Quantitative Analysis of Exopolysaccharide Production in A Stirred Tank Bioreactor

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Abstract

Xanthan gum is an extracellular microbial heteropolysaccharide that is produced efficiently by *Xanthomonas campestris* TISTR 1100 through the fermentation process. This research is to study the effect of agitation rate and temperature on xanthan gum production. A 3.7 litre fermenter with a 2.7 litre of working volume was used throughout this experiment. The ranging of agitation rates from 300 to 700 rpm and the ranging of temperatures from 30 to 35 ^{0}C were investigated. Results showed that under the temperature of 33 ^{0}C when the agitation rates at 300, 500 and 700 rpm were tested, the maximum viscosity of the fermenter broth were 77.5, 252.5 and 877.5 centripoises. The fermentation kinetics showed the maximum of biomass concentration were 1.04, 2.40 and 2.19 g/L, the maxminum specific growth rate were 0.133, 0.173 and 0.178 h^{-1}, the maximum of xanthan concentration were 0.380, 0.318 and 0.883 g/g/h, the maximum of substrate utilization were 22.29, 26.15 and 23.29 g/L, the maximum of substrate utilization were 11.800, 8.889 and 6.407 g/g/h, respectively. The optimum agitation rate at 700 rpm was chosen as a fixed parameter, while the temperature conditions at 30, 33 and 35 ^{0}C were evaluated. Results revealed that the maximum viscosity of fermentation broth were 430, 887.5 and 420 centripoises. The fermentation kinetics indicated that the maximum specific growth rate were 0.224, 0.178 and 0.154 h^{-1}, the maximum specific rate of xanthan production were 0.447, 0.883 and 0.883g/g/h, the maximum specific of substrate utilization were 5.842, 6.407 and 10.913 g/g/h, respectively.

1. Introduction

The coconut juice is uneconomical agricultural product which usually sales in the lower value. The juice is considered as valuable sources of substrate such as carbon (glucose and fructose), nitrogen sources (arginine, alanine, cystine and others), minerals (P, Na, Ca, Pb, Cu, S and Cl) for microbial growth. As the high content of nutritional nutrient for microbial growth, it is used as the substrate for *Acetobacter xylinum* culture to produce the bacterial cellulose for many commerical food products application which are commonly sale in the Southeast Asia. The predominant microbial bacteria, *Xanthomonas campestris*, is the efficient gram-negative bacteria that can make use of a coconut juice to synthesis the extracellular polysaccharide, xanthan gum. This gum is recognized as an important economic interest since it is widely used in variety of industries, such as pharmaceuticals, agriculture, textile and petroleum production [1,2]. In the course of the fermentation, the secretion of the polysaccharide results in a highly viscous broth. This behavior of the broth causes serious problems of mixing, heat transfer and oxygen supply, resulting in lower the xanthan gum yield. Environmental parameters affecting polymer synthesis still need the understanding and controlling the important variables in order to advantage in the design of an economic process. Therefore, in this study we present an alternative strategy for the xanthan production by using the cheap substrate, coconut juice. This approach might result in a lower cost of final product. To improve on this process, we investigated the factors that dictate the productivity in terms of the temperature and

agitation in order to construct the kinetic analysis of growth and xanthan production in the batch bioreactor for optimization.

2. Materials and Methods

2.1 Microorganism and inoculum preparation

Xanthomonas campestris TISTR 1100 (ATCC 13951) was obtained from Thailand Institute of Scientific and Technological Research, Bangkok, Thailand. This strain was used throughout this experiment. Inoculum preparation was transferred from the stock solution to YM agar slants [3] and incubated for 2 days at 28°C. Following this period, single colonies were transferred into the broth media according to Pons, 1989 [4], which contained the following: yeast extract (3 gL^{-1}), malt extract (3 gL^{-1}), peptone (5 gL^{-1}), glucose (10gL^{-1}); 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 exact composition of culture in chemical defined medium was 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 $CaCl_2$. 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 bioreactor. For the coconut medium, the ripen coconut juice was passed through Whatman No.1 filter. The solution is then adjusted the pH to 7.0 with 1 N NaOH. This adjusted solution was used as the culture media.

2.2 Fermentations

Batch fermentations were carried out in a 3.7 litre Benchtop Fermenter (Type KLF 2000, Bioengineering AG, Wald, Switzerland)). The working volume was 2.7 L for coconut juice medium. Experiments were conducted at 33 ^{0}C with aeration rate 150 L/h resulting in 15% of the dissolved oxygen in the fermentation broth under three the agitation rates at 300, 500 and 700 rpm. The fermentor was equipped with all monitors and controllers for pH, temperature and dissolved oxygen. After the agitation speeds were evaluated, three set of the experiments were carried out stepwise at a different temperature. The temperature evaluation, 30, 33 and 35 ^{0}C were chosen to study under 700 rpm agitation rate and 150 L/h aeration rate. Runs were terminated after 35 h of culture. All runs were carried out in replicate and averaged values were presented in this work. Aliquots of approximately 12 mL were withdrawn aseptically from the cultures every 5 h. The pH was maintained at 7.0 by automatic addition of 4 N NaOH/ 4 N H_2SO_4 during xanthan fermentation.

2.3 Analytical methods

2.3.1 Determination of biomass concentration

Broth samples were taken at regular intervals. 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. Aliquots of 5 mL was added 1% KCl to reduce the viscosity and then the cell were collected after centrifugation at 12,000 rpm for 30 min at 4 ^{0}C. The supernatant was collected for 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. All test were done in triplicate.

2.3.2 Determination of xanthan gum concentration

The collected exopolysaccharide was recovered from the cell-free supernatant by precipitation with two volumes of 95% ethylalcohol . The solution was then centrifuged at 14,000 rpm at 4 ^{0}C for 30 min. The supernatant was saved for total sugar determination. The precipitates were further rinsed with 95% ethylalcohol and passed through 0.45 µm cellulose nitrate membrane. The residues were dried in an oven at 60°C for 48 h and weighed.

2.3.3 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 Anthrone method [5].

3. Results and Discussion

3.1 Production medium

The kinetics of growth and xanthan production by *Xanthomonas campestris* TISTR 1100 in batch culture were studied in stirred tank bioreactor with a pH control. Fermentations were carried out over a range of stirrer speeds from 300 to 700 rpm, which covered the entire range of stirrer speed practical for the bioreactor used. The comparison of agitation rates on xanthan production, including the agitation rates of 300, 500 and 700 rpm by using the coconut juice as the solely substrate are illustrated in Fig. 1.

