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KINETIC MODELS FOR XANTHAN GUM PRODUCTION USING *Xanthomonas campestris* FROM MOLASSES

The effects of media temperature, agitation rate and molasses concentration on the yield of fermentation in xanthan gum production process were investigated. Xanthan gum was produced in batch fermentation by Xanthomonas campestris PTCC 1473 from molasses. At 32 °C, 500 rpm and media with 30 g/l of total sugar, maximum production of xanthan gum (17.1 g/l) was achieved. For the purity of the xanthan FTIR spectrum was obtained. The identified spectrum was compared with the commercial product. In batch culture, several kinetic models for the biochemical reactions were extensively studied. The growth kinetic parameters were evaluated by unstructured model and derived from the related equations. Based on Malthus and Logistic rate equations, the maximum specific growth rate, μ_{max} , and initial cell dry weight, X_0 , were defined. Luedeking-Piret and Modified Luedeking-Piret models were applied for the product formation and substrate consumption rates. In batch experiments, the kinetic parameters for the growth associated (m , a) and non-growth associated (n , b) parameters were determined.

Key words: xanthan gum; molasses; Xanthomonas campestris; growth rate; kinetic model.

Xanthan gum is an industrial biopolymer with wide applications, produced by *Xanthomonas campestris*. The natural sources of the polysaccharide originated from a cabbage plant bacteria, *Xanthomonas species*. Xanthan gum is a hetero-polysaccharide with repeated pentasaccharide units consisting of two molecular structures of glucose, mannose and one unit of glucuronic acid. The presence of organic acids in xanthan gum structure produces an anionic polysaccharide type. Interaction of xanthan gum with other polymer molecules may generate a complex structure of biopolymer [1-3].

Xanthan gum has unique physical properties with number of industrial applications (food industries, cosmetics and petroleum industries) such as emulsifiers and syrup thickeners. Xanthan gum is often used in food industries in small amount for economical reasons as food additives and stabilizers. Even use of xanthan at low concentration in food products gives highly viscous solution [1,4-7].

In batch production of xanthan gum and often for large-scale production glucose is considered as a suitable substrate with high production yield [2,8,9]. However, the increasing market price and demand suggest that glucose may no longer be economically feasible as a raw material. In order to reduce the costs of raw material for xanthan gum production low costs of carbon sources were recommended. There is a number of research studies devoted to identifying a suitable substrate with low costs [10-12]. Molasses is a by-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. Use and bioconversion of molasses to value added biological products such as xanthan gum is also reported in literature [13]. Several research studies have been conducted on xanthan production using low costs carbon sources such as whey, waste sugar beet pulp, citrus waste pulp and olive-mill wastes [11-14].

Growth kinetic models are important in projection of substrate utilization and biomass production. Several models have been suggested to estimate the concentration of xanthan in fermentation broth [15]. Malthus, logistic, Luedeking-Piret and modified Luedeking-Piret kinetic models have been discussed for sub-

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strate utilization, cell growth rate and xanthan gum production [9].

In the present research paper, batch xanthan gum production at optimal operating conditions from sugar beet molasses was performed. In addition, mathematical models were proposed for kinetics of xanthan gum production, substrate consumption rate and cell growth rate. In order to obtain suitable kinetic parameters, the substrate concentrations were varied from 20 to 40 g/l. The obtained data were correlated in the projected models.

MATERIALS AND METHODS

Microorganism and inoculums preparation

Xanthomonas campestris PTCC 1473 was supplied by Iranian Research Organization for Science and Technology (IROST), Tehran, Iran. The microorganism was grown on agar medium containing (g/l): yeast extract 5.0, peptone 5.0, glucose 10.0, and agar 20.0 (Merck, Germany). The propagated cells for the inoculums were grown in a medium (the stated compositions excluding agar) with neutral pH in 500 ml flasks with 100 ml of culture medium in an incubator shaker (Stuart, S1500 series, USA) for 24 h. The incubator shaker was set at 200 rpm and 28 °C to enhance oxygen mass transfer rate into the media. Thus, maximum cell growth was obtained and the revived fresh inoculum of the bacteria was used for each experimental run. The stock culture was stored on slant agar was maintained at 4 °C and the subcultures were revived for every two weeks to avoid strain degradation.

Preparation of molasses

Initially, the concentrated sugar beet molasses were diluted with distilled water (300 g thick molasses were diluted to one liter). Dilute acid hydrolysis was carried out to enhance the dextrin and monomeric carbohydrate contents of molasses. Concentrated HCl (2.5 ml) was added to one liter of the diluted molasses. The pretreatment was prolonged for 24 h. The pretreated molasses were autoclaved at 121 °C for 15 min, neutralized and the reduced sugar was measured using DNS in colormetric method [16].

