$ = Specific rate of substrate utilization (g/g/h)

Table 2 Maximum values of biomass and xanthan concentration, substrate utilization, specific growth rates and xanthan production during growth of *Xanthomonas campestris* in the sugar cane medium with the aeration rate of 0, 5, 10 and 15 ppm at 28 °C.

Aeration Rate (ppm)	Biomass Conc. (g/L)	Xanthan Conc. (g/L)	Substrate Utilization (g/L)	Growth Kinetic Parameters											
				$^{a}r_x$ (g/L/h)		$^{b}\mu$ (g/g/h)		$^{c}r_s$ (g/L/h)		$^{d}q_s$ (g/g/h)		$^{e}r_p$ (g/L/h)		$^{f}q_p$ (g/g/h)	
				Max.	Period (h)	Max.	Period (h)	Max.	Period (h)	Max.	Period (h)	Max.	Period (h)	Max.	Period (h)
0	4.0	70.40	4.75	0.12	35	0.03	35	0.17	10	0.11	10	1.36	35	0.11	10
5	5.0	84.60	1.16	0.20	35	0.04	35	0.17	20	0.18	30	2.64	35	0.06	20
10	5.9	104.80	1.01	0.28	35	0.05	35	2.43	30	0.54	30	3.78	35	0.54	30
15	6.8	117.30	0.58	0.44	35	0.07	35	1.42	20	0.50	20	5.74	35	0.51	20

$^{a}r_x$ = Growth rate (g/L/h); $^{b}\mu$ = Specific growth rate (h^{-1}) ; $^{c}r_p$ = Rate of xanthan production (g/L/h)
$^{d}q_p$ = Specific rate of xanthan production (g/g/h); $^{e}r_s$ = Rate of substrate utilization (g/L/h); $^{f}q_s$ = Specific rate of substrate utilization (g/g/h)

The fermentations of the profiles of each aeration rates from control, 5, 10, 15 ppm of synthetic and sugar cane medium were reported in Figure 3 and 4. The phenomenon of increased productivity in the production phased is expected in xanthan fermentations, where the production of the gum is partly growth associated as reported in the time courses of fermentation in Figure 3 (a, b, c and d). With the development of increasing dissolved oxygen leading to an increasing of xanthan yield, it is shown that the superior mixing achieved at 15 ppm, compared with control and low aeration rates (5, 10 ppm). Results were in agreement with the sugar cane medium as illutrated in Figure 4 (a, b, c and d).