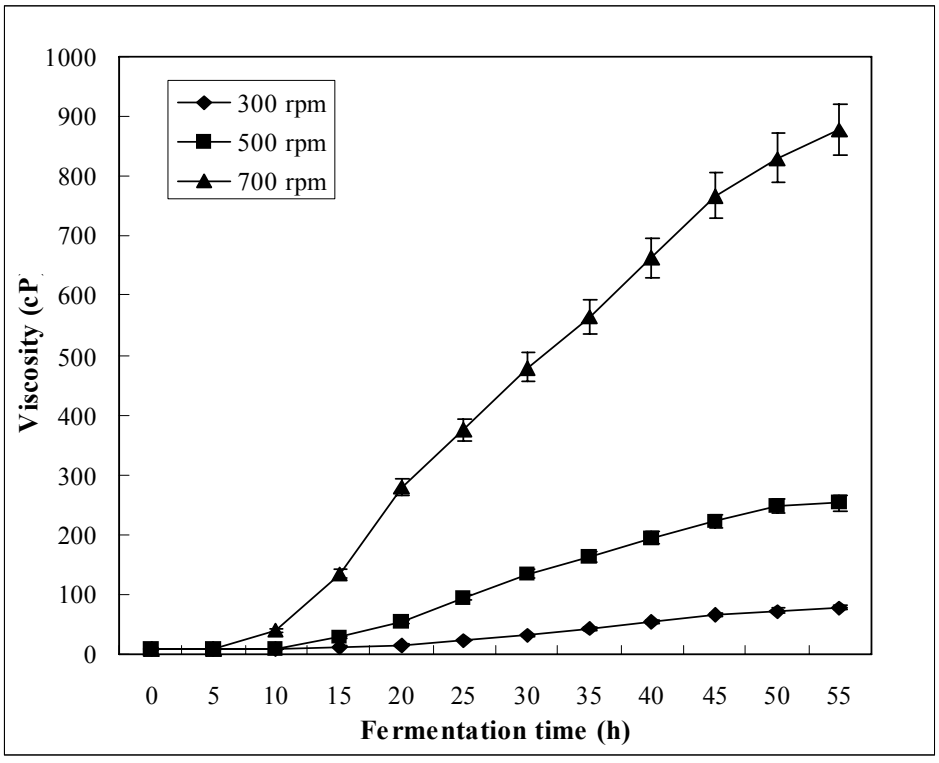

Figure 1. Time-courses comparison of agitation rates at 300, 500 and 700 rpm on viscosity.

Result showed a significantly increase in the viscosity when the fermentation broth were subjected to the agitation at 700 rpm. The time-courses as increasing of agitation rates on the changing of biomass concentration, substrate concentration and xanthan gum production over a time-course of 55 hours at 33 ^{0}C are shown in Figs. 2 and 3.

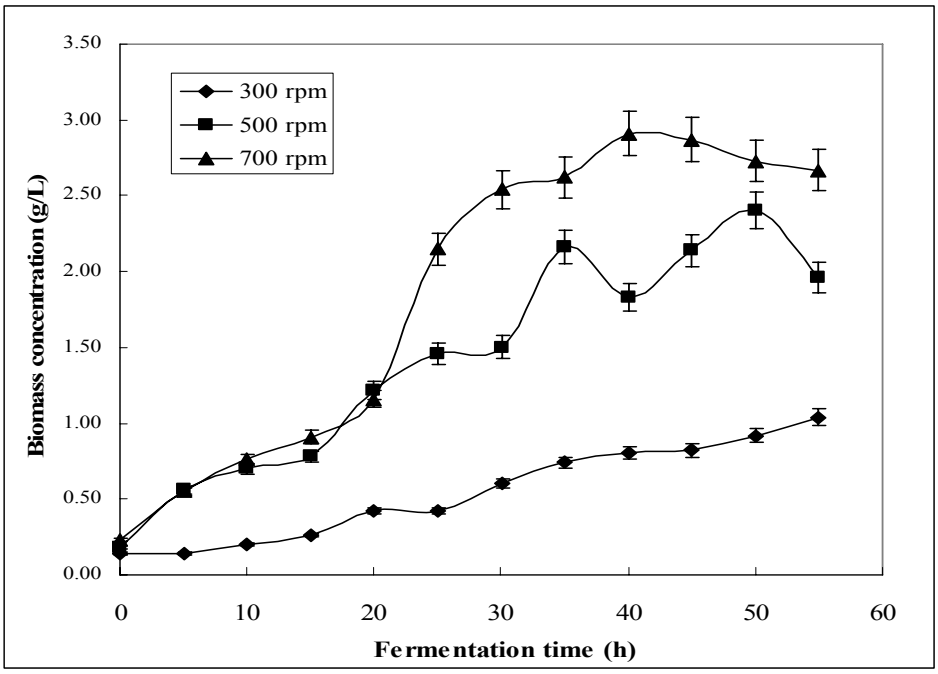

Figure 2. Time-courses comparison of agitation rates at 300, 500 and 700 rpm on biomass concentration.

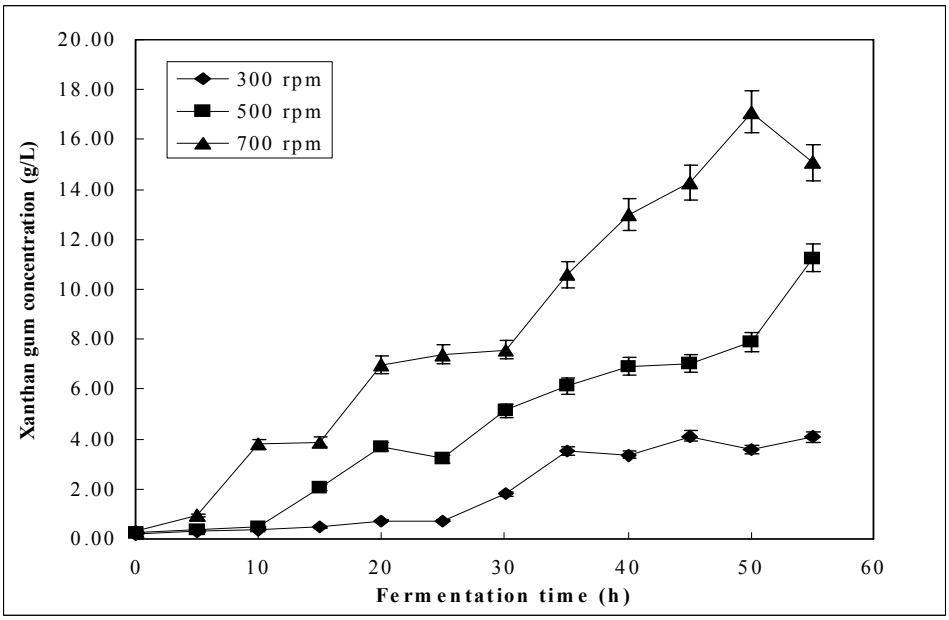

Figure 3. Time-course comparison of agitation rates at 300, 500 and 700 rpm on xanthan concentration.

