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Genetic Algorithm Optimization of the Parameters Involved in Biosurfactant Production from Beet Peel as Substrate

Ana Luiza Campos[a], Júlia Nogueira[a],Filipe A. Coelho[b], Ana M. F. Fileti[b], Brunno F. Santos[a]*

[a] Department of Chemical and Materials Engineering, Pontifical Catholic University of Rio de Janeiro, Rua Marquês de São Vicente, 225, Gávea. Rio de Janeiro, RJ 22453-900, Brazil.
[b] School of Chemical Engineering (FEQ), Department of Chemical Systems Engineering (DESQ), University of Campinas (UNICAMP). Rua Albert Einstein, 500 – Cidade Universitária, Campinas – SP, 13083-852, Brazil.
bsantos@puc-rio.br

Biosurfactants, synthesised by microorganisms, are surface-active compounds capable of reducing surface tension and increasing system's emulsification. Several factors, such as the use of waste instead of synthetic substrate, can influence biosurfactant production. Hence, modelling and optimization are extremely important to find an economic route for its application in industrial scale.The classical numerical methods based on gradient usually fail to obtain nhe optimum kinetic parameters because they often converge to local minima.Stochastic global search algorithms, such as the Genetic Algorithm (GA), have been showing a great potential to detect optimal solutions in complex systems as bioprocesses. This work aims to evaluate the procedure that employs GA for estimating the kinetic parameters involved in biosurfactant production from agro-industrial waste using *Bacillus subtilis*. Three different models were proposed to describe biomass growth, substrate consumption, biosurfactant synthesis and dissolved oxygen in the medium. The technique's quality was evaluated from the normalized sum of squared errors (SSE) and correlation coefficient (R^2), calculated by the software MATLAB 2017a for each model. Two of the tested models have to be considered to achieve the optimal solution, once both presented are remarkable performance reproducing the dynamics of most variables, obtaining R^2 values superior to 0.9 and normalized SSE near to 0.
Keywords: Genetic Algorithm; Parameter Estimation; Global optimization; Biosurfactant production; Agro-industrial Waste.

1. Introduction

Most surfactants used worldwide are derived from petroleum and represent a potential threat to the environment due to their recalcitrant nature (Aparna et al., 2012). It is in this context that emerge the biosurfactants, surface active molecules produced by microorganisms that can be used in petrochemical, food, cosmetics, and pharmaceutical industries (Santos et al., 2014). They also have several applications in environmental protection that include EOR, oil spills control, biodegradation, and detoxification of oil contaminated industrial effluents and soil (Khopade et al., 2012).

Moreover, biosurfactants are biodegradable, less toxic and can be synthesized from renewable sources by a wide variety of microorganisms, which makes each of them unique. The recently reported renewable sources most included agro-industrial waste such as ground nut and soybean oil refinery residue, distillery and whey wastes, potato peels and rice straw (Amodu et al., 2016). Agro-industrial wastes have a great potential to be used as substrates in biosurfactant production due to their high nutritional power (Santos, 2015). It became clear on Secato's et al (2016) work that *Bacillus subtilis* is capable of producing biosurfactant in industrial waste growth medium.

Although many studies reported biosurfactant synthesis, very limited information is available about its kinetic production, as well as renewable substrate's consumption by microorganisms. Asit deals with the metabolism of living organisms, the system's behaviour is slightly predictable, complicating the math modelling. In these cases, numerical methods for model fitting based on gradient are not applied because of non-convexity of the

error landscape, with several local minima being present. On the other hand, Artificial Intelligence has been employed to model and optimize high complexity systems, as biochemistry processes, where the use of exacts methods are considerably restricted (Link & Weuster-Botz, 2006, Pappu & Gummadi, 2017, Dhanarajan et al., 2017).

Stochastic approaches are the most proper way to find the ideal solution or optimum point because they are based on probability rules, which make them be considerably strong and effective for complex system's optimization (Chowdhury & Garai, 2017).One of these methods is Genetic Algorithm (GA), founded in Charles Darwin's evolution theory. It relies on genetic operators, as mutation and crossing-over, to generate new possible solutions, and natural selection mechanism, to privilege the most adapted individuals. Thus, the algorithm increases the probability of convergence to the global optimum point.