Xanthan gum production

The production of xanthan gum was carried out in 1000 ml Erlenmeyer flasks with 300 ml of medium. That containing (g/l): glucose 30.0, yeast extract 5.0 and additive solution 3.0 ml (consist of: KH_2PO_4 (3 g/l), MgCl_2 (0.6 g/l), Na_2SO_4 (0.1 g/l), H_3BO_3 (0.006 g/l), ZnO (0.006 g/l), $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (0.02 g/l), CaCO_3 (0.02 g/l)). The glucose was obtained from hydrolyzed and pretreated molasses.

Batch experiments were conducted for xanthan gum production with variation of temperature in the range of 25–36 °C. The glucose concentration and agitation rate were 30 g/l and 450 rpm, respectively. The fermentations were commenced with inoculums size of 5% (v/v), experiments were conducted at five different agitation rates (200, 300, 400, 500 and 800 rpm) on a magnetic hot-plate stirrer (VELP, Italy). In a medium with glucose concentration of 30 g/l, the desired agitation rate and optimal temperature were 500 rpm and 32 °C, respectively. Runs were terminated after 72 h of incubation. The pH was initially neutral and was not controlled by any titrants throughout the runs. All experimental runs were replicated and averaged values are reported in this work. Error analysis was conducted to obtain standard deviation for the collected data. The standard deviations for the reported data representing deviations from the mean values of pH, product yield, agitation rate, temperature and viscosity were 1, 3, 5, 6 and 9%, respectively.

Analytical methods

For determining the surplus sugar of medium during fermentation, DNS method was implemented according to standard method. The total carbon of medium was determined by phenol-sulfuric acid method in according to standard methods [17,18].

During fermentation, for evaluating the xanthan gum, it was recovered by centrifuging at (10000 g) for 30 min at 4 °C in order to sediment the cells. Xanthan gum in the supernatant was precipitated using ethanol, methanol, or acetone (1:3, v/v) (Fulka, Germany). The solution was maintained at 5 °C for 24 h and re-centrifuged at 10000 g, for 30 min at 4 °C. The precipitate was diluted in distilled water and dried at 50 °C in a conventional oven (Binder, Germany) until constant weight, to determine the xanthan gum content. Cell dry weight was also determined using a cellulosic filter, 25 mm diameter and 0.25 pore sizes (Wathman, USA), then the filter was dried in an oven at 80 °C for 24 h and weighed [19].

The medium pH and viscosity were measured by pH meter, Hanna, model 21 (Italy) and Siemens glassy viscometer at 40 °C, respectively. FTIR spectra were obtained. The dried powder of xanthan was analyzed with Fourier transform infrared (FTIR, Bruker, Model Vector 22, Germany) to define the functional group of synthesized xanthan gum. The dry sample powder was mixed with KBr and pressed into pellets under reduced pressure. The FTIR spectra were obtained by scanning between 4000 and 500 cm^{-1} .

RESULTS AND DISCUSSION

In this study, for production of xanthan gum, *X. campestris* was grown on diluted and pretreated molasses with total reduced sugar concentration of 30 g/l. Several process variables such as temperature, agitation rate and substrate concentrations for xanthan gum productions were investigated. The media pH, viscosity of the product in broth and the product yield were monitored. The inoculated culture of *X. campestris* was incubated within the temperature range of 25–36 °C since the organism was known as mesophile. Figure 1 shows the effect of incubated culture temperature on xanthan gum production. The optimum temperature for maximum growth was obtained, since *X. campestris* showed the desired biological activities for xanthan production. Effect temperature on xanthan production has been investigated by others; the optimum reported temperature was in the range 30–33 °C [15,20,21]. Maximum xanthan gum production was obtained at 32 °C.

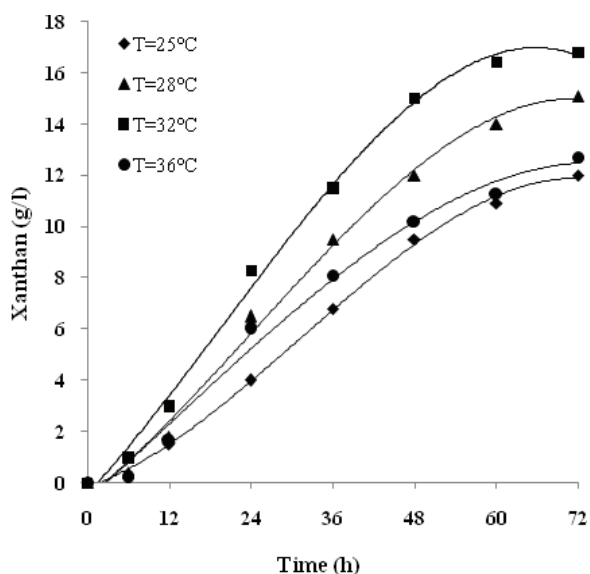


Figure 1. Effect of fermentation temperature on xanthan gum production (450 rpm, S: 30 g/l).