This significant data showed the confirmation that the coconut juice was an excellent source of growth. Substrate was completely utilized by the microorganism, the concentration being practically zero from 35 hours of fermentation carried out at 700 rpm (Fig. 6) as an increasing of xanthan production.

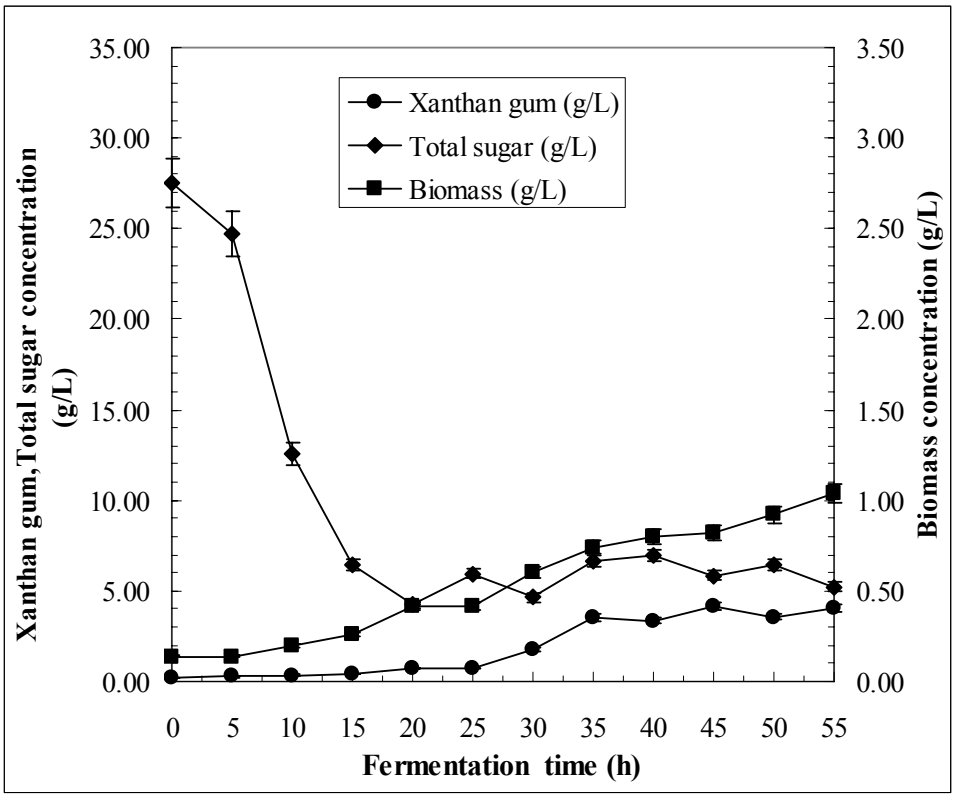

Figure 4. Fermentation profiles for *Xanthomonas campestris* TISTR 1100 grown on coconut juice. Profile showing biomass, substrate, and xanthan gum concentration (g/L). Fermentation condition was at 33 ^{0}C with an agitation rate of 300 rpm.

The schematic diagrams of the corresponding time course of xanthan production as accounting for the biomass and the substrate utilization are shown in Figs. 4, 5, and 6. All figures showed the same profile of the growth-associated product. Since xanthan gum constitutes the bacterial capsule, the concentration of the carbon source affects the efficiency of the bacteria growth to convert substrate into polysaccharide. It is reported in the lituratures that glucose concentration of 1-5% give the best xanthan yield, while at higher glucose concentration, product yield decrease [6,7]. To produce xanthan gum, *X. campestris* needs several nutrient, including and macronutrient such as carbon and nitrogen and micronutrients such as potassium, iron, and calcium salts. Glucose and sucrose are the most frequently used carbon sources. The concentration of carbon source affects the xanthan yield; a concentration of 2-4 % is prefered [8,9]. A carbon source concentration, coconut juice of 2-3 % was used as the medium of this present work. *X. campestris* was able to produce copious amount of xanthan even using a sole substrate without other supplements. Therefore, this alternative method in replacing a high value synthetic media would allow the use of a really low-cost media, coconut juice for effective xanthan production.

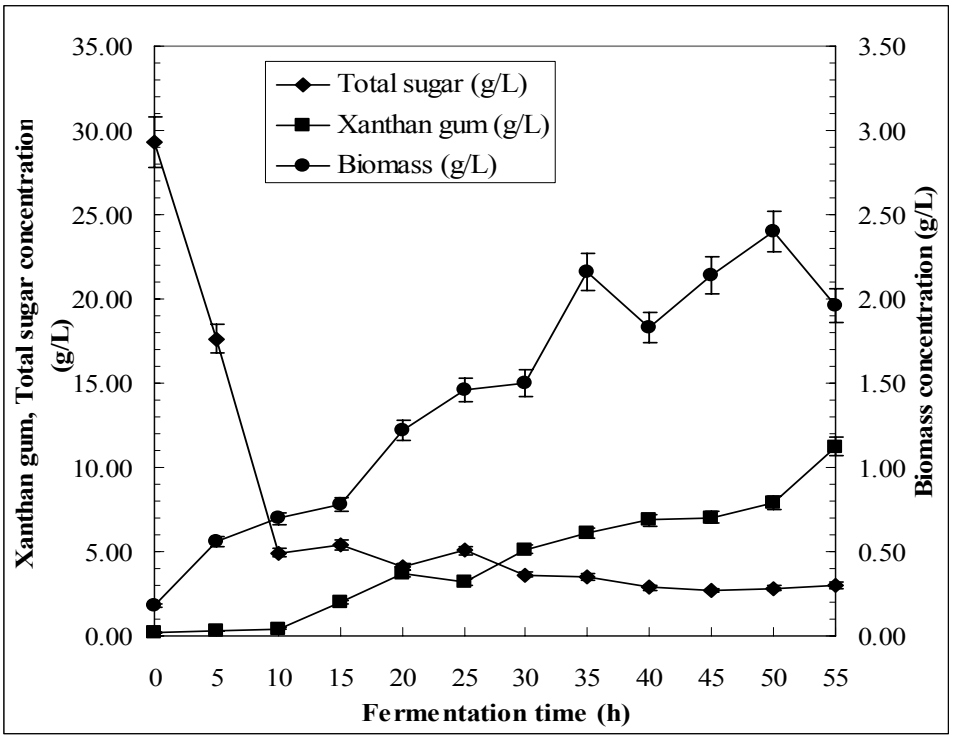

Figure 5. Fermentation profiles for *Xanthomonas campestris* TISTR 1100 grown on coconut juice. Profile showing biomass, substrate, and xanthan gum concentration (g/L). Fermentation condition was at 33 ^{0}C with an agitation rate of 500 rpm.