This work aims to model the kinetics of renewable substrate consumption, biomass growth, product formation and dissolved oxygen in the culture medium, as well as optimize the parameters involved in biosurfactant production from *Bacillus subtilis* using GA as the global optimum search mechanism.

2. Process Description

The experimental procedure was developed by Santos (2015) and the data was used to model and optimize system's behaviour. Substrate consumption, biomass growth, biosurfactant production and dissolved oxygen concentration were monitored for 24 h in a batch fermentation process. The microorganism utilized was *Bacillus subtilis*. The inoculum was prepared in a broth medium, which mostly contained peel beet and residual glycerine. Peel beet was the sugar source while glycerine was added for microorganism maintenance during sucrose hydrolysis into glucose.

The inoculum was taken to a jacketed stirred bioreactor with 7 L of maximum volume capacity and temperature, pH and dissolved oxygen sensors. System's aeration was measured online while the remaining variables were determined by sample's collection from reactional medium. Biomass growth was observed through spectroscopy measures every 3 h. Glucose concentration was inferred from a calibration curve, established by a laboratory biochemical test kit.

The process optimization was performed in MATLAB R2017a using the GA functions available in the Global Optimization Toolbox. The population size, number of generations, selection and mutation functions were defined as 350, 100, stochastic uniform ('selectionstochunif') and gaussian ('mutationgaussian'), respectively. The crossover function and fraction, as well as migration direction, interval and probability were set to scattered ('crossoverscattered'), 0.8, 'both', 20 and 0.3, respectively. The kinetic parameters' initial values were defined as a vector 'vr' and restricted by a lower bound vr*0.05 and upper bound vr*6.

The optimization function aims to minimize the normalized sum of the squared errors (SSE) between the experimentally determined concentrations and those calculated throughout the simulation. The tested models were Aiba-Shonda's (1969), Levenspiel's (1999) and Andrews' (1968) original proposals for specific growth rate. At the end of the simulation, the code exhibits the optimized parameters; the normalized SSE and variable's graphical behaviour. The program runtime is estimated between 30 and 60 minutes.

2.1 General Equations

The mass balance equations in batch bioreactor that describe the biomass growth (Gaden, 1955), substrate consumption (Jurecicet al., 1984) and dissolved oxygen (Pirt, 1975) are listed by Eq. (1) to Eq. (4).The balance for product formation can be mathematically expressed by several proposals. Three of them are presented in 'Individual Model Equations' section.

$$\frac{dX}{dt} = \mu_X X \tag{1}$$

$$\frac{dS}{dt} = -\mu_S X + \frac{1}{2} k_1 S_C^{\,k} - \mu_{mO2}\, S\, P^n \tag{2}$$

$$\frac{dC_{O2}}{dt} = k_L a (C_{O2S} - C_{O2}) - Q_{O2} X \tag{3}$$

$$Q_{O2} = m_O + \frac{\mu_X}{Y_O} \tag{4}$$

Where X, P, S, and $S_c$are biomass, biosurfactant, glucose and sucrose concentration (g L^{-1}), respectively. Additionally, μ_x and μ_S are the specific growth and substrate rates (h^{-1}), μ_{mO2} is the maximum oxygen consume rate (h^{-1}) and n, k and k_1 are kinetic parameters. The variable $k_L a$ refers to the oxygen transfer volumetric coefficient (h^{-1}),C_{O2} is this gas' concentration (g L^{-1}),C_{O2S} is the saturated oxygen concentration (g L^{-1}), Q_{O2} is the specific consumption rate (g_{O2} g_{cells}^{-1} h^{-1}).m_o is related to the maintenance coefficient for oxygen (g_{O2} g_{cells}^{-1} h^{-1}) and Y_o is the gas conversion factor to cells (g_{O2} g_{cells}^{-1}). Notice that substrate's

equation has a positive term even though glucose is consumed during all the experiment. It is due to peel beet's sucrose hydrolysis into glucose, contributing to substrate's concentration rise.