Xanthan production with respect to agitation rate of 200 to 800 rpm was investigated. Figure 2 depicts agitation rates that may enhance mass transfer coefficients, as the xanthan concentration increased with agitation rate up to 500rpm. Maximum xanthan production (17.1 g/l) was obtained for incubation time of 72 h. As the agitation increased to 800 rpm, mass transfer limitation occurred while there was limited soluble oxygen available to the living cell. This means the rate oxygen diffusion was also limited. Therefore very high shear rate did not improve xanthan production. Similar

results were reported by other researchers; they have found the suitable agitation rate was 500 rpm. It was noticed that at the same agitation rate with the same organism, yield of xanthan with molasses (30 g/l) was about 7% higher than the xanthan produced with 40 g/l of sucrose [6].

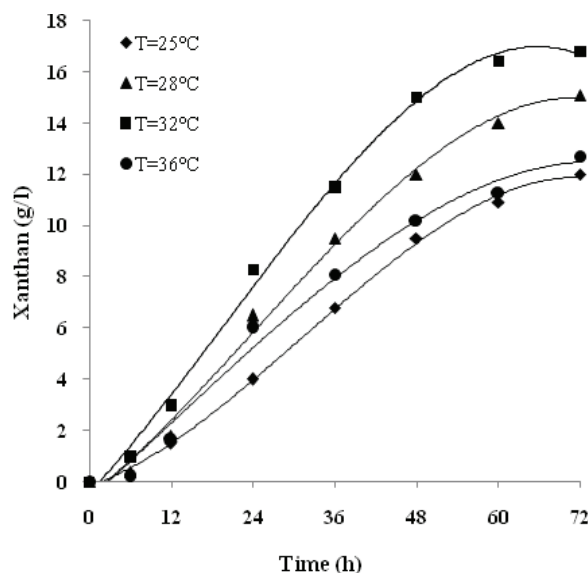


Figure 2. Effect of agitation rates on xanthan gum production (S: 30 g/l, 32 °C).

Figure 3 represents the ratio of xanthan to cells with respect to incubation time. The value for xanthan/cells ratio nearly reached a stable condition after 24 h of incubation. The substrate concentrations were in the range of 20 to 40 g/l for the conducted experiments. An optimum value for substrate was 30 g/l while the maximum value for the ratio was obtained. The yield of fermentation at optimum temperature and agitation rate with respect to time was calculated with several glucose concentrations, the yield is stated in the following equation [17]:

$$Y\left(\frac{P}{S}\right) = -\frac{\Delta P}{\Delta S} \quad (1)$$

Maximum yield of 0.57 g xanthan/g reduced sugar was obtained with 30 g/l of substrate concentration.

The culture pH was just monitored without use of any controlling unit. In the early stage of fermentation the value for pH was gradually in decreasing trend while in the second day of incubation the pH value was sharply decreased. This is due to formation of organic acids build-up in the system. The organic acids may cause negative impact on xanthan production; therefore use pH controller is strongly recommended. Figure 4 illustrates the value of pH for the whole fermentation period.

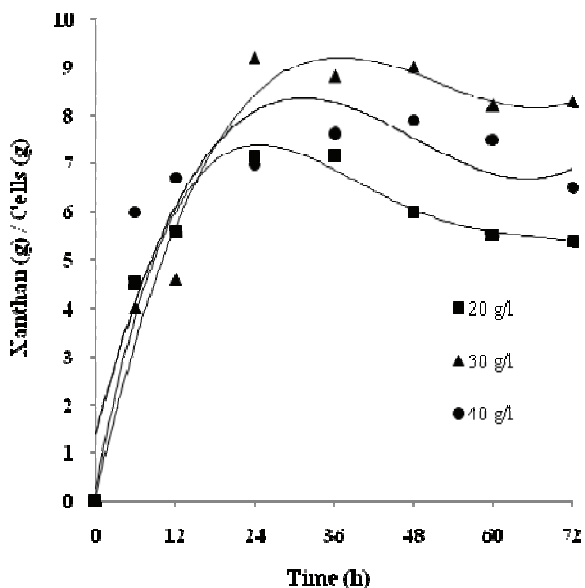


Figure 3. Change of the ratio of xanthan gum and cells with time at 20 (■), 30 (▲) and 40 (●) g/l glucose concentration (500 rpm, 32 °C).

