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A Comparison Between Shaker and Bioreactor Performance Based on the Kinetic Parameters of Xanthan Gum Production

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Abstract Xanthan gum production was studied using sugarcane broth as the raw material and batch fermentation by *Xanthomonas campestris* pv. *campestris* NRRL B-1459. The purpose of this study was to optimize the variables of sucrose, yeast extract, and ammonium nitrate concentrations and to determine the kinetic parameters of this bioreaction under optimized conditions. The effects of yeast extract and ammonium nitrate concentrations for a given sucrose concentration (12.1–37.8 g L^{-1}) were evaluated by central composite design to maximize the conversion efficiency. In a bioreactor, the maximum conversion efficiency was achieved using 27.0 g L^{-1} sucrose, 2.7 g L^{-1} yeast extract, and 0.9 g L^{-1} NH_4NO_3. This point was assayed in a shaker and in a bioreactor to compare bioreaction parameters. These parameters were estimated by the unstructured kinetic model of Weiss and Ollis (Biotechnol Bioeng 22:859–873, 1980) to determinate the yields ($Y_{P/S}$), the maximum growth specific rate (μ_{max}), and the saturation cellular concentration (X^*). The parameters of the model (μ_{max}, X^*, m, λ, α, and β) were obtained by nonlinear regression. For production of xanthan gum in a shaker, the values of μ_{max} and $Y_{P/S}$ obtained were 0.119 h^{-1} and 0.34 g g^{-1}, respectively, while in a bioreactor, they were 0.411 h^{-1} and 0.63 g g^{-1}, respectively.

Keywords Fermentation · Optimization · Xanthan gum · Kinetic · Sugarcane broth

Introduction

The most recent gums introduced to the market have been produced by microorganisms. These polysaccharide-based gums are of great industrial interest and possess properties that in some cases are superior to vegetable-, algae-, and synthetic-based gums. Xanthan gum is

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a natural polysaccharide and an important industrial biopolymer, is classified as a ramified, anionic hetero-exopolysaccharide, and is produced by fermentation using the bacterium *Xanthomonas*. Among the biogums, xanthan has been extensively studied due to its uncommon rheological properties, such as high pseudoplasticity, high viscosity even at low concentrations, compatibility and stability with most metallic salts, excellent solubility and stability in both acidic and basic solutions, and resistance to degradation at high temperatures and pH oscillations. This gum exhibits several advantages as a thickener, stabilizer of emulsions and suspensions, and dispersing agent. It is used in pharmaceutical formulations, cosmetics, textile printing pastes, ceramic glazes, slurry explosive formulations, rust removers, and agricultural products [1–3]. High viscosity of solutions and water solubility of the polymer have created important applications for xanthan in the petroleum industry, where it is commonly used in drilling fluids and in enhanced oil recovery processes [3].

The main producers of xanthan are Merck, Kelco, and Pfizer in the USA; Rhône Poulenc, Mero-Rousselot-Santia, and Sanofi-Elf in France; Saidy Chemical in China; and Jungbunzlauer in Austria [3, 4]. World production of xanthan gum is 30,000 tons/year [4, 5], while its consumption in the USA has an annual growth rate between 5% and 10% [6]. Xanthan has an actual market of approximately US $270 million and is expected to reach US $400 million with a production of 80,000 tons/year in 2015 [4].

Xanthan production is usually modeled by unstructured models as described by Moraine and Rogovin [7], De Vuyst et al. [8], and Pons et al. [9]. These authors do not consider in their equations all the essential nutrients of the system. For example, nitrogen and oxygen have been omitted. García-Ochoa et al. [10] and Letisse et al. [11] have proposed and applied unstructured kinetic models describing biomass, carbon source, nitrogen source, and dissolved oxygen evolution. The unstructured kinetic models of Cadmus et al. [12], De Vuyst et al. [8], Margaritis and Zajic [13], Moraine and Rogovin [7], Pinches and Pallent [14], and Souw and Demain [15], which have been proposed for the modeling of xanthan gum production, can be classified into two groups: (1) models that consider growth and production to be dependent upon medium nutrients and (2) models that express growth and production only as a function of temporal changes in biomass.

As reported by García-Ochoa et al. [10], xanthan production is influenced by several factors, such as the type of reactor used, the mode of operation employed—batch and continuous—the medium composition, and the operational conditions. Although most authors have used a stirred-tank reactor with batch fermentation operation, there is no uniformity with regard to the operational conditions employed. Therefore, in our study, the xanthan production was optimized by means of central composite design aiming at reduction of the process costs. Industrially, the xanthan gum production using starch–maize hydrolysis to glucose has been reported by the literature but many studies with respect to replace this raw material has been carried out.

Sugarcane broth constitutes an interesting alternative to the conventional process and has the potential to emerge as a raw material to xanthan biosynthesis once that Brazil is a major producer worldwide of sugarcane, it is advantageous for researchers there to create new biotechnological processes. The high sucrose content and the presence of several mineral salts in the cane broth classify it as a promising production medium because it requires low supplementation. These aspects have been evaluated and it has been determined that the inexpensive cost of this raw material, compared to the raw materials commonly used, was decisive in the selection of the carbon source adopted in this study.