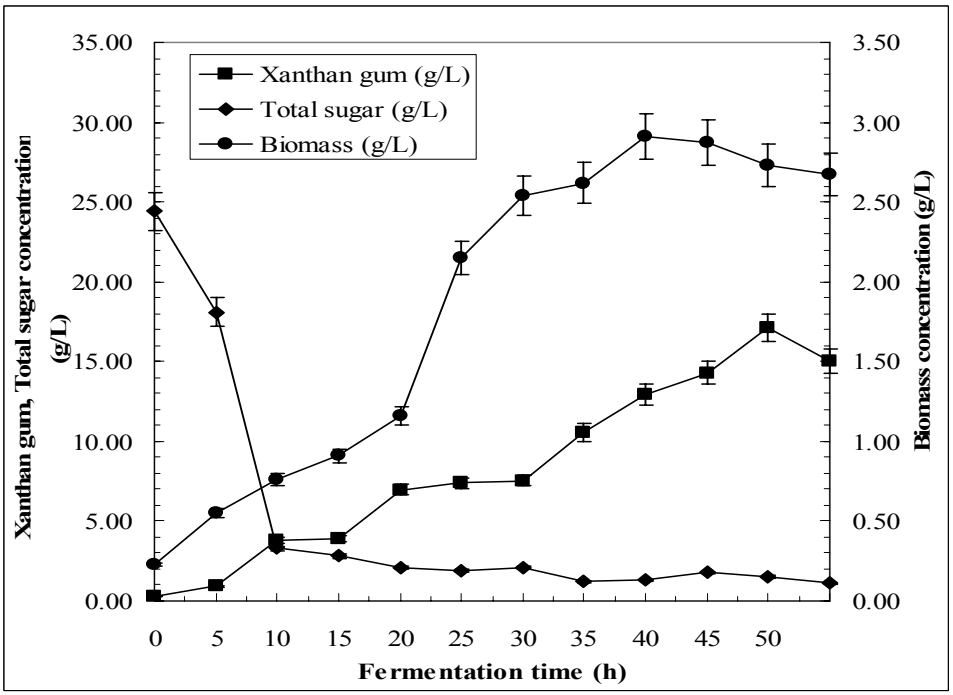

Figure 6. Fermentation profiles for *Xanthomonas campestris* TISTR 1100 grown on coconut juice. Profile showing biomass, substrate, and xanthan gum concentration (g/L). Fermentation condition was at 33 ^{0}C with an agitation rate of 700 rpm.

3.2 Agitation rate

Table 1 summarizes the maximum values of biomass and xanthan concentrations, substrate utilization, specific growth rates and xanthan production during growth of *Xanthomonas campestris* TISTR 1100 in the coconut juice with the agitation rate of 300, 500, 700 rpm as the temperature was fixed at 33 ^{0}C. The examination of the influence of stirrer speed on culture performance by comparison of the rate constant obtained for the xanthan production was reported. Results indicated that at 700 rpm has a maximum both specific growth rate and specific xanthan production (0.109 h^{-1} and 0.883 g/g/h). The aeration rate comparsions shown in specific growth rate, specific rate of xanthan production and specific rate of substrate utilization were higher at 700 rpm agitation rate when compared with those of the lower speeds in Figs. 7, 8 and 9.

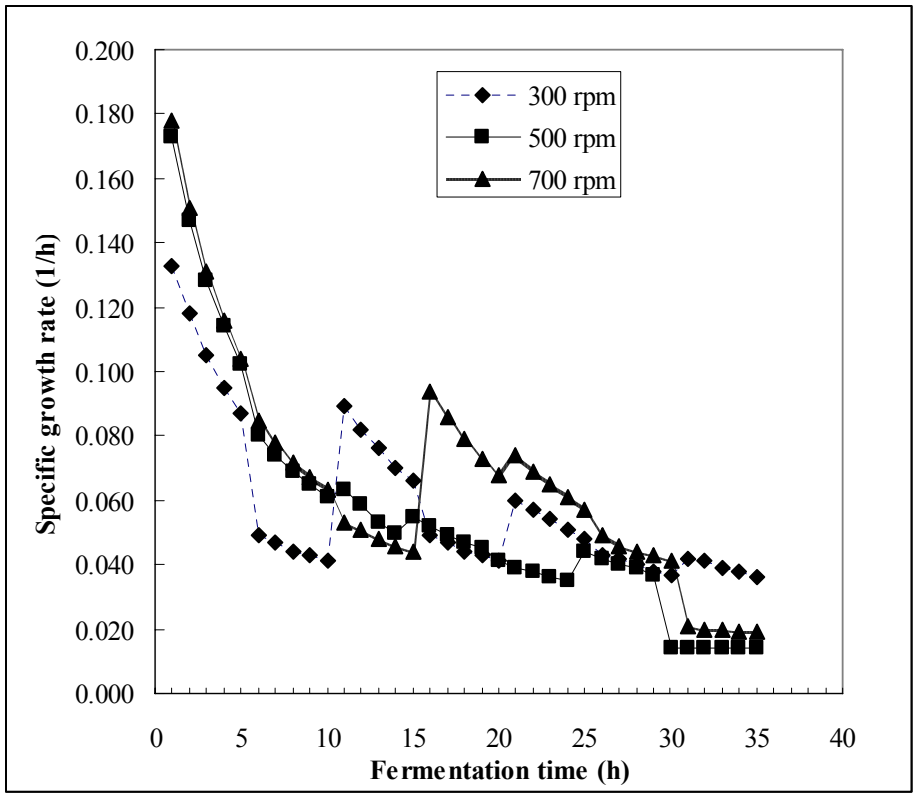

Figure 7. Time-courses comparison of the specific growth rate of *Xanthomonas campestris* TISTR 1100 grown on coconut juice at 300, 500 and 700 rpm.