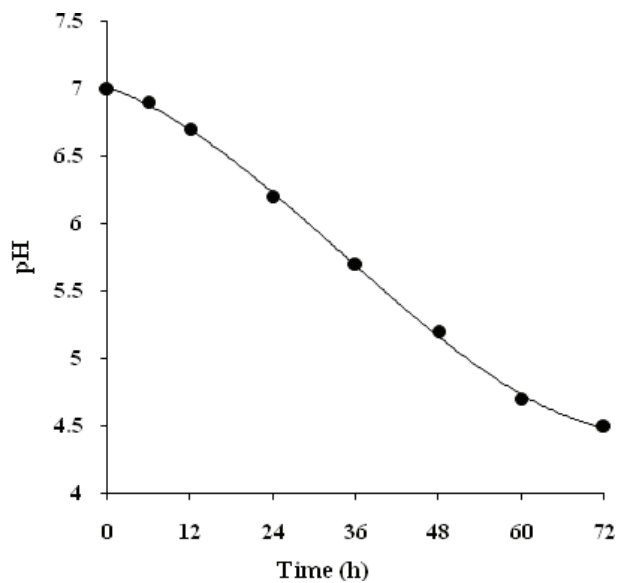


Figure 4. pH changes of broth during fermentation at the optimum condition.

Figure 5 demonstrates the apparent viscosity of the fermentation broth with respect to incubation time. For the optimal substrate concentration (30 g/l) and agitation rate (500 rpm) the broth viscosity was measured for the entire period of fermentation. The broth apparent viscosity was progressively increased. The starting value for the viscosity at inoculation time was nearly 1 cP, then reached to 160 cP. At the third day of incubation, maximum value for viscosity was obtained.

Figure 6 depicts the values of xanthan gum concentration, cell dry weight and glucose consumption

versus time. Data was obtained at the optimum conditions. At 72 h incubation time, the highest amount of xanthan gum and cell dry-weight were defined to be 17.1 and 2.5 g/l, respectively. It was reported that, with 30 g/l reduced sugar concentration from sugarcane molasses 15.5 g/l xanthan was produced [22]. Additional xanthan concentration (1.6 g/l) in this work was most probably due to use of the pretreated molasses.

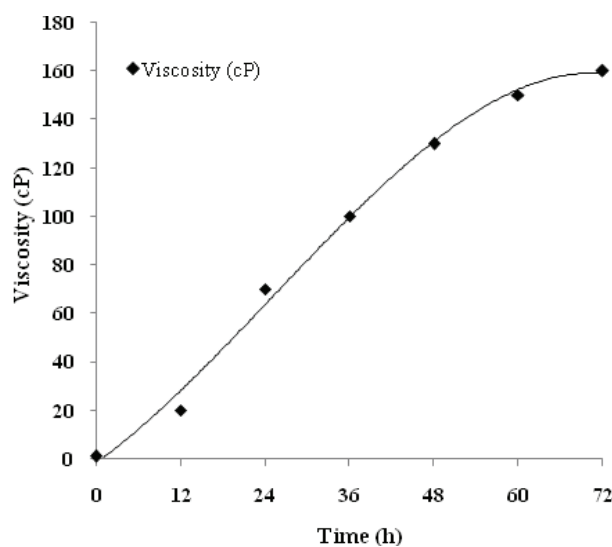


Figure 5. Apparent viscosity of the broth during fermentation at the optimum condition.

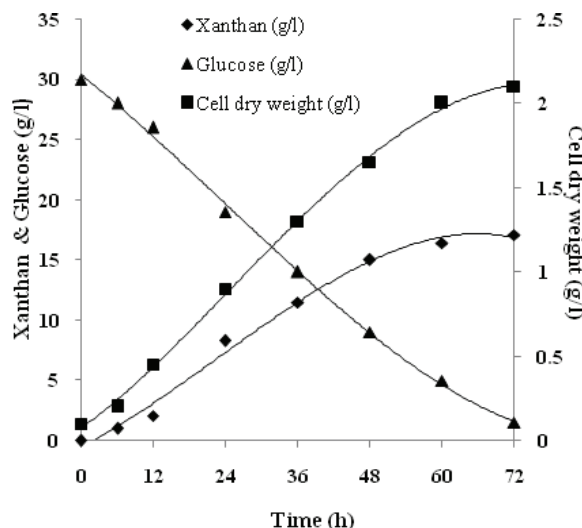


Figure 6. Xanthan gum (◆), cell dry-weight production (■) and amount of glucose utilization (▲) at the optimum condition.