The purpose of this study was to optimize the process variables of sucrose, yeast extract, and ammonium nitrate concentrations. Response surface methodology was used for optimization, and the kinetic parameters (μ_{max}, X^*, m, λ, α, and β) regarding the

bioprocess, using either a shaker or a bioreactor, were obtained by applying the unstructured kinetic model.

Materials and Methods

Organism and Maintenance

Xanthomonas campestris pv. *campestris* NRRL B-1459 was supplied by the Tropical Collection Culture of the Fundação André Tosello, Brazil and was grown and maintained in yeast extract malt agar [16] with the following composition: 10.0 g L^{-1} glucose, 3.0 g L^{-1} yeast extract, 3.0 g L^{-1} malt extract, 5.0 g L^{-1} peptone, and 18.0 g L^{-1} agar. The pH of the medium was adjusted to 6.0 and the culture media was sterilized at 110 °C for 20 min. After 48 h of growth at 30±1 °C, the culture was maintained under sterile conditions at 5±1 °C.

Raw Material

The experiments were carried out using cane broth as the sucrose source. The cane was collected, milled, and the broth filtrate was stored at −5 °C during the experimental phase under aseptic conditions.

Optimization of Selected Variables Using Response Surface Methodology

Table 1 displays the levels and the variables selected for the optimization process. A central composite design, with 16 experiments, defined for the experimental matrix of the codified values of the variables under investigation was generated by Software Statistica 7.0. The response of interest that was monitored during the process was the relative yield to product formation $Y_{P/S}$ (g g^{-1}).

The established range of values for the variables X_1, X_2, and X_3 in the experimental design were attributed intervals of $-\alpha$ the $+\alpha$ as described in the literature [3, 11, 17–20]. The levels $-\alpha$ (−1.287 codified) corresponding to the yeast extract and ammonium nitrate concentrations were set at zero. These values were adopted to verify if such conditions would influence or not the system behavior.

Experiments: Comparison of Xanthan Gum Production in a Shaker and a Bioreactor

The inoculum contained 10% (*v*/*v*) cultivation medium and was agitated (150 rpm) in a shaker at 28±1 °C for 24 h. The medium used for preparation of the inoculum consisted of 20.0 g L^{-1} sucrose, 3.0 g L^{-1} yeast extract, 0.86 g L^{-1} NH_4NO_3, 2.5 g L^{-1} Na_2HPO_4, and

Table 1 Studied variables and their levels in the central composite design.

Variables	Variable levels				
	$-\alpha$ (1.287)	−1.0	0	+1.0	$+\alpha$ (1.287)
X_1	12.1	15.0	25.0	35.0	37.8
X_2	0.0	0.67	3.0	5.33	6.0
X_3	0.0	0.19	0.86	1.52	1.71

X_1 [sucrose (g L^{-1})]; X_2 [yeast extract (g L^{-1})]; X_3 [NH_4NO_3 (g L^{-1})]

2.5 g L^{-1} KH_2PO_4 [17, 20, 21]. The average cellular concentration of the inoculum was 0.29±0.05 g L^{-1}.

For optimization of the process variables, fermentations were carried out in a Biostat-B fermentor with a 5.0-L capacity, containing 4.0 L of production medium. The production medium corresponding to the central composite design consisted of sucrose (12.1, 15.0, 25.0, 35.0, or 37.8 g L^{-1}), yeast extract (0, 0.67, 3.0, 5.33, or 6.0 g L^{-1}), NH_4NO_3 (0, 0.19, 0.86, 1.52, or 1.71 g L^{-1}), Na_2HPO_4 (2.2 g L^{-1}), KH_2PO_4 (2.2 g L^{-1}), and antifoam (0.5 mL L^{-1}). During the process, the agitation speed was 800 rpm, the aeration was 0.5 vvm [20, 22], the temperature was 28±1 °C, and the process time was 24 h as preliminary tests. All experiments were confirmed in duplicate and pH was controlled by the addition of 1 N NaOH. The pH *set point* was maintained constant and equal to 7.2. To ensure that foreign microorganisms were not introduced into the fermentation mixture, the production medium (without the carbon source) was sterilized while in the fermentation vessel. The carbon source was sterilized separately and then aseptically introduced into the vessel. During the process, the concentrations of cells, sucrose, and xanthan gum were measured in the culture medium and microscopic observations using the Gram method were done in order to determine if contaminants might be present.

The fermentations carried out in a rotatory shaker were done in 500 mL Erlenmeyer flasks without pH control at 150 rpm and a process time of 60 h. A volume of 75.0 mL of culture medium was used in all tests with the following composition: sucrose (25.0 or 27.0 g L^{-1}), yeast extract (2.7 g L^{-1}), NH_4NO_3 (0.9 g L^{-1}), Na_2HPO_4 (2.2 g L^{-1}), and KH_2PO_4 (2.2 g L^{-1}). The temperature was maintained at 28±1 °C. The sterilization procedure was similar to that described using the bioreactor.

Analytical Determinations

Sucrose consumption, after acidic hydrolysis, was assayed using a glucose-oxidase (GPO-PAP) kit [23]. For biomass determination, the fermented broth samples were diluted 1:1 with NaCl solution (0.85% *w*/*v*) [18], and the cells were separated by centrifugation at 18,900×*g* for 40 min. The cells were then washed in a 0.85% saline solution to remove any remaining medium and metabolites. Cellular biomass was quantified by drying the washed cell mass at 90±1 °C until constant weight was achieved.