2.2 Individual Model Equations

This study works with different proposals for microorganism behaviour. The particular equations of each model are exhibited in Table 1.All the tested models indicate an inhibitory factor in the system: Andrews suggests that substrate may be interfering in microorganism growth, while Levenspiel and Aiba-Shonda propose that it is the product formation. Levenspiel's model considers the maximum product concentration achieved experimentally whereas Aiba-Shonda's contemplate only the instantaneous product concentration.
The variable μ_m express the maximum specific growth rate (h^{-1}), Y_{XS} is the theoretical biomass yield and K_S and m_S are the substrate's saturation constant and maintenance coefficient (g L^{-1}). P_{max} represents the maximum biosurfactant concentration achieved experimentally, μ_{O2} is the oxygen consumption rate (h^{-1}) and K_{O2} is the gas saturation constant (g L^{-1}). Finally, k_2, k_3, m, n and N are kinetic parameters and K_p and K_i are the inhibition constant for product and substrate (g L^{-1}), respectively.

Table 1: Individual equations for each tested model.

	Aiba-Shonda	Levenspiel	Andrews
μ_x	$\mu_m \frac{S}{(K_S + S)} \frac{K_p}{(P + K_p)}$	$\mu_m \frac{S}{(K_S + S)} \left(1 - \frac{P}{P_{max}}\right)$	$\mu_m \frac{S}{K_S + S + \frac{S^2}{K_i}}$
μ_s	$Y_{xs}\, \mu_{O_2} \frac{S}{(m_S + S)}$	$\frac{\mu_x}{Y_{xs}} + m_S$	$Y_{xs}\, \mu_{O_2} \frac{S}{(m_S + S)}$
μ_{O_2}	$\frac{C_{O_2}}{(K_{O_2} + C_{O_2})}$	-	$\frac{C_{O_2}}{(K_{O_2} + C_{O_2})}$
$\frac{dP}{dt}$	$k_3 \mu_x X - k_2 S P^n$	$\mu_{O_2} k_3 e^{-m S} X - k_2 S P^N$	$\mu_{O_2} k_3 e^{-m S} X - k_2 S P^n$

3. Results and Discussion

The kinetic performance of substrate consumption, product formation, cell growth and dissolved oxygen for biosurfactant production from *Bacillus subtilis* in renewable medium are reported in Figures 1 to 4, respectively. The asterisks represent the values measured experimentally by Santos (2015) and the curves show the dynamic trend calculated by the GA for each variable.
Figure 1 shows the initial increase of substrate's concentration due to sucrose hydrolysis into glucose in the beginning of the experiment. Then, glucose concentration presents the expected downward trend, once it is consumed by the microorganism in order to grow and synthesize products. Figure 2 reveals that the biosurfactant production initially increases but around 2 to 10 h, rely on the model, it switches to a decay and/or stabilization behaviour. At the same time, Figure 3 illustrates the fast cell concentration raise until 10 h of experiment, when the growth velocity is reduced, achieving steady state in some tested models. This performance is characteristic of exponential and stationary phases of microorganism growth curve. Figure 4 displays the awaited drop pattern of dissolved oxygen in culture medium, reaching zero or close concentrations since 10 h of simulated data.
The squared correlation coefficients (R^2) between the simulated and experimental data for each variable and model are shown in Table 2. The normalized SSE displayed by GA in the end of the simulations can also be observed in Table 2.
Levenspiel's and Andrews' models weren't able to represent the development of experimental glucose concentration. On the other hand, Aiba-Shonda's equation exhibited a conduct very similar to the reality and the best estimated substrate behaviour, achieving $R^2 = 0.9717$.
With regard to biosurfactant production, none of the tested models successfully predicted the experimental behaviour, implying that additional studies are necessary to predict the synthesis process. The system's complexity, heterogeneity of growing medium, limited comprehension of microorganism metabolism and little information about biosurfactant production in renewable mediums denote some of the obstacles to mathematically express and simulate the biosurfactant's concentration pattern.

