

Avanços Científicos e Tecnológicos em Bioprocessos

Alberdan Silva Santos
(Organizador)



Atena
Editora

Ano 2018

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(Organizador)

Avanços Científicos e Tecnológicos em Bioprocessos

Atena Editora
2018

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Diagramação e Edição de Arte: Geraldo Alves e Natália Sandrini

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Dados Internacionais de Catalogação na Publicação (CIP) (eDOC BRASIL, Belo Horizonte/MG)

A946 Avanços científicos e tecnológicos em bioprocessos [recurso eletrônico] / Organizador Alberdan Silva Santos. – Ponta Grossa (PR): Atena Editora, 2018.

Formato: PDF

Requisitos de sistema: Adobe Acrobat Reader

Modo de acesso: World Wide Web

Inclui bibliografia

ISBN 978-85-85107-47-5

DOI 10.22533/at.ed.475180110

1. Bioprocessos. 2. Bioquímica. 3. Biotecnologia. I. Santos, Alberdan Silva.

CDD 553.7

Elaborado por Maurício Amormino Júnior – CRB6/2422

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2018

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APRESENTAÇÃO

Avanços Científicos e Tecnológicos em Bioprocessos é uma obra que reúne vinte e três capítulos com temas em pesquisas científicas realizadas no campo da biotecnologia, e que envolve agentes biológicos e bioquímicos na geração de produtos ou processos. Nesta obra se concentram diversos avanços descritos nas metodologias e nos resultados, distribuídos em quatro tópicos principais, envolvendo: processos químicos e biotecnológicos no aproveitamento de resíduos; produção de metabólitos e enzimas; métodos analíticos e de simulação; e biotratamentos envolvidos na geração de energias. Esta obra foi escrita por jovens pesquisadores brasileiros que estão desenvolvendo suas teses e/ou dissertações em instituições nacionais. Por este motivo, os aspectos inovadores e o alcance dos resultados apresentados podem ser um grande estímulo para aqueles que visam conhecer com maior amplitude alguns dos aspectos biotecnológicos estudados em algumas das instituições de nosso país.

Alberdan Silva Santos

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MATHEMATICAL MODELING OF GLUCOSE ACCUMULATION DURING ENZYMATIC HYDROLYSIS OF CARRAGEENAN WASTE

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ABSTRACT: A simple mathematical model based on the experimental data was developed and the best values of kinetic parameters were determined for the enzymatic hydrolysis of the glucan fraction contained in the waste generated by the carrageenan processing of algal biomass (*Kappaphycus alvarezii*) aiming at the glucose accumulation. This residue was subjected to hydrolysis with several commercial enzymatic extracts containing different enzyme loads. A detailed sequence of model development, parameter estimation, and model validation is presented. The temporal profiles of glucose concentration were derived by direct analytical integration of the mathematical model,

obtaining an explicit equation over time. The model adjustment goodness was evaluated by different statistical criteria, including the Fisher's F-test, R^2 -value, and p-value and the accuracy of the model was judged acceptable. The good performance presented by the mathematical model recommends its use in further studies aimed at improving the enzymatic hydrolysis process.

KEYWORDS: mathematical modeling, enzymatic hydrolysis, carrageenan production waste, algal biomass

1 | INTRODUCTION

The third generation of biofuels production bioprocesses are based on the use of algal biomass. *Kappaphycus alvarezii* is a seaweed of high industrial interest since it is used as raw material for carrageenan production. Although there are many industrial applications for carrageenan its main use is in the food industry as a thickening, gelling, stabilizing and suspending agent in water and milk systems (WEBBER et al., 2012; MASARIN et al., 2016; OLIVEIRA et al., 2017).

Enzymatic hydrolysis of the residue generated in the carrageenan production from the macroalgae *K. alvarezii* is a promising

process to produce fermentable monomeric sugars aiming biofuels production. The replacement of fossil fuels by biofuels derived from algae reduces GHG emissions from fuel-driven vehicles. Moreover, the cultivation of algae offers environmental advantage since biomass growth captures CO₂, a greenhouse gas (GHG), from the atmosphere via photosynthesis. These carbon sinks can help to mitigate global warming (GW). Thus, algal biomass is poised to provide many environmental and economic benefits (MASARIN et al., 2016).

However, to render the biofuel production bioprocess economically viable, increases in hydrolysis rates and yields are necessary and require improvement both in enzyme engineering and processing by optimization of reaction conditions, reactor design, enzyme and substrate cocktail compositions, enzyme recycling and recovery strategies (BANSAL et al., 2009). In this scenario, optimization studies, based on mathematical models, become necessary to overcome the technological bottlenecks involved in the entire enzymatic process (OLIVEIRA, et al., 2017).