Recovery of Xanthan Gum

The fermented broth was diluted 1:1 with deionized water and centrifuged in a Beckman Coulter Avanti J-25 centrifuge at 18,900×*g* for 40 min to remove cells. The supernatant was filtered and treated with a saturated solution of KCl, and the polymer was recovered by precipitation with ethanol [24]. Finally, the product was dried under vacuum system at 30±1 °C.

Rheologic Behavior Evaluation of Xanthan Gum Solution

The polymeric rheology of a 1% (*w*/*v*) gum solution was determined by a Brookfield RVDVIII rheometer using shear stress data. This was measured from the shear rate, following the Ostwald Waele model or *power law* [25]. Then, the apparent viscosity for a fluid using the *power law* was determined by Eq. 2.1.

$$\mu_a = \frac{\tau}{\gamma} = K(\gamma)^{n-1}. \tag{2.1}$$

Modeling of Xanthan Gum Production: Identification of Parameters and Constants

The experimental values of the various treatment parameters (μ_{max}, X_{max}, m, λ, α, and β) were identified through the assays using different initial sucrose levels. Results were generated by nonlinear regression, using a multiresponse algorithm [26]. The integration of the set of differential equations forming the model was completed using a fourth-order Runge–Kutta algorithm [27]. The residues among the experimental and calculated model values were obtained by the minimization of the sum of squares of residues (SRS), as defined by Eq. 2.2.

$$\mathrm{SRS} = \sum_{i=1}^{N} \left(y_{\mathrm{exp}} - y_{\mathrm{theor}}\right)^2. \tag{2.2}$$

Kinetic Model

The unstructured kinetic model used in this investigation [28] expresses the growth rate only as a function of the biomass, by utilization of the Verhulst–Pearl equation (Eq. 2.3) and the Luedeking–Piret equations (Eqs. 2.4 and 2.5) [29].

$$\frac{dX}{dt} = \mu_{max} X \left(1 - \frac{X}{X_{max}}\right) \tag{2.3}$$

$$\begin{aligned} &\tfrac{\mathrm{d}P}{\mathrm{d}t} = m\,\tfrac{\mathrm{d}X}{\mathrm{d}t} + \lambda X \\ &\text{where } m = \tfrac{1}{Y_{X/P}} \end{aligned} \tag{2.4}$$

$$\begin{aligned} &-\tfrac{\mathrm{d}S}{\mathrm{d}t} = \alpha\,\tfrac{\mathrm{d}X}{\mathrm{d}t} + \beta X \\ &\text{where } \alpha = \tfrac{1}{Y_{X/S}} \end{aligned} \quad . \tag{2.5}$$

The parameters (μ_{max}, X_{max}, m, λ, α, and β) of this model were determined using a nonlinear regression, multiresponse technique applied to Eqs. 2.3, 2.4, and 2.5.

Results and Discussion

Experimental Design and Optimization by Response Surface Methodology

The yields of xanthan gum produced using variable sucrose, yeast extract, and ammonium nitrate concentrations (X_1, X_2, and X_3, respectively) are presented in Table 2. Analysis of all 16 experiments reveals that experiment 3 gave the smallest substrate to product conversion, while experiment 16 provided the highest conversion. Comparing these two experiments shows that when X_1 passed the level of −1.0 (15.0 g L^{-1}) to 0.0 (25.0 g L^{-1}), a marked gain in the conversion was produced. However, this increase was not solely due to cellular concentration as cellular growth was 2.44 g L^{-1} in experiment 3 and 2.66 g L^{-1} in

Table 2 Effect of the independent variables X_1, X_2, and X_3 in the $Y_{P/S}$ conversion.

Experiment	X_1	X_2	X_3	Biomass concentration (g L^{-1})	Xanthan concentration (g L^{-1})	$Y_{P/S}$ (g g^{-1})
1	15.0	0.67	0.19	1.77	3.10	0.218
2	15.0	0.67	1.52	1.84	3.30	0.223
3	15.0	5.33	0.19	2.44	2.70	0.180
4	15.0	5.33	1.52	2.24	3.00	0.200
5	35.0	0.67	0.19	2.18	13.0	0.403
6	35.0	0.67	1.52	2.35	13.4	0.414
7	35.0	5.33	0.19	2.24	12.4	0.374
8	35.0	5.33	1.52	2.30	12.7	0.397
9	12.1	3.0	0.86	1.45	3.00	0.247
10	37.8	3.0	0.86	2.31	14.1	0.409
11	25.0	0.0	0.86	2.13	13.5	0.540
12	25.0	6.0	0.86	2.63	11.9	0.476
13	25.0	3.0	0.0	2.37	10.5	0.420
14	25.0	3.0	1.71	2.79	13.9	0.556
15	25.0	3.0	0.86	2.57	15.2	0.608
16	25.0	3.0	0.86	2.66	15.8	0.632

X_1 [sucrose (g L^{-1})]; X_2 [yeast extract (g L^{-1})]; X_3 [NH_4NO_3 (g L^{-1})]

experiment 16. This indicates that the initial concentration of the carbon source may have an upper limit. Experiment 10 supported this hypothesis because increasing the sucrose concentration from 25.0 to 37.8 g L^{-1} did not generate a higher yield.