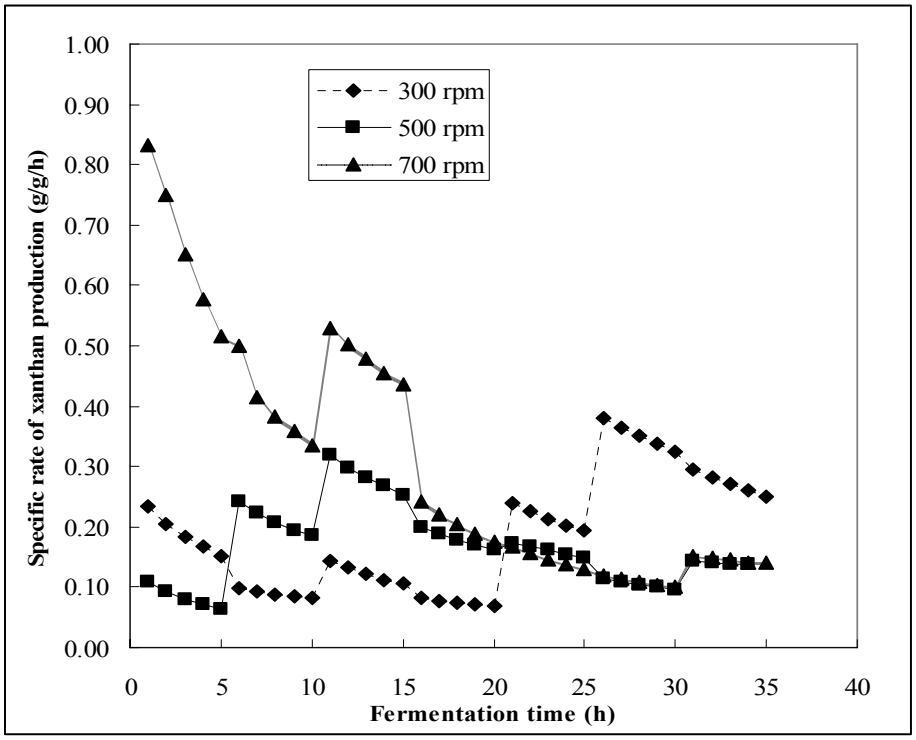

Figure 8. Time-courses comparison of the specific rate of xanthan production by *Xanthomonas campestris* TISTR 1100 grown on coconut juice at 300, 500 and 700 rpm.

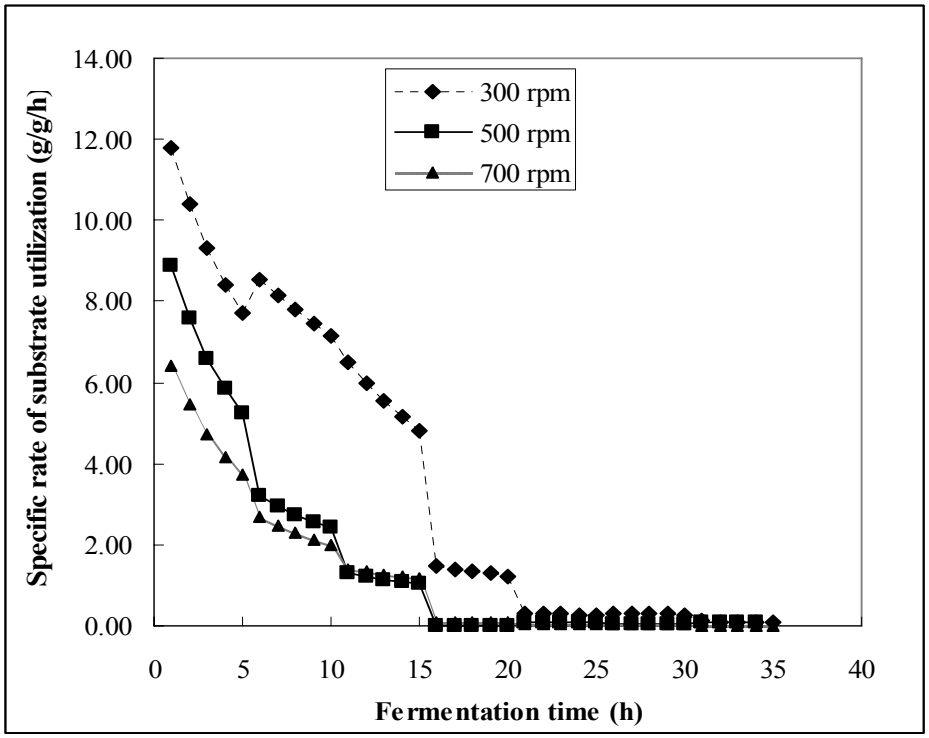

Figure 9. Time-courses comparison of the specific rate of substrate utilization by *Xanthomonas campestris* TISTR 1100 grown on coconut juice at 300, 500 and 700 rpm.

The data in Table 1 is also revealed that the increasing in agitation levels resulted in higher production levels. When the stirrer speed was increased from 300 to 500 rpm, the production of xanthan was triple increased. Agitation facilitated the maintenance of homogenous condition with in the bioreactor, especially with respect to the temperature and the dissolved oxygen ; therefore, xanthan was produced more effectively at 700 rpm, at which speed, a good level of aeration was ensured. This is because in the stirred tank reactors the rate of oxygen mass transfer are influenced by the stirred speed and the air flow rate resulting in the high xanthan yield. As a chemiorganotropic microorganism, *X. campertris* is an obligate aerobe with a strictly repiratory type of metabolism that requires oxygen as the terminal electron acceptor [1]. The beneficial effects of increased agitation was attributed by some investigators to a thinning of the slime layer, enhancing this way the transfer of nutrients and oxygen necessary for xanthan production. Although various types of bioreactor have been used to produced xanthan gum including stirred tank, bubble column, airlift and plugging jet reactor, the sparged stirred tank is frequently employed [10-14]. In all, a stirred tank bioreactor leads other bioreactors under certain operational conditions; both the xanthan yield and the final xanthan concentration are high, and also the fermentation time required to attain these values is relatively short.

3.3 Growth temperature

Temperatures employed for xanthan production in the coconut juice range from 30 to 35 ^{0}C are summarized in Table 2. The comparison of the appararent viscosity as the indicator of xanthan production is illustrated in Fig. 10. Time courses illustrating the results from this set of experiments are presented in elaborated details in Figs. 11, 12 and 13. Included in these figures are a biomass concentration, substrate utilization and xanthan production. Results indicated that the culture temperature at which xanthan is produced has a significant impact on xanthan yield. In this case the temperature at 33 ^{0}C was the best condition for xanthan production. The highest productivity was observed at 33 ^{0}C with 94% and 80% yield higher than at 30 and 35 ^{0}C. These results agreed with those of García-Ochoa et al., 2000 [1] that the temperatures normally used for xanthan production range from 25 to 34 ^{0}C. Furthermore, Thonart et al., 1985 [15] reported an optimum process temperature of 33 ^{0}C. However, the optimal temperature for xanthan production depended on the production medium used. For a high xanthan yield, a temperature between 31 and 33 ^{0}C was recommended.