Unstructured kinetic model

Theoretically, cell growth rate is expressed in Eq. (2). Malthus equation represents the exponential growth in a batch culture as follows [9]:

$$\frac{dX}{dt} = \mu X \quad (2)$$

where X is the cell dry weight concentration (g/l), μ is the specific growth rate (h^{-1}) and t (h) is related to incubation.

Another projected model that explains growth kinetics is known as the Logistic equation, which is a quite fair kinetic equation to predict the growth rate. Also, the specific growth rate was predicted by the Malthus equation as expressed by the following equation (3):

$$\mu = \mu_{\max} \left(1 - \frac{X}{X_{\max}} \right) \quad (3)$$

where X_{\max} is the maximum cell dry weight concentration (g/l) and μ_{\max} is the maximum specific growth rate (h^{-1}). Substituting Eq. (3) into Eq. (2) followed by integration, resulted in the following equation for the cell concentration [15]:

$$X(t) = \frac{X_0 \exp(\mu t)}{1 - \left(\frac{X_0}{X_{\max}} \right) (1 - \exp(\mu t))} \quad (4)$$

where X_0 is the initial cell concentration after inoculation (g/l). The above equation was used to predict the cell growth in batch experiments. Finally, by the use of simple algebraic manipulations, Eq. (4) can be written as follows:

$$\ln \left(\frac{X_{\max}}{X_0} - 1 \right) + \ln \left(\frac{X(t)}{X_{\max} - X(t)} - 1 \right) = \mu_{\max} t \quad (5)$$

Figure 7 shows the plot of the logistic equation, which was linearly fitted with experimental data. Maximum specific growth rate was obtained from the slope of the drawn line from the illustrated plot.

Also, Luedeking-Piret and modified Luedeking-Piret equations were used to describe product formation rate and substrate consumption rate, respectively [9]. Luedeking-Piret kinetic expression describes growth and non-growth associated model. The relation is given by Eq. (6) as follows:

$$\frac{dP}{dt} = m \frac{dX}{dt} + nX \quad (6)$$

where the parameters m and n are considered as growth associated and non-growth associated constants, respectively. The values of m and n are empirical constants that may vary with fermentation conditions such as temperature, pH and agitation rate. Integration of Eq. (6) with initial condition at $t = 0$, $P = P_0$, by incorporating Eq. (4) into Eq. (6) yields:

$$P(t) = P_0 + m [X(t) - X_0] + n (X_{\max} / \mu) \ln [1 - (X_0 / X_{\max}) (1 - \exp(\mu t))] \quad (7)$$

In addition, after rearrangement the resulted equation is as follows:

$$\frac{P(t) - P_0 - n (X_{\max} / \mu) \ln [1 - (X_0 / X_{\max}) (1 - \exp(\mu t))]}{m [X(t) - X_0]} = \quad (8)$$

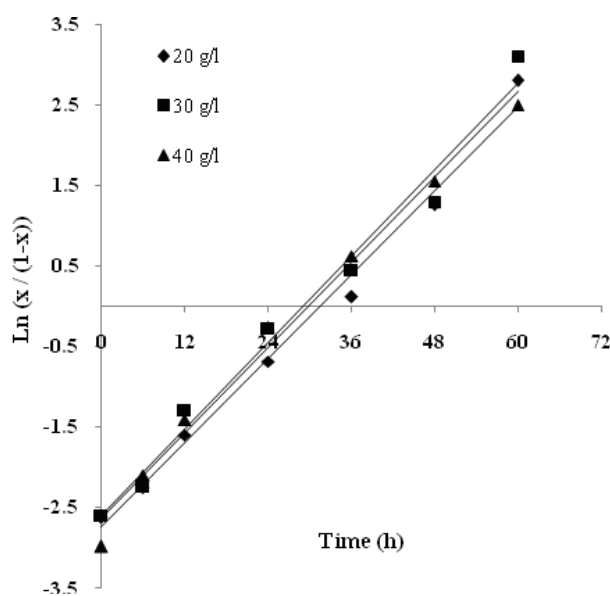


Figure 7. Linear plot of Eq. (5) and experimental data with logistic model at 20 (♦), 30 (■) and 40 (▲) g/l substrate concentrations.

Since n is non-growth associated constant, it is not dependent on growth phase therefore it should be evaluated from the stationary-phase data, where $dX/dt = 0$, as given below:

$$n = \frac{\left(\frac{dP}{dt} \right)_{\text{stationary phase}}}{X_{\max}} \quad (9)$$

It was understood that at the beginning of the biochemical reaction there was absolutely no xanthan gum in the media at initial phase, which means P_0 was equal to zero. In order to determine the value of m a plot of left-hand side of Eq. (8) versus $[X(t) - X_0]$ was drawn and from the slope of the line, the value of m was obtained.