Determining the ideal concentration of yeast extract and nitrogen source was also important. One of the largest cost in the industrial production of xanthan gum is due to the yeast extract. In addition, yeast extract can become unviable. Therefore, the use of yeast extract is only justified if an economical gain in the product recovery is obtained, or it preserves some fundamental characteristic of high value to the product [30]. The concentration of nitrogen in the cane broth can oscillate and frequently nitrogen salts are added to the culture medium. Fermentation processes that use sugarcane broth as the raw material, in general, are less onerous and require low medium supplementation.

Experiments 11 and 13 showed the effects of omitting the yeast extract or ammonium nitrate, respectively. Moderate $Y_{P/S}$ values were obtained in both cases. However, when compared to experiments 15 and 16 (central point), it was verified that changing the yeast extract and ammonium nitrate concentrations from the codified level (−1.287 to 0.0) caused the yields to increase significantly.

Alves [17] reported that an optimum carbon/nitrogen relationship does not exist for all cases. However, for each initial substrate concentration used, there is a carbon/nitrogen relationship that is a consequence of an optimum nitrogen concentration contributing to the formation of biomolecules.

Experiments 15 and 16 were done with the values of the variables set at the central point (Table 2). The central point represents the best conditions obtained for the responses, xanthan gum concentration (15.8 g L^{-1}), $Y_{P/S}$ (0.632 g g^{-1}), and viscosity of the gum solution at 1% concentration (22,800.0 cP). Statistical analysis using the response surface technique confirmed that the maximum point was close to the central point. All 16 experiments were carried out in duplicate. For statistical analysis of the responses, the

parameters with significance levels larger than 10%, using a *t* Student hypothesis test, were not considered relevant.

Figure 1 illustrates the responses surfaces for X_1 and X_2 and X_1 and X_3. Equation 3.1 refers to the effects of the isolated variables X_1, X_2, and X_3 and the quadratic interactions effects that compose the adjusted statistical model.

$$Y_{P/S} = 0.618 + 0.0862X_1 - 0.174(X_1)^2 - 0.0167X_2 - 0.0656(X_2)^2 + 0.0207X_3 - 0.0777(X_3)^2 \quad (3.1)$$

The maximum point (27.0 g L^{-1} sucrose, 2.70 g L^{-1} yeast extract, and 0.90 g L^{-1} NH_4NO_3) was found after canonical analysis of surface response (Software Maple V Release 4). The optimized conversion $Y_{P/S}$ calculated from Eq. 3.1 was 0.62 g g^{-1} when the maximum point was substituted. The conversion $Y_{P/S}$ was confirmed experimentally, where xanthan production reached 0.59 g g^{-1} and the viscosity was 22,500.0 cP for the 1% xanthan gum solution.

The fit model was checked by the correlation coefficient (R^2), which was evaluated to be 0.975. This suggested that the model was very adequate in approximating the response surface of the experimental design.

The results of the second-order response surface model in the form of analysis of variance (ANOVA) are given in Table 3. The Fisher *F* test [$F(9, 6)=26.3$] with a very low probability value demonstrate a high significance for the regression model. The goodness of fit of the model was checked by the determination coefficient (R^2). In this case, the value of the determination coefficient ($R^2=0.975$) indicates that only 2.50% of the total variations are not explained by the model. The value of the adjusted determination coefficient (adjusted $R^2=0.938$) is also high to advocate for a high significance of the model. A higher

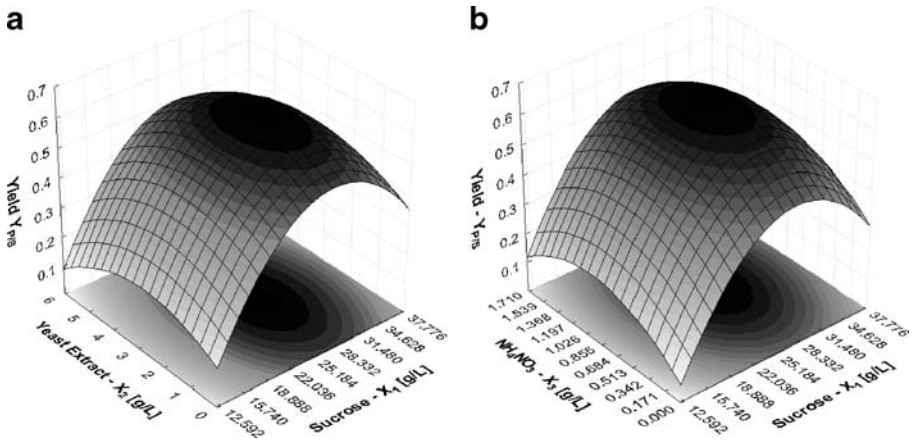

Fig. 1 3D plots of response surface optimization experiment results: **a** the effect of supplementary yeast extract concentration, sucrose concentration, and their mutual interaction on xanthan production and **b** the effect of supplementary NH_4NO_3 concentration, sucrose concentration, and their mutual interaction on xanthan production

Table 3 ANOVA for the quadratic model

Source of variations	Sum of squares	Degrees of freedom	Mean square	F value (calculation)	Probability (p)
Regressions	0.315766	9	0.035085	26.3	0.000379
Residual	0.008004	6	0.001334		
Total	0.323770	15			

R^2 =0.975; R=0.987; adjusted R^2 =0.938

value of the correlation coefficient (R=0.987) justifies an excellent correlation between the independent variables.