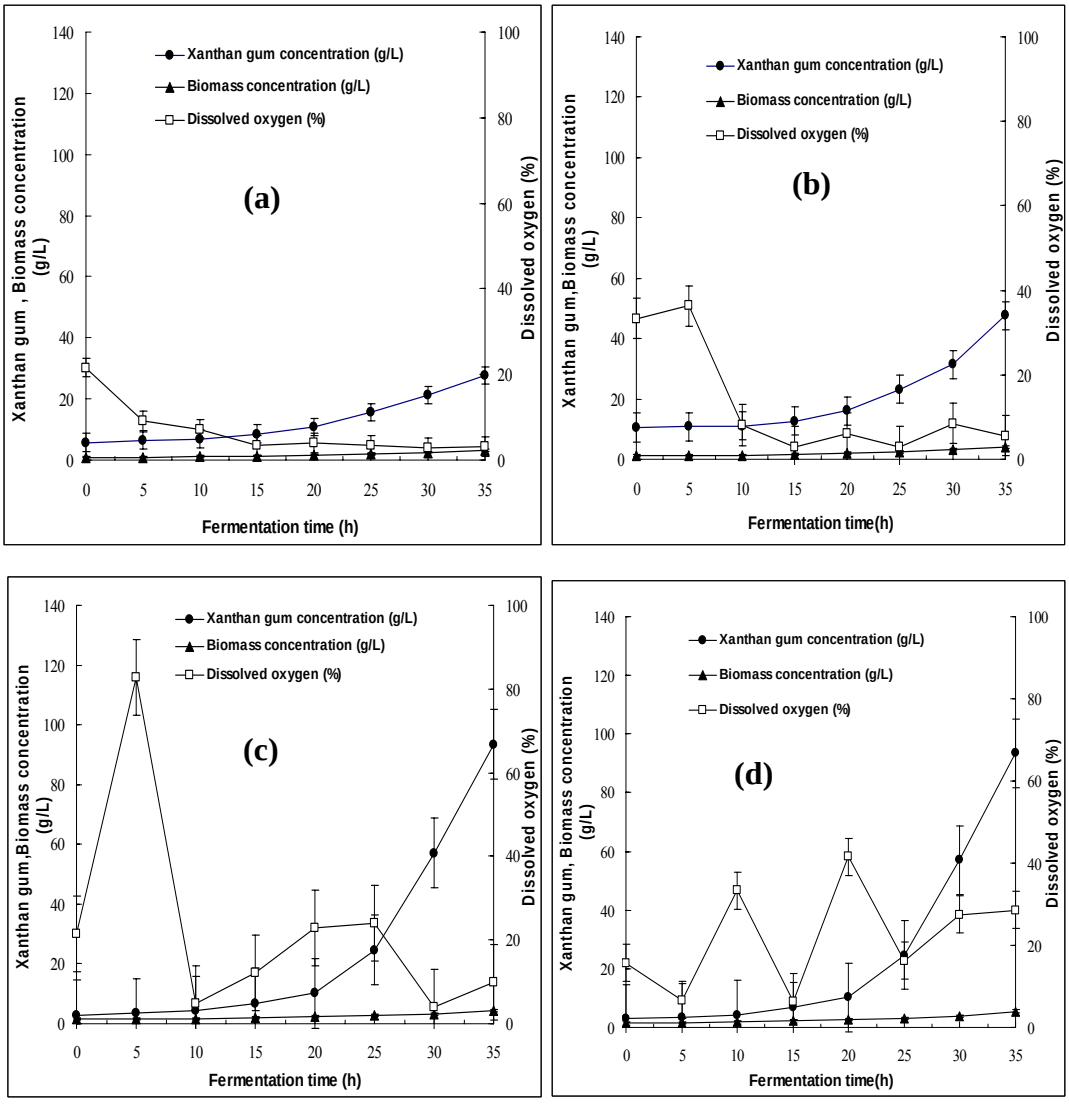

Figure 3 Fermentation profiles for *Xanthomonas campestris* TISTR 1100 grown on synthetic medium. Profile showing biomass and xanthan gum concentration (g/L) versus the dissolved oxygen (%) at (a) 0 ppm, (b) 5 ppm (c) 10 ppm, and (d) 15 ppm.