Mathematical modeling aims to better understand the kinetic behavior of polysaccharides (polymers) hydrolysis in order to develop hydrolytic enzymatic processes that can achieve high yields in glucose and other sugars (monomers) (XIANG et al., 2004). Polysaccharide hydrolysis reactions are very complex, being affected by a series of factors of different natures, starting with the fact that the substrate is in a solid phase and the biocatalyst (enzyme) in a liquid phase, thus comprising a heterogeneous catalytic system (AGUILAR et al., 2002). The past years have seen a significant increase in the number of studies on the kinetics of enzymatic hydrolysis (AGUILAR et al., 2002). Different assumptions regarding rate limiting factors and basic substrate–enzyme interaction mechanisms were employed to develop and validate kinetic models for hydrolysis reactions (AGUILAR et al., 2002).

In this study, the hydrolysis kinetics of glucan (polysaccharide) present in the waste generated in the carrageenan production from the macroalgae *K. alvarezii* was followed by monitoring the concentration of monomer (glucose) formed during reaction and a mathematical model was proposed for description of the concentration temporal profiles experimentally observed.

2 | DEVELOPMENT, IDENTIFICATION, AND VALIDATION OF THE MATHEMATICAL MODEL

Enzymatic hydrolysis of glucan involves the use of cellulase enzymes to convert this polymer into hexoses (glucose). Cellulases are a mixture of three different cellulolytic enzymes, including endoglucanase (1,4-β-D-glucan glucohydrolase), exoglucanase (1,4-β-D-glucan cellobiohydrolase), and cellobiase (β glucosidase), that act synergistically to convert glucan into glucose (WANG et al., 2011).

Due to the difficulty in finding a strict mechanism for hydrolysis reactions, it is

usual to use simplified models, based on a pseudo-homogeneous approach, to describe the kinetics of hydrolysis (AGUILAR et al., 2002). Such a model is that describing the enzymatic hydrolysis as an irreversible first-order reaction, represented by (decomposition of glucose is not considered due to mild reaction conditions):



According to concepts of enzymatic kinetics, the reaction rate (r) can be expressed in terms of either the change of the substrate or the product concentrations (G and g , respectively) as follows:

$$r = -dG/dt = dg/dt \quad (2)$$

Although the rate of enzymatic reactions (r) is generally given as a function of substrate concentration and of enzyme load, according to a Michaelis-Menten type equation, a semi-empirical approach, based on direct inspection of the experimental data, can be used to derive a kinetic expression as follows.

The enzymatic hydrolysis reaction was monitored following the accumulation of glucose over time (Figure 1). The glucose accumulation can be adequately described by a curve that tends to an asymptotic maximum value (Fig. 1). Thus, the rate of glucose accumulation (dg/dt) was assumed to be proportional to the difference between the maximum concentration of glucose (g_{max}), which could potentially be obtained when the reaction time tends to infinite, and that (g) actually present in the reaction medium at a given time t , characterizing a saturation kinetics as follows:

$$dg/dt = r = k(g_{max} - g) \quad (3)$$

In Equation (3), the kinetic constant k incorporates the parameter \hat{k} as well as the enzyme load E_0 used in the hydrolysis reaction. Thus, this parameter can be decomposed into these two factors, i.e., $k = \hat{k}E_0$, where \hat{k} can be interpreted as the specific rate constant per unit of enzymatic load. By introducing this relation into Equation (3) and integrating, one obtains the temporal profile of glucose concentration (g):

$$g = g_{max}(1 - e^{-\hat{k}E_0 t}) \quad (4)$$

The residue generated in the carrageenan production from algal biomass was hydrolyzed with different commercial extracts of cellulases using enzyme loads of 10 and 100 FPU/g-substrate. All hydrolysis trials were conducted in 50 mL Falcon tubes containing a polysaccharide suspension at 2% (w/v) of consistency in 50mM sodium acetate buffer, pH 4.8 under rotary stirring at 120 rpm and temperature of 45°C for 72 h.