Determination of Parameters (μ_{max}, X_{max}, m, λ, α, and β)

The kinetic study was accomplished for the central point of the design and the maximum point found experimentally. A fourth order Runge–Kutta algorithm was coupled to the nonlinear regression method to derive the solutions for the differential Eqs. 2.3, 2.4, and 2.5. The parameters (μ_{max}, X_{max}, m, λ, α, and β) were obtained for the optimized conditions (Table 4).

The calculated sum of squares of the residues for each of the model calculations was relatively low (Table 4), suggesting a good model fit. Indeed, when the experimental data are compared to the quantitative responses predicted by the Weiss and Ollis model [28], there is a high degree of correspondence, regardless of the initial sucrose concentrations (Figs. 2 and 3). The experimental results and the model predictions of the concentrations of cells, sucrose, and xanthan gum at initial sucrose concentrations (27.0 g L^{-1} shaker and bioreactor) show that the Weiss and Ollis model [28] represents the changes in concentrations during the fermentations.

The kinetic parameters presented in Table 4, mainly those associated with growth terms (m and α), were largely affected by the xanthan production rate and substrate consumption. Using a bioreactor, the terms (m and α) were less than using a shaker. The operational conditions defined in the reactor differed substantially from those adopted in the shaker, promoting larger cellular growth in the exponential phase due to the accentuated substrate consumption in the same period. The nonassociated growth terms (λ and β) were also compared using either a bioreactor or a shaker that their values were less than the associated growth terms.

El-Salam et al. [31] found that xanthan gum production by *X. campestris* E-NRC-3 from cane broth syrup using 3.0% of total sugar in the fermented medium reached 15.5 g L^{-1} of xanthan and a substrate conversion to product ratio of 0.58 g g^{-1}. These results are similar to those obtained in this work.

It is always advisable to check the coherence between the experiment and the model, within this application area, before the model can be considered to be "valid". The established model was validated by simulating a culture carried out under different initial conditions and by comparing the results of this simulation with those obtained experimentally.

The conditions chosen used a medium in which the initial sucrose concentration in sugarcane broth was the central point conditions (e.g., 25.0 g L^{-1} reactor and shaker). The simulation results indicate that for this concentration of sucrose, a good correlation between the experimental values and the values given by the model was observed (Figs. 4 and 5) validating the model.

Table 4 Comparison of kinetic constant values under optimized conditions (shaker and bioreactor) from modeling and the literature.

Parameters	Shaker[b]	Bioreactor[b]	Weiss and Ollis [25]	Pinches and Pallent [14]	García-Ochoa et al. [32]	Serrano-Carreón et al. [21]	Letisse et al. [11]
μ_{max} (h^{-1})	$0.119\pm7.60\times10^{-2}$	$0.411\pm2.50\times10^{-2}$	0.152	0.29	0.110	0.25	0.38
X_{max} (g L^{-1})	$1.95\pm4.20\times10^{-2}$	$2.84\pm5.00\times10^{-2}$	2.450	2.15	1.790	4.42	3.30
α (gS gX^{-1})	$4.90\pm9.18\times10^{-1}$	$1.38\pm3.93\times10^{-1}$	2.00	1.24	14.286	3.03	3.23
β [gS gXh^{-1}]	$0.197\pm2.17\times10^{-2}$	$0.411\pm4.67\times10^{-2}$	0.284	0.24	0.120	0.26	0.36
m (gP gX^{-1})	$1.72\pm2.98\times10^{-1}$	$0.881\pm1.47\times10^{-1}$	1.830	0.47	10.00	2.00	0.50
λ [gP gXh^{-1}]	$0.071\pm0.69\times10^{-2}$	$0.265\pm2.50\times10^{-2}$	0.155	0.13	0.003	0.25	0.23
$Y_{P/S}$ (exp.)	0.34	0.63					
$Y_{P/S}$ (model)[a]	0.35	0.64					
SRS	7.89	23.03					

[a] $Y_{P/S}$—yield parameter obtained from Weiss and Ollis model [28]

[b] Kinetic parameters for shaker and bioreactor under the optimized conditions: 27.0 g L^{-1} sucrose, 2.7 g L^{-1} yeast extract, and 0.90 g L^{-1} NH_4NO_3