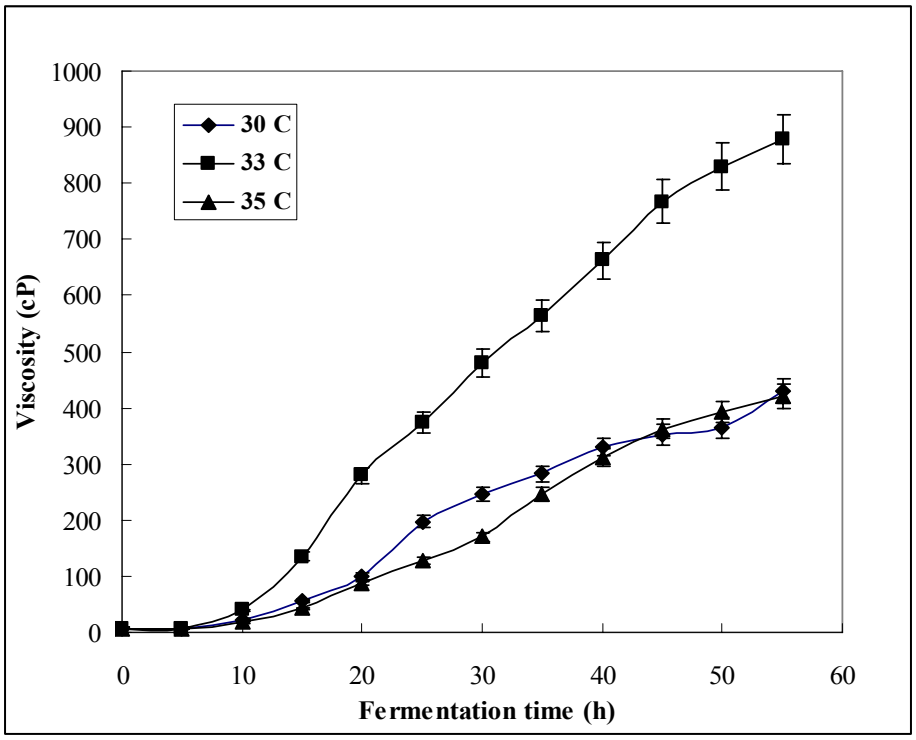

Figure 10. Time-courses comparison of the temperatures at 30, 33 and 35 ^{0}C on viscosity.

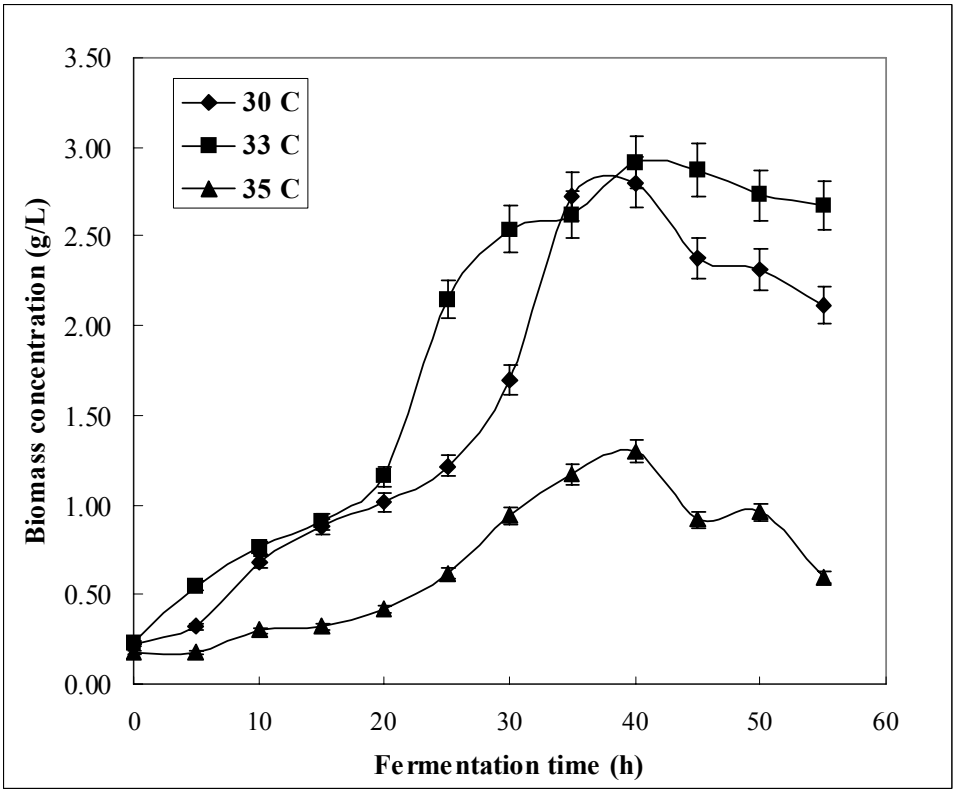

Figure 11. Time-courses comparison of the temperatures at 30, 33 and 35 ^{0}C on biomass concentration.