Modified Luedeking-Piret equation predicts substrate with respect to growth. The model also included two parameters, a and b . In this model similar to Luedeking-Piret the two terms are considered as growth associated and non-growth associated constants, respectively. The modified define equation is given as follows:

$$-\frac{dS}{dt} = bX + a \frac{dX}{dt} \quad (10)$$

Similarly, integration of Eq. (10) with initial condition at $t = 0$, $S = S_0$ implementing Eq. (4) into equation (10), resulted in the following equation:

$$S_0 - S(t) = a[X(t) - X_0] + b(X_{\max} / \mu) \ln[1 - (X_0 / X_{\max})(1 - \exp(\mu t))] \quad (11)$$

with rearrangement of the above equation yields:

$$\frac{S_0 - S(t) - b(X_{\max} / \mu) \ln[1 - (X_0 / X_{\max})(1 - \exp(\mu t))]}{a} = X(t) - X_0 \quad (12)$$

The coefficient b is the non-growth associated constant and does not dependent on cell growth phase, which was evaluated from the stationary-phase data, where $dX/dt = 0$; the coefficient is given as follows:

$$b = \frac{-\left(\frac{dS}{dt}\right)_{\text{stationary phase}}}{X_{\max}} \quad (13)$$

The plot of left-hand side of Eq. (12) *versus* $[X(t) - X_0]$ was also drawn where the value of a , was slope of the line. Figure 8 depicts the plotted models fitted with experimental. The modified Luedeking-Piret shows the bioconversion of substrate to xanthan gum product.

In order to consider the effect of substrate concentration changes on the growth parameters as mentioned above the fermentation was performed using 20, 30 and 40 g/l glucose in batch culture. Other process parameters kept at same conditions (32 °C, 500

rpm). The obtained results from these operations were summarized in Table 1.

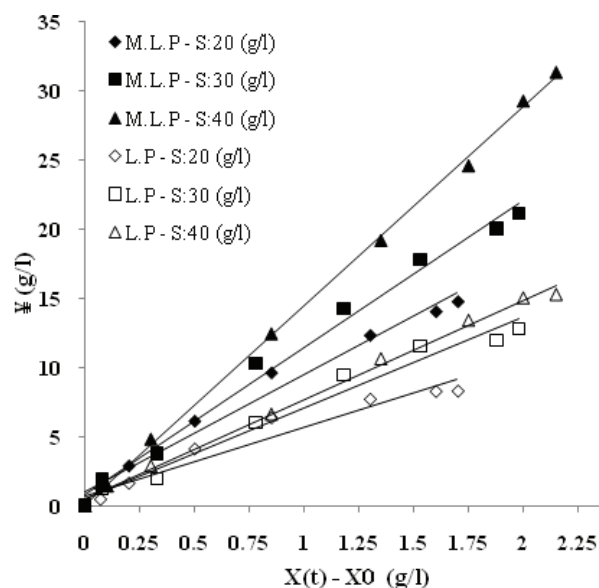


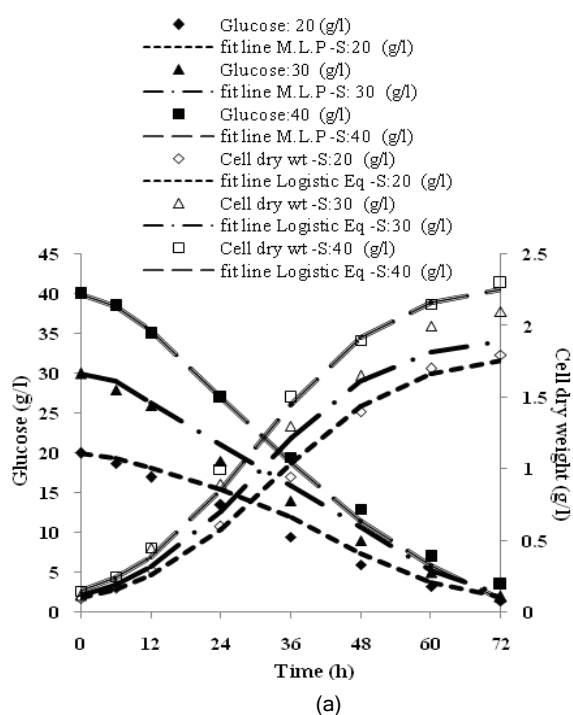
Figure 8. Linear plots of Eqs. (8) and (12) and experimental data with Luedeking-Piret (LP) and modified Luedeking-Piret (MLP) at 20 (◆,◇), 30 (■,□) and 40 (▲,△) g/l substrate concentrations.