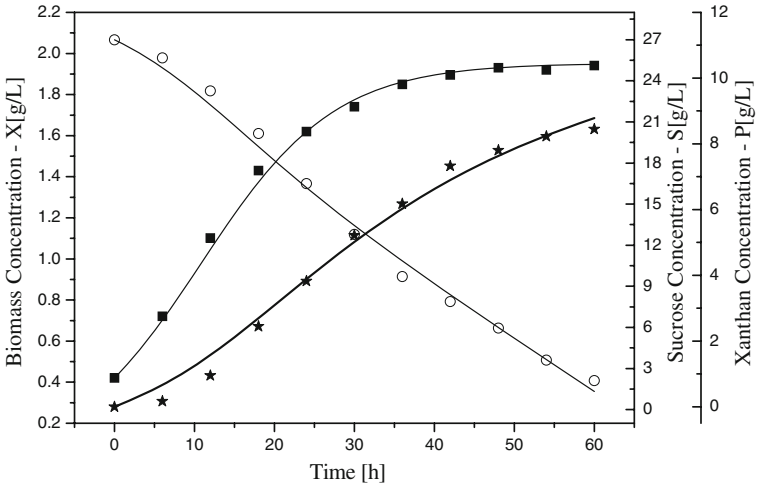

Fig. 2 Change in the concentration of microbial biomass (*closed square*), sucrose (*open circle*), and xanthan gum (*closed star*) when the initial sucrose concentration was 27.0 g L^{-1} (shaker). *Symbols* represent the experimental results; *lines* represent the Verhulst–Pearl and Luedeking–Piret kinetic model data

Bibliographical and Experimental Comparison

The kinetic parameters of the optimized conditions of this study using the bioreactor were compared to those obtained using the shaker and to values reported in the literature. As shown in Table 4, a wide range of variability was observed for the parameter values in this study compared to other reports. This may be due to differences in the microorganism cultivation conditions and xanthan gum production. Weiss and Ollis [28] modeled the Moraine and Rogovin [7] experimental data, using the strain *X. campestris* pv. *campestris*

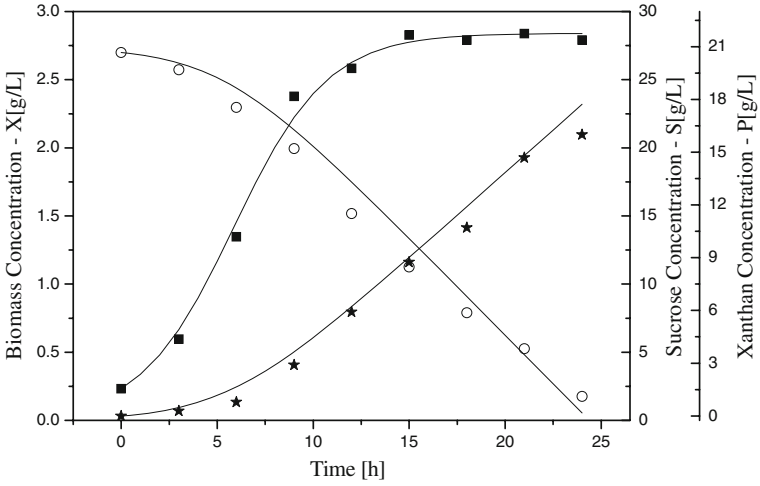

Fig. 3 Change in the concentration of microbial biomass (*closed square*), sucrose (*open circle*), and xanthan gum (*closed star*) when the initial sucrose concentration was 27.0 g L^{-1} (bioreactor). *Symbols* represent the experimental results; *lines* represent the Verhulst–Pearl and Luedeking–Piret kinetic model data

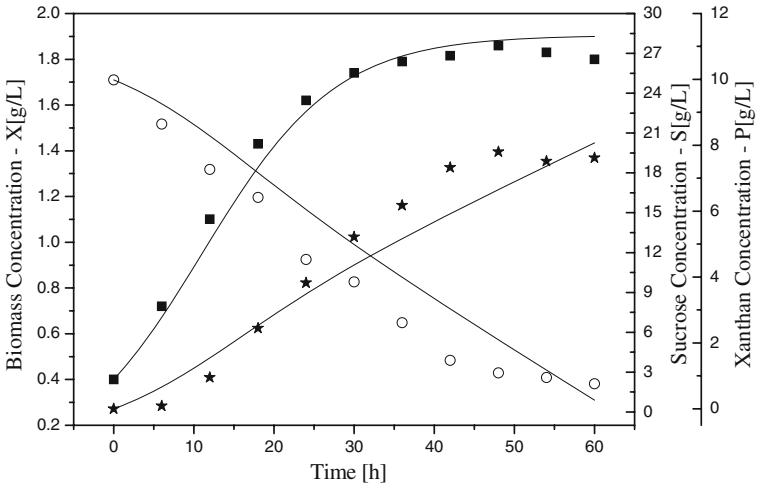

Fig. 4 Change in the concentration of microbial biomass (*closed square*), sucrose (*open circle*), and xanthan gum (*closed star*) when the initial sucrose concentration was 25.0 g L^{-1} (shaker). *Symbols* represent the experimental results; *lines* represent the Verhulst–Pearl and Luedeking–Piret kinetic model data using the parameters values obtained in the optimized conditions in the shaker (27.0 g L^{-1})

NRRL B-1459 in production medium consisting of 5% glucose and 0.06% nitrogen, at pH 7.1±0.1, a temperature of 28±1 °C, and aeration of 1 vvm. Pinches and Pallent [14] used the same Moraine and Rogovin [7] strain and production medium broth composed of 22.5 g kg^{-1} glucose and nitrogen (sodium L-glutamate or peptone) at pH 7.0±0.5, a temperature of 30±0.5 °C, and aeration of 0.4 vvm. Serrano-Carreón et al. [21] also used *X.*