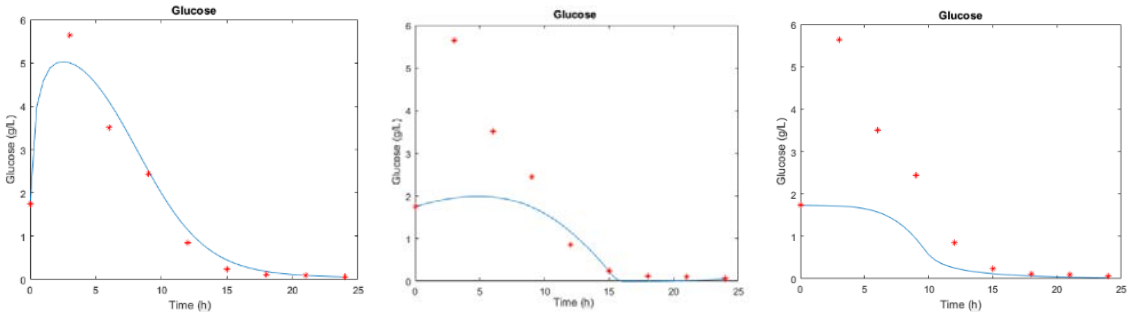

Figure 1: Glucose concentration results for Aiba-Shonda,Levenspiel and Andrews models.

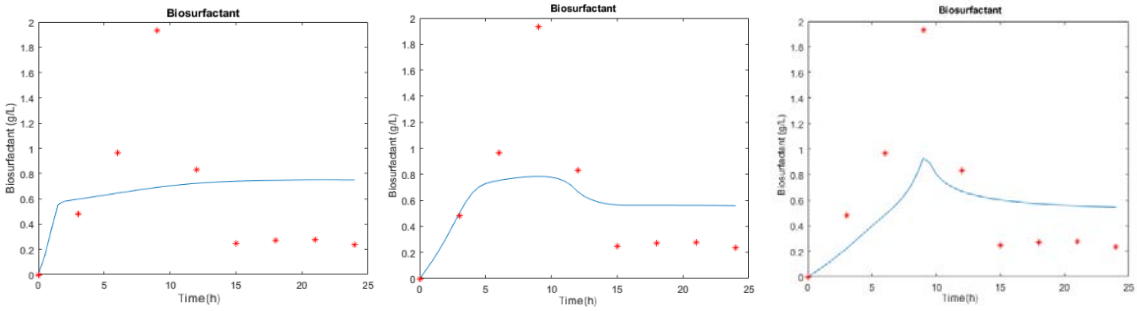

Figure 2: Biosurfactant concentration results for Aiba-Shonda, Levenspiel and Andrews models.

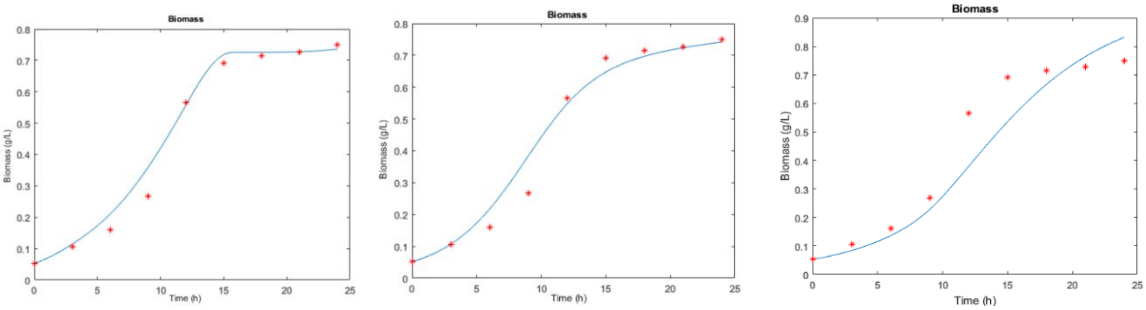

Figure 3: Biomass concentration results for Aiba-Shonda, Levenspiel and Andrews models.