It was noticed that the growth associated constants, m and a , were higher than the non-growth associated coefficients. The magnitude of parameter a increases as glucose concentration increases. Similarly, the values of b increased from 20 to 40 g/l glucose concentration where the ratio of glucose utilization depends mainly on the cell concentration in the stated range. The values of parameter m also in-

TABLE 1. Kinetic parameters calculated by: modified Luedeking-Piret and Luedeking-Piret equations and logistic model (a , g glucose/g cells, b , g glucose/(g cells h), n , g xanthan/(g cells h), m , g xanthan/g cells, μ_{\max} , h^{-1} , X_0 , g cells/l, X_{\max} , g cells/l)

Kinetic parameters	Glucose concentration, g/l		
	20	30	40
Modified Luedeking-Piret equation			
b (gS/gX)	0.052	0.079	0.090
a (gS/gX h)	8.45	10.60	14.38
R^2	0.980	0.990	0.999
Luedeking-Piret equation			
n (gP/gX h)	0.034	0.041	0.036
m (gP/gX)	4.98	6.55	7.15
R^2	0.943	0.975	0.995
Logistic model			
μ_{\max} / h^{-1}	0.087	0.090	0.089
X_0 / g l ⁻¹	0.10	0.12	0.15
R^2	0.989	0.985	0.993
X_{\max} / g l ⁻¹	1.8	2.1	2.3

creased at high glucose concentration levels and they are always larger than the values of n . At the time of maximum cell growth rate (exponential phase), glucose utilization was higher than stationary phase. The above calculated values lead us to the conclusion that glucose consumption depends mainly on the rate of cell growth and is higher in exponential than in stationary phase. The values of growth associated parameters reveal that they are largely affected by the rate of xanthan gum production and glucose consumption. The values of μ_{\max} were nearly constant in all experiments when the growth were kept at the same conditions.



Figures 9a and 9b present the substrate, cell dry weight and product concentration profiles for 20, 30 and 40 g/l of glucose. The trends for substrate consumption and product formation for all substrate concentrations are about the same. For substrate concentration of 30 g/l, maximum xanthan gum production was obtained. The obtained data were fitted to linear plot of logistic equation, Luedeking-Piret and modified Luedeking-Piret models.

FTIR spectra of xanthan gum

The FTIR spectra of synthesized xanthan gum for identification functional groups were obtained (Figure 10). The x-axis represents wavelength (cm^{-1})

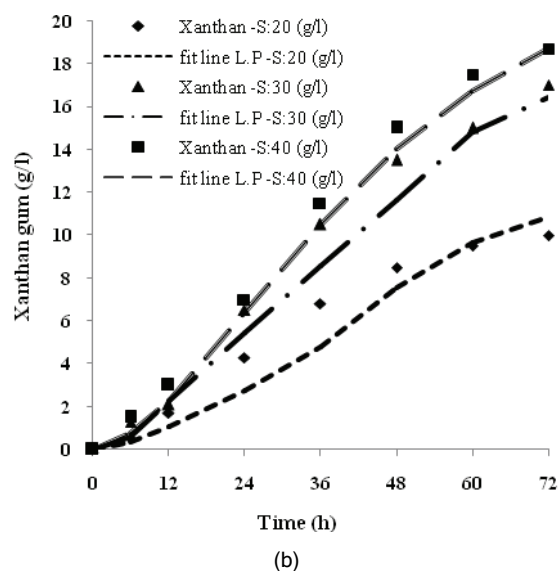


Figure 9. a) Logistic and modified Luedeking-Piret (MLP) model and b) Luedeking-Piret (LP) model fitted with experimental data at 20 (◆), 30 (▲) and 40 (■) g/l substrate concentrations.