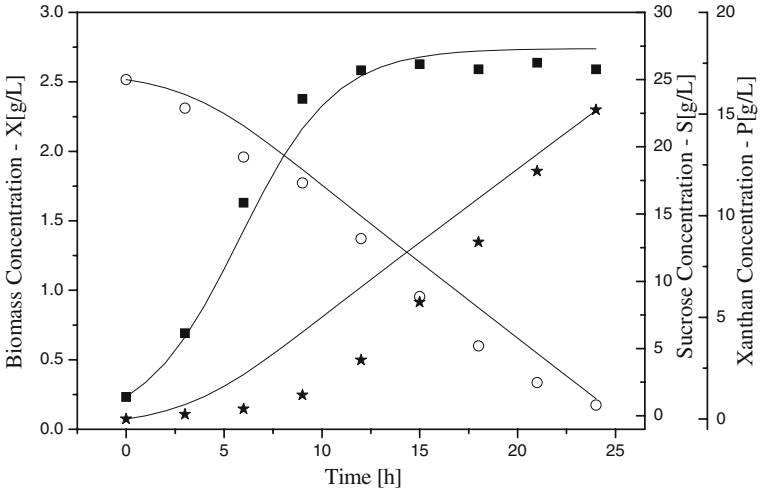

Fig. 5 Change in the concentration of microbial biomass (*closed square*), sucrose (*open circle*), and xanthan gum (*closed star*) when the initial sucrose concentration was 25.0 g L^{-1} (reactor). *Symbols* represent the experimental results; *lines* represent the Verhulst–Pearl and Luedeking–Piret kinetic model data using the parameters values obtained in the optimized conditions in the reactor (27.0 g L^{-1})

campestris pv. *campestris* NRRL B-1459, but sucrose (40.0 g L^{-1}) was the substrate employed and the nitrogen source was NH_4Cl (2.0 g L^{-1}) at pH 7.0±0.1, a temperature of 29 °C, and aeration of 0.5 vvm. García-Ochoa et al. [32] and Letisse et al. [11] used sucrose as the carbon source and the pH was not controlled. Letisse et al. [11] worked with *X. campestris* ATCC 13951, using sucrose (42 g L^{-1}), and NH_4NO_3 (1.125 g L^{-1}) at pH 7.0 and 28 °C. The substrate, the source of nitrogen, and the conditions in this work were different from those reported in the cited literature.

The kinetic parameter values determined in this study were within the ranges reported in the literature, as shown in Table 4. Using the bioreactor, the parameters were closest to those cited by Letisse et al. [11]. The variability between the studies can be explained hypothetically by differences in correlated factors, including the strain selected, C/N relationship, nitrogen source, agitation speed, pH control in the medium, and the use of complex mediums, such as sugarcane broth [30]. In addition, it is difficult to characterize complex fermentation processes.

Conclusion

The employed optimization process for the biosynthesis of xanthan gum using cane broth as the raw material determined $Y_{P/S}$ values from the variable concentrations of sucrose (X_1), yeast extract (X_2), and ammonium nitrate (X_3) between the values of $-\alpha$ and $+\alpha$, as defined by the response surface methodology. The kinetic model of Weiss and Ollis described the cellular growth, xanthan production, and carbon source consumption satisfactorily, as shown in Figs. 2 and 3. Therefore, this model accurately predicts production performance and describes the kinetic behavior of such fermentations carried out in either a shaker or a bioreactor, under the specific conditions of these experiments (Figs. 4 and 5).

Nomenclature

SRS	sum of squares of residues
t	time (h)
$Y_{P/S}$	yield of xanthan per amount of sucrose (gP gS^{-1})
$Y_{X/P}$	yield of biomass per amount of xanthan (gX gP^{-1})
$Y_{X/S}$	yield of biomass per amount of sucrose (gX gS^{-1})
y_{exp}	experimental values to xanthan, carbon source and biomass concentration (g L^{-1})
y_{theor}	calculated values to xanthan, carbon source and biomass concentration (g L^{-1})
m	Weiss and Ollis [28] model parameter of xanthan production due to growth (gP gX^{-1})
P	xanthan concentration (g L^{-1})
S	carbon source concentration (g L^{-1})
X	biomass concentration (g L^{-1})

Greek Symbols

β	Weiss and Ollis [28] model parameter of carbon source consumption due to biomass (gS gX^{-1} h^{-1})
α	Weiss and Ollis [28] model parameter of carbon source consumption due to growth (gS gX^{-1})

μ_{max} maximum specific growth rate (h^{-1})
λ Weiss and Ollis [28] model parameter of xanthan production due to biomass ($gP\ gX^{-1}\ h^{-1}$)