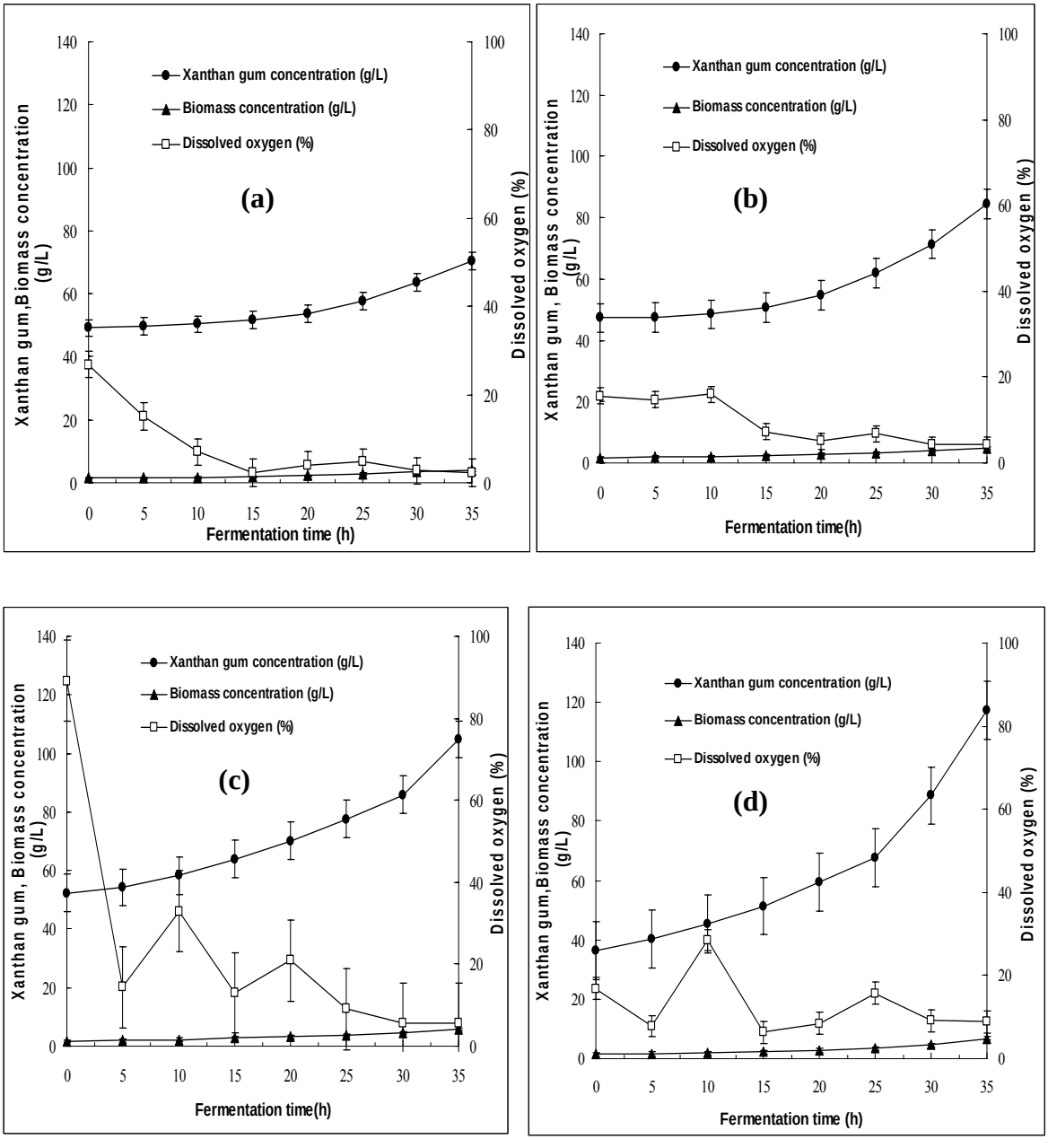

Figure 4 Fermentation profiles for *Xanthomonas campestris* TISTR 1100 grown on sugar cane medium. Profile showing biomass and xanthan gum concentration (g/L) versus the dissolved oxygen (%)at (a) 0 ppm, (b) 5 ppm (c) 10 ppm, and (d) 15 ppm.

It is shown that the growth rates in synthetic medium increased 3 and 5 times as the aeration rates were increased by 2 and 3 times. The increment in the growth was resulting in the greater rate of xanthan production. Data revealed that an increasing of aeration rates from 2 and 3 times was influenced the rate of xanthan

production by 3 and 3.5 times. In the sugar cane medium, the growth rates multiplied from 2 to 4 times as 2 and 3 times increasing in aeration rates. To the same amount of this aeration rates, the greater rates of xanthan production were obtained by 3 and 3.5 times.This information has clearly highlighted the importance of a well mixed and oxygenated region for providing the capacity for high microbial oxygen uptake rates, which ultimately governs xanthan productivity. Such a medium is also a useful way to maintain a restrained oxygen demand without complex feed strategy in the later period of the culture, when viscosity may lead to gas-liquid transfer problems.These results suggest that, provided sufficient oxygen can be supplied to maintain cell viability throughout the stationary phase, xanthan can continue to be produced at a level governed by a specific oxygen uptake rate, unless there are biological limitations preventing the formation of the product. At the same time, the medium should be sufficiently characterized to facilitate eventual optimization procedure, taking into account the economic balacing volumetic productivity against yield value. The medium used here achieves such an objective, since biomass accumulation is rapidly initiated by the use of an organic carbon source with the high product transformation.

CONCLUSION

The uneconomical agricultural product, sugar cane medium, can be used as an excellent substrate source to replace the synthetic medium for xanthan gum production. The operational factors should be performed at 28 °C, pH 7, 250 rpm of the shaking speed with the 15 ppm aeration rate supplement. Under these controlled parameters, the optimization of the xanthan gum productivity is acheived.

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