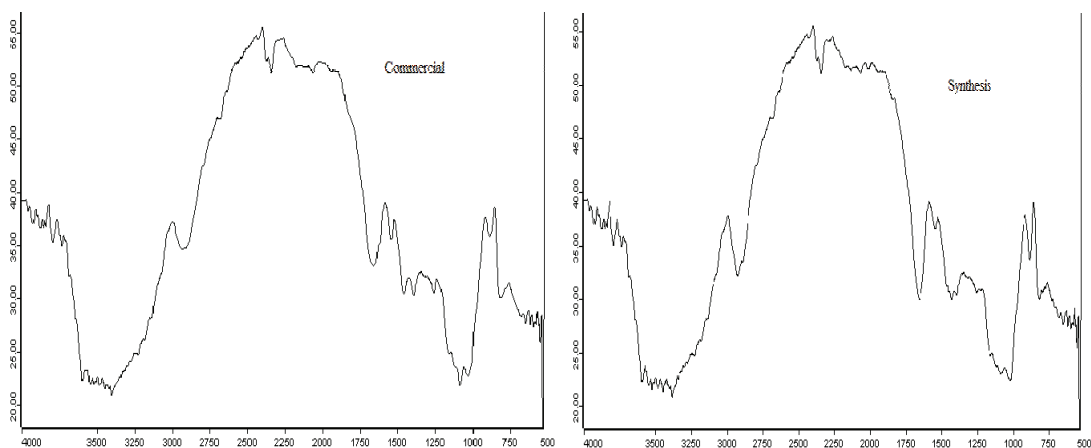


Figure 10. FTIR spectra of commercial and synthesized xanthan gum.

and y-axis shows the light transmittance through the sample. The FTIR spectrum of the sample shows the carboxyl, carbonyl, acetal, and the other groups that carboxyl, carbonyl, and acetal groups are very important owing to these groups were formed during the production of xanthan gum from glucose. Xanthan gum with high acetal content was easily soluble in water, which was due to high hydrophilic properties of the produced xanthan. The produced xanthan had slightly higher proportion of acetal group than the commercial product. The spectra of functional groups are summarized in Table 2. These results indicate the accommodating between synthesized and commercial xanthan gum.

Table 2. FTIR spectral data for standard and sample product

Functional group	Hydroxyl	Carbonyl	Carboxyl	Acetal
Standard	3386	1627	1529	1160
Product	3386	1630	1535	1167

CONCLUSION

Xanthan gum samples were produced from molasses by *X. campestris* and the bioproduct functional groups were compared with the commercial xanthan by FTIR spectra. The results show that maximum amounts of xanthan gum, biomass, and maximum yield of xanthan gum were produced from 30 g/l glucose at optimum fermentation temperature 32 °C and agitation rate of 500 rpm.

The growth kinetic of *X. campestris* was investigated. The growth kinetic parameters were evaluated by unstructured model and derivative equations. These parameters such as maximum specific growth rate, initial cell dry weight, growth associated (m , a) and non-growth associated (n , b) were obtained. The results showed that all of the growth parameters almost increased with the increase of substrate concentration. The obtained data demonstrated that the xanthan biopolymer was growth related; this means the product and cell concentration profiles had almost similar increasing trends.

Acknowledgments

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Nomenclature

a [g glucose/g cells] - growth-associated constant in substrate consumption rate

b [g glucose/(g cells h)] - Non-growth-associated constant in substrate consumption rate

m [g xanthan/g cells] - growth-associated constant in product formation rate

n [g xanthan/(g cells h)] - Non-growth-associated constant in product formation rate

P [g/l] - concentration of product

P_0 [g/l] - initial concentration of product

S [g/l] - concentration of substrate

S_0 [g/l] - initial concentration of substrate

t [h] - Time

T [°C] - Temperature

$Y_{p/s}$ [g xanthan/g glucose] - yield coefficient

X [g/l] - biomass concentration

X_0 [g/l] - initial biomass concentration

X_{max} [g/l] - maximum biomass concentration at stationary phase.

Greek symbols

μ_{max} [h⁻¹] - specific growth rate.

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NAUČNI RAD

KINETIČKI MODELI ZA PRODUKCIJU KSANTANA POMOĆU *Xanthomonas campestris* IZ MELASE

U radu je ispitivan uticaj temperature, brzine mešanja i koncentracije melase na prinos ksantana u šaržnom procesu pomoću Xanthomonas campestris PTCC 1473. Maksimalni prinos ksantana od 17,1 g/l je postignut na 32 °C, 500 min⁻¹ i 30 g/l ukupnih šećera. Čistoća ksantana je ocenjena na osnovu FTIR spektra, koji je upoređen sa spektrom komercijalnog proizvoda. Nekoliko kinetičkih modela za bioemijske procese pri šaržnom gajenju je široko proučavano. Parametri kinetike mikrobnog rasta su određeni iz jednačina nestrukturiranog modela. Maksimalna specifična brzina rasta, μ_{max} , i početna suva biomasa, X_0 , su definisane na osnovu Malthus-ove i logističke jednačine brzine rasta. Brzine sinteze proizvoda i potrošnje supstrata definisane se pomoću Luedeking-Piret-ovog i modifikovanog Luedeking-Piret-ovog modela. Određeni su kinetički parametri za sintezu proizvoda zavisne (m, a) i nezavisne (n, b) od mikrobnog rasta.

Ključne reči: ksantan; melasa; Xanthomonas campestris; brzina rasta; kinetički model.