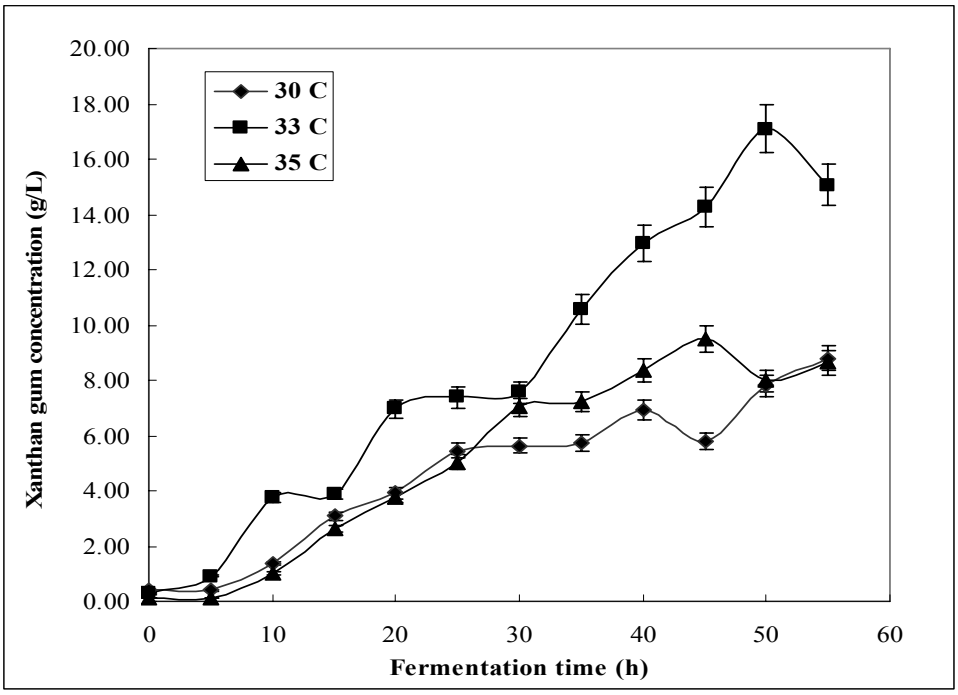

Figure 12. Time-course comparison of the temperatures at 30, 33 and 35 ^{0}C on xanthan concentration.

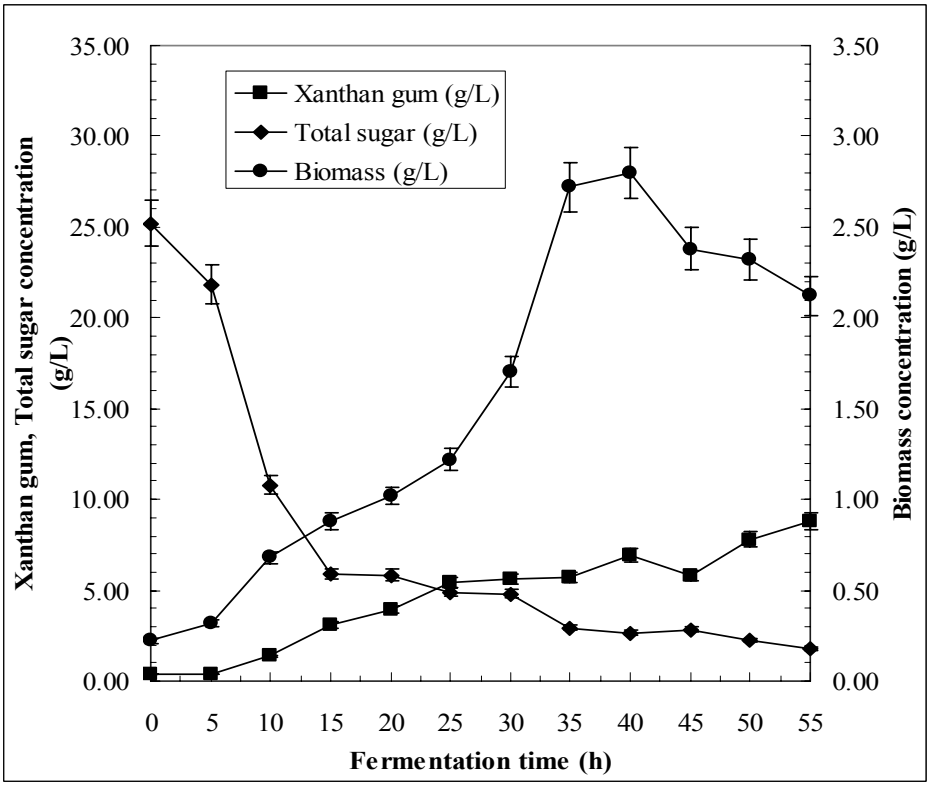

Figure 13. Fermentation profiles for *Xanthomonas campestris* TISTR 1100 grown on coconut juice. Profile showing biomass, substrate, and xanthan gum concentration (g/L).Fermentation condition was at 30 ^{0}C with an agitation rate of 700 rpm.

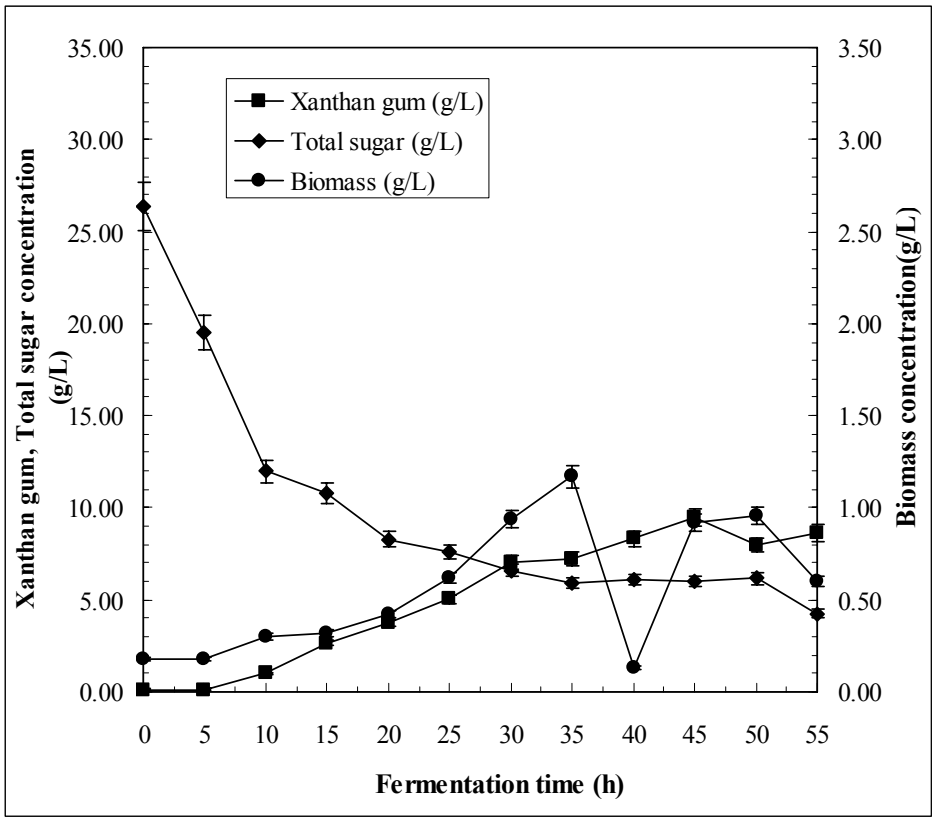

Figure 14. Fermentation profiles for *Xanthomonas campestris* TISTR 1100 grown on coconut juice. Profile showing biomass, substrate, and xanthan gum concentration (g/L).Fermentation condition was at 35 ^{0}C with an agitation rate of 700 rpm.