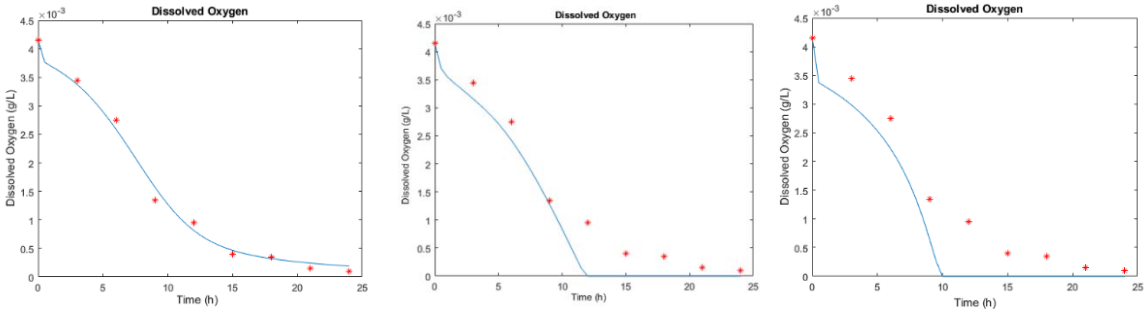

Figure 4: Dissolved oxygen concentration results for forAiba-Shonda, Levenspiel and Andrews models.

Nevertheless, Andrews' model turned up to be promising for further studies in this area because it showed adequate performance, predicting a peak at the same time as the experimental data. In addition, it was able to predict biosurfactant's concentration increase, decrease and steadiness times in accordance with experimental data. Yet, improvements in the equations are necessary to achieve better R^2 fit and better understanding of the microorganism metabolism.

Every tested model indicated the biomass raise until 14 h of experiment. However, Andrew's equation matched the experimental data only until 10 h, not corresponding to the expected behaviour afterwards. On the other hand, Aiba-Shonda's and Levenspiel's models expressed conducts very close to the laboratory measures. Even though both models could perceive the inflection point in biomass growth, Aiba-Shonda's presented the best R^2 value, reaching 0.9883.

The reduction in the dissolved oxygen concentration in growth medium can be observed in all three tested models. This trend is expected since *Bacillus subtilis* synthesizes biosurfactants via an aerobic fermentation route. Yet, only Aiba-Shonda's did not predict a premature decrease and stabilization in zero between 10 h and 24 h. Hence, it was the best model to predict dissolved oxygen's real evolution, obtaining $R^2 = 0.9919$.

Table 2: R^2 and normalized SSE for each model

		Aiba-Shonda	Levenspiel	Andrews
R^2	Glucose	0.9717	0.7239	0.7129
	Biosurfactant	0.0868	0.4205	0.4252
	Biomass	0.9883	0.9731	0.9333
	Dissolved Oxygen	0.9919	0.9693	0.9540
Normalized SSE		0.7758	1.3974	1.5432

Table 3: Optimum parameters calculated by GA for Andrews' model

k_1*	Y_o ($g_{O_2} g^{-1}_{células}$)	Y_{xs} (-)	m_S (g L^{-1})	K_S (g L^{-1})	μ_{mO2} (h^{-1})	K_{O2} (g L^{-1})	μ_m (h^{-1})
0	1.6019	0.0895	0.8622	0.3188	0.5031	0	0.3426
k_3*	m_o ($g_{O_2} g^{-1}_{células}$ h^{-1})	k_La (h^{-1})	n (-)	k (-)	k_2*	m (-)	Ki (g L^{-1})
4.0784	0.0324	9.3024	3.0349	0.1629	0.3184	0.7772	1.7375

* These units may vary according to the reaction order.

Table 4: Optimum parameters calculated by GA for Aiba-Shonda's model

k_1*	Y_o ($g_{O_2} g^{-1}_{células}$)	Y_{xs} (-)	m_S (g L^{-1})	K_S (g L^{-1})	μ_{mO2} (h^{-1})	K_{O2} (g L^{-1})	μ_m (h^{-1})
0	16.9210	21.3293	21.3057	9.3691	7.9544	0.0068	0.7494
k_3*	m_o ($g_{O_2} g^{-1}_{células}$ h^{-1})	k_La (h^{-1})	n (-)	k (-)	k_2*	K_P (g L^{-1})	
29.151	0.0379	7.1342	10.3120	5.0589	32.1017	27.6360	

* These units may vary according to the reaction order.