Due to the parameters g_{max} and \hat{k} to be derived from a pseudo-homogeneous approach, they incorporate several intervening factors in the hydrolysis kinetics and, for this reason, were estimated for each trial, aiming to incorporate the variations resulting from the different reaction conditions used (enzyme load (EL), extract nature, initial concentrations, etc.). These parameters were estimated for each hydrolysis trial by non-linear regression, minimizing the sum of the squares of the residuals between the experimental values and those calculated by the model, according to the algorithm of Marquardt (FROMENT et al., 2010). Six data points were used for each model

adjustment. The model was validated by means of specific statistical tests, which are described as follows:

- Fisher's F -test: It is based upon the regression sum of squares and the residual sum of squares:

$$F_c = \frac{\sum_{i=1}^n \frac{\hat{y}_i^2}{p}}{\sum_{i=1}^n \frac{(y_i - \hat{y}_i)^2}{n-p}} > F(p, n-p; 1-\alpha) \quad (5)$$

In Equation 5, y_i and \hat{y}_i are respectively the experimental and calculated values of glucose concentration at time i , n is the number of experimental data ($n=6$) and p is the number of model parameters ($p=2$). If the calculated value of F (F_c) is greater than the corresponding tabulated value for degrees of freedom p and $n-p$, and probability $1-\alpha$ ($F(p, n-p; 1-\alpha)$), the regression is considered statistically significant, being 0.05 the value commonly adopted for the significance level (α). For all the adjustments performed, the tabulated value of F is $F(2,4; 0.95) = 6.9443$.

- p -value test: According to which, the model is rejected if this value is greater than the significance level (α) set for the test.
- Coefficient of determination (R^2): Provides a fair first indication of how much of the variance in the experimental data is explained by the model.

$$R^2 = 1 - \left[\frac{\sum_{i=1}^n (y_i - \hat{y}_i)^2}{\sum_{i=1}^n (y_i - \bar{y})^2} \right] \quad (6)$$

3 | RESULTS AND DISCUSSION

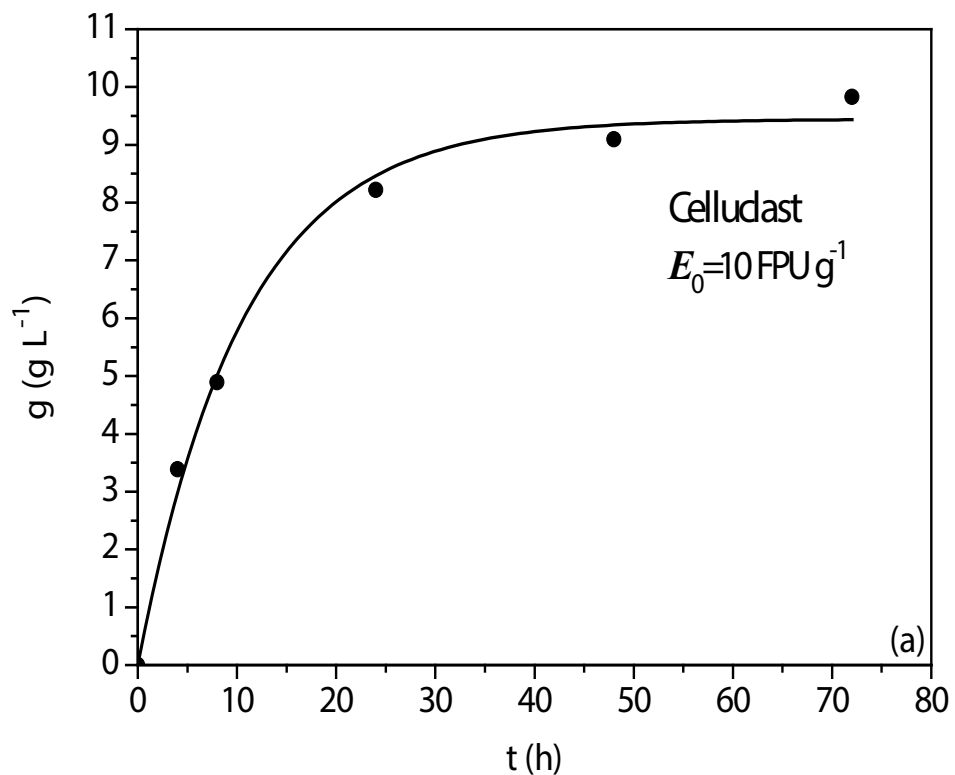
Table 1 presents the parameter estimates and statistics indicators for the kinetic model adjustments to the experimental data obtained for each enzymatic hydrolysis trial.