Conclusion

The aim of this work was to generate information pertaining to xanthan production on the cheap substrate, coconut juice. As discussed, the yield are influence by the agitation rate and temperature. However, other environmental factors contributing a significant effect on xanthan production, for example, microbial strain and growth medium have to look insight into. Further approaches for improving of xanthan production can be achieved through optimization all these parameters and modeling the fermenation process.

Acknowledgements

We are indebted to Oranut Phowchinda for initiation of the xanthan gum reserach. This work was supported by grants from the Thailand Research Fund and Facutly of Applied Science, King Mongkut's Institute of Technology, North Bangkok.

References

[1] García-Ochoa, F., Santos, V.E., Casas, J.A., Gómez, E. Xanthan gum: production, recovery, and properties. Biotechnology Advances. 2000; 18: 549-579.

[2] Morris E.R. Molecular origin of xanthan solution properties. Extracellular polysaccharides. ACS Symp. 1997;45: 81-89.

[3] Jeanes, A., Rogovin, P., Cadmus, M.C., Silman, R.W., Knutson, C.A. Polysaccharide (xanthan) of *Xanthomonas campestris* NRRL B-1459: procedures of culture maintenance and polysaccharide production purification and analysis. ARS-NC-51. Agricultural Reserach Service, US Department of Agriculture, Peoria, Illinois, 1976.

[4] Pons, A., Dussap, C.G., and Gros, J.B. Modelling *Xanthomonas campestris* batch fermentation in a bubble column. Biotechnol. Bioeng. 1989; 33: 394-405.

[5] Herbert, P.J., Phipps, P.J., and Strange, R.E. Chemical analysis of microbial cells. In: Methods in Microbiology, vol. 5 B. Norris, J. R. and Ribbon, D.W. (Eds.), Academic Press, London. 1971, 266-272.

[6] Papagianni, M., Psomas, S.K., Batsilas, L., Paras, S.V., Kyriakidis, D.A. Liakopoulou-Kyriakides, M. Xanthan production by *Xanthomonas campestris* in batch cultures. 2001; 37: 73-80.

[7] Pace, G.W. In: Kristiansen, B., Bu'lock, J. editors. Basic Biotechnology. London: Academic Press.1987, 449-462.

[8] Souw, P., Demain, A.L. Role of citrate in xanthan production by *Xanthomonas campestris*. J. Ferment. Technol. 1980,58: 441-446.

[9] Funahashi, H., Yoshida, T., and Taguchi, H. Effect of glucose concentration on xanthan gum production by *Xanthomonas camperstis*. J. Ferment. Technol. 1987, 65: 603-606.

[10] Cadmus M.C., Knutson, C.A., Lagoda, A.A., Pittsley, J.E., and Burton, K.A. Synthetic media for production of quality xanthan gum in 20 litre fermentors. Biotechnol. Bioeng. 1978; 20: 1003-1014.

[11] Pons A., Dussap, C.G., and Gros, J.B. Xanthan batch fermentations: compared performance of a bubble column and stirred tank fermentor. Bioprocess Eng. 1990, 5: 107-114.

[12] García-Ochoa, F., Santos, V.E., and Alcon, A. Xanthan gum production in a laboratory aerated stirred tank bioreactor. Chem. Biochem. Eng. 1997, 11: 69-74.

[13] Suh I-S, Schumpe, A., Deckwer, W-D. Xanthan production in bubble column and air lift reactors. Biotechnol. Bioeng. 1992, 39: 85-94.

[14] Zaidi, A., Ghosh, P., Schumpe, A., and Deckwer, D-W. Xanthan production in a plunging jet reactor. Appl. Microbiol. Biotechnol. 1991, 35: 330-333.

[15] Thonart, Ph, Paquot, M., Hermans, L., Alaoui, H., d'Ippolito, P. Xanthan production by *Xanthomonas campestris* NRRL B-1459 and interfacial approach by zeta potential measurement. Enzyme Microbiol. Technol. 1985;7:235-8.

Table1. Maximum values of biomass and xanthan concentrations, substrate utilization, specific growth rates and xanthan production during growth of *Xanthomonas campestris* TISTR 1100 in the coconut juice with the agitation rate of 300, 500, 700 rpm at 33 ^{0}C.

Agitation Rate (rpm)	Biomass Conc. (g/L)	Xanthan Conc. (g/L)	Substrate Utilization (g/L)	Growth Kinetic Parameters											
				r_x[a]		μ[b]		r_p[c]		q_p[d]		r_s[e]		q_s[f]	
				Max.	period (h)	Max.	period (h)	Max.	period (h)	Max.	period (h)	Max.	period (h)	Max.	period (h)
300	**1.04**	**4.12**	**23.26**	**0.025**	**33**	**0.133**	**1**	**0.187**	**28**	**0.380**	**26**	**1.403**	**8**	**11.800**	**1**
500	**2.40**	**11.24**	**26.61**	**0.063**	**28**	**0.173**	**1**	**0.245**	**33**	**0.318**	**11**	**2.400**	**3**	**8.889**	**1**
700	**2.91**	**17.10**	**23.29**	**0.109**	**23**	**0.178**	**1**	**0.403**	**13**	**0.883**	**1**	**1.828**	**3**	**6.407**	**1**

[a]r_x = Growth rate (g/L/h); [b]μ = 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 concentrations, substrate utilization, specific growth rates and xanthan production during growth of *Xanthomonas campestris* in the coconut juice with the ranging of temperature at 30, 33 and 35 ^{0}C with the agitation rate of 700 rpm.

Temperature (^{0}C)	Biomass Conc. (g/L)	Xanthan Conc. (g/L)	Substrate Utilization (g/L)	Growth Kinetic Parameters											
				r_x[a]		μ[b]		r_p[c]		q_p[d]		r_s[e]		q_s[f]	
				Max.	period (h)	Max.	period (h)	Max.	period (h)	Max.	period (h)	Max.	period (h)	Max.	period (h)
30	**2.80**	**8.80**	**22.41**	**0.113**	**28**	**0.224**	**1**	**0.272**	**18**	**0.447**	**1**	**1.287**	**8**	**5.842**	**1**
33	**2.91**	**17.10**	**22.29**	**0.109**	**23**	**0.178**	**1**	**0.403**	**13**	**0.883**	**1**	**1.828**	**3**	**6.407**	**1**
35	**1.30**	**9.48**	**22.14**	**0.050**	**28**	**0.154**	**1**	**0.292**	**23**	**0.883**	**11**	**1.419**	**3**	**10.913**	**1**

[a]r_x = Growth rate (g/L/h); [b]μ = 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)