Subscripts

max maximum value

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References

1. Mulchandani, A., Luong, J. H. T., & Leduy, A. (1988). *Biotechnology and Bioengineering*, *32*, 639–646. doi:10.1002/bit.260320508.
2. Ashtaputre, A. A., & Shah, A. K. (1995). *Applied and Environmental Microbiology*, *61*(3), 1159–1162.
3. García-Ochoa, F., Santos, V. E., Casas, J. A., & Gómez, E. (2000). *Biotechnology Advances*, *18*, 549–579. doi:10.1016/S0734-9750(00)00050-1.
4. Pradella, J. G. C. (2006) Biopolímeros e Intermediários Químicos, Centro de Gestão e Estudos Estratégicos, Relatório Técnico no 84 396–205, Laboratório de Biotecnologia Industrial—LBI/CTPP, São Paulo, pp. 119.
5. Skaracis, G. N., Kalogiannis, S., & Iakovidou, G. (2003). *Process Biochemistry*, *39*, 249–256. doi:10.1016/S0032-9592(03)00067-0.
6. Harcum, S. W., & Yoo, S. D. (1999). *Bioresource Technology*, *70*, 105–109 short communication. doi:10.1016/S0960-8524(99)00013-9.
7. Moraine, R. A., & Rogovin, P. (1971). *Biotechnology and Bioengineering*, *13*, 381–391. doi:10.1002/bit.260130305.
8. De Vuyst, L., Vermiere, A., Van Loo, J., & Vandamme, E. J. (1987). *Mededelingen van de Faculteit landbouwwetenschappen—Rijksuniversiteit Gent*, *52*, 1881–1900.
9. Pons, A., Dussap, C. G., & Gros, J. B. (1990). *Bioprocess Engineering*, *5*, 107–114. doi:10.1007/BF00388188.
10. García-Ochoa, F., Santos, V. E., & Alcon, A. (1995). *Enzyme and Microbial Technology*, *17*, 206–217. doi:10.1016/0141-0229(94)00009-G.
11. Letisse, F., Lindley, N. D., & Roux, G. (2003). *Biotechnology Progress*, *19*, 822–827. doi:10.1021/bp0257168.
12. Cadmus, M. C., Knutson, C. A., Lagoda, A. A., Pittsley, J. E., & Burton, K. A. (1978). *Biotechnology and Bioengineering*, *20*, 1003–1014. doi:10.1002/bit.260200703.
13. Margaritis, A., & Zajic, J. E. (1978). *Biotechnology and Bioengineering*, *20*, 939–1001. doi:10.1002/bit.260200702.
14. Pinches, A., & Pallent, L. J. (1986). *Biotechnology and Bioengineering*, *28*, 1484–1496. doi:10.1002/bit.260281006.
15. Souw, P., & Demain, A. L. (1980). *Journal of Fermentation Technology*, *58*, 411–416.
16. Nakajima, S., Funahashi, H., & Yoshida, T. (1990). *Journal of Fermentation and Bioengineering*, *70*(6), 392–397. doi:10.1016/0922-338X(90)90120-L.
17. Alves, R. S. A. (1991), Dissertação, Escola de Química, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brasil.
18. De Vuyst, L., & Vermeire, A. (1994). *Applied Microbiology and Biotechnology*, *42*, 187–191.
19. Umasankar, H., Annadurai, G., Chellapandian, M., et al. (1996). *Bioprocess Engineering*, *14*(6), 307–309. doi:10.1007/BF00369474.
20. Lima, M. A. G. A. (1999) Tese de doutorado, Escola de Química, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brasil.
21. Serrano-Carreón, L., Corona, R. M., Sánchez, A., & Galindo, E. (1998). *Process Biochemistry*, *33*(2), 133–146. doi:10.1016/S0032-9592(97)00039-3.
22. Amanullah, A., Satti, S., & Nienow, A. W. (1998). *Biotechnology Progress*, *14*, 265–269. doi:10.1021/bp9800079.
23. Shu, C.-H., & Yang, S.-T. (1990). *Biotechnology and Bioengineering*, *35*, 454–468. doi:10.1002/bit.260350503.

24. Ramírez, M. E., Fucikovsky, L., García-Jiménez, F., et al. (1988). *Applied Microbiology and Biotechnology*, *29*, 5–10. doi:10.1007/BF00258343.
25. Chhabra, R. P., Richardson, J. F. (1999). *Non-Newtonian flow in the process industries fundamentals and engineering applications* p. 436. Great Britain: Butterworth Heinemann.
26. Marquardt, D. W. (1963). *Journal of the Society for Industrial and Applied Mathematics*, *11*, 431–441. doi:10.1137/0111030.
27. Gill, S. (1951). A process for the step-by-step integration of differential equations in an automatic computing machine. *Proceedings of the Cambridge Philosophical Society*, *47*, 96–108. doi:10.1017/S0305004100026414.
28. Weiss, R. M., & Ollis, D. F. (1980). *Biotechnology and Bioengineering*, *22*, 859–873. doi:10.1002/bit.260220410.
29. Luedeking, R., & Piret, E. L. (1959). *Journal of Biochemical and Microbiological Technology and Engineering*, *1*, 393–412. doi:10.1002/jbmte.390010406.
30. Borzani, W. (2001). Engenharia bioquímica. In W. Borzani, U. A. Lima, E. Aquarone, & W. Schmidell (Eds.), *Biotecnologia Industrial, v. 2*(1st ed.). São Paulo: Blücher.
31. El-Salam, M. H. A., Fadel, M. A., & Murad, H. A. (1994). *Journal of Biotechnology*, *33*, 103–106. doi:10.1016/0168-1656(94)90103-1.
32. García-Ochoa, F., García-León, M. A., & Romero, A. (1990). *Chemical and biochemical engineering*, *4*, 15–20.