Enzymatic Extract	E_0 (FPU g ⁻¹)	g_{max} (g L ⁻¹)	\hat{k} (g h ⁻¹ FPU ⁻¹)	k (h ⁻¹)	F_c	p -value	R^2
Cellic CTec2	10	9.63393	0.017734	0.1773	438.997	3.067x10 ⁻⁵	0.979
		(± 0.38007)	(± 0.002774)				
Celluclast	10	9.44459	0.009446	0.0945	1221.606	3.999x10 ⁻⁶	0.994
		(± 0.23912)	(± 0.000851)				
Cellulase from <i>Trichoderma</i>	10	8.48358	0.008242	0.0824	595.822	1.671x10 ⁻⁵	0.988
		(± 0.31863)	(± 0.001070)				
Cellic CTec2	100	8.93941	0.0050265	0.5027	1181.391	4.275x10 ⁻⁶	0.990
		(± 0.21264)	(± 0.0009364)				

Table 1 - Parameter estimates and statistical indicators for the kinetic model adjustments

It is observed that the value of \hat{k} is around $0.01 \text{ g h}^{-1} \text{ FPU}^{-1}$, except for that estimated for the trial performed with the enzymatic extract Cellic CTec2 ($E_0=100 \text{ FPU g}^{-1}$), for which the value of \hat{k} was $0.005 \text{ g h}^{-1} \text{ FPU}^{-1}$, a value about two times smaller than those verified for the other extracts. However, this value of \hat{k} , when linked to the high enzymatic loading used in this trial ($E_0=100 \text{ FPU g}^{-1}$), provided the highest value of k , which significantly increased the reaction rate. The parameter g_{max} was independent on the experimental conditions used, presenting small variations around the average value of 9.12 g L^{-1} .

According to the applied tests, the proposed kinetic model was validated for all trials, presenting statistical indicators that were very favorable to its validation at a probability level above 95%. Based on the R^2 value, graphs below illustrate the best (a) and the worst (b) model fits, which were obtained for the enzyme extracts Celluclast ($E_0=10 \text{ FPU g}^{-1}$) and Cellic CTec2 ($E_0=10 \text{ FPU g}^{-1}$), respectively. The high values assumed by the determination coefficient provide an indication of the suitability of the theoretical approach followed and of the reliability of the related kinetic equations obtained.



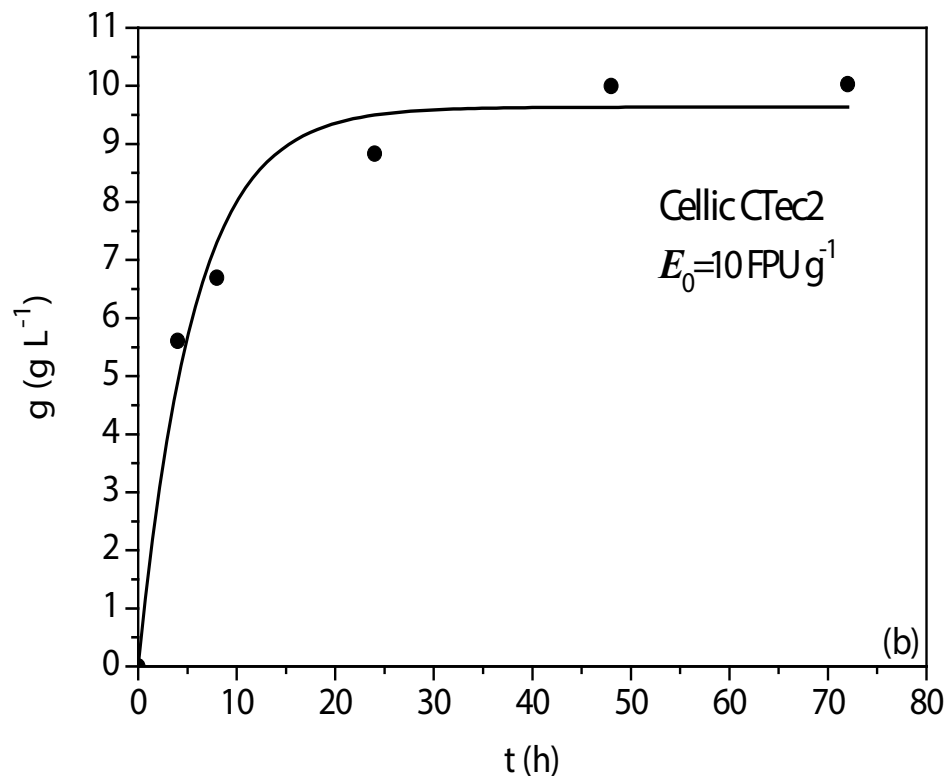


Figure 1 – Illustration of the best (a) and worst (b) adjustments produced by the mathematical model

4 | CONCLUSION

A simple mathematical model was developed to describe the kinetics of enzymatic hydrolysis of the polysaccharide fraction of the residue generated in the carrageenan production process from algal biomass. The proposed model provided adjustments to the experimental data having excellent statistical indicators, thus recommending its use for optimization studies of the enzymatic hydrolysis process. However, the model needs to be further tested against additional experimental data to validate or disprove any underlying hypothesis.

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SOBRE O ORGANIZADOR

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Agência Brasileira do ISBN
ISBN 978-85-85107-47-5