The graphical results, observed in Figures 1 to 4, show that the best model to predict biosurfactant formation in agro-industrial waste growth medium is Andrew's. Although modifications in the kinetic equations have to be made, it presented satisfactory correlation coefficients for all the variables analysed. On the other hand, Aiba-Shonda's model was able to accurately anticipate the evolution of substrate consumption, biomass growth and dissolved oxygen concentration. Both models were partially successful and should be considered in further studies to optimize biosurfactant production in renewable mediums. The optimum parameters calculated by GA for these models are exhibited in Tables 3 and 4.

4. Conclusions

The simulated data based on Aiba-Shonda's model presented the expected behaviour for three out of four analysed variables, reaching R^2valuessuperior to 0.97as well as normalized SSE smaller than 1. Because of the system's significant complexity, it could not predict the biosurfactant formation process. However, Andrews' equation expressed an acceptable conduct for its production, even when it is the most difficult variable to estimate. The proposed equation showed an appreciable potential to describe the biosurfactant concentration evolution throughout the fermentation process, achieving the highest R^2. Both models should be considered in further studies in order to better understand the microorganism metabolism in complex growth

medium and improve the results. Nevertheless, the optimization strategy was satisfactory to provide the models' parameters.

Reference

Aiba S., Shonda M., 1969, Reassessment of Product Inhibition in Alcohol Fermentation, J. Ferment. Technol., v. 47, p.790-794.

Amodu O.S., Ojumu T.V., Ntwampe S.K.O., 2016, Kinetic Modelling of Cell Growth, Substrate Utilization, and Biosurfactant Production from Solid Agrowaste (*Beta vulgaris*) by *Bacillus licheniformis* STK 01, The Canadian Journal of Chemical Engineering, 94, 2268-2275.

Andrews J.F., 1968, A Mathematical Model for the Continuous Culture of Microorganisms Utilizing Inhibitory Substrate, Biotechnology and Bioengineering, v.10, p.707-723.

Aparna A., Srinikethan G., Hedge S., 2012, Production and Characterization of Biosurfactant Produced by aNovel Pseudomonas sp. 2B, Colloids and Surfaces B: Biointerfaces, 95, 23-29.

Chowdhury B., Garai G., 2017, A review on multiple sequence alignment from the perspective of genetic algorithm, Genomics, 109, 419-431.

Dhanarajan G., Rangarajan V., Bandi C., Dixit A., Das S., Ale K., Sen R., 2017, Biosurfactant-biopolymer driven microbial enhanced oil recovery (MEOR) and its optimization by an ANN-GA hybrid technique, Journal of Biotechnology, 256, 46-56.

Ganden, E.L.Jr., 1955, Fermentation Kinetics and Productivity, Chemistry and Industry, February 12, p.154-9.

Jurecic, R., Berovic, M., Steiner, W., Koloini, T., 1984, Mass Transfer in Aerated Fermentation Broths in a Stirred Tank Reactor, The Canad. J. Chem. Eng., vol. 62, p.334-9.

Khopade A., Ren B., Liu XY, Mahadik K., Zhang L., Kokare C., 2012, Production and Characterization of Biosurfactant from Marine *Streptomyces* species B3, Journal of Colloid and Interface Science, 367, 311-318.

Levenspiel O., 1999, Chemical Reaction Engineering. Department of Chemical Engineering, Oregon State University, John Wiley & Sons, Third Edition.

Link H., Weuster-Botz D., 2006, Genetic algorithm for multi-objective experimental optimization, Bioprocess and Biosystems Engineering, 29(5-6), 385-390.

Pappu S.M.J., Gummandi S.N., 2017, Artificial neural network and regression coupled genetic algorithm to optimize parameters for enhanced xytol production by Debaryomyces nepalensisin bioreactor, Biochemical Engineering Journal, 120, 136-145.

Pirt, S.J., 1975, Principles of Microbe and Cell Cultivation, London, Blackwell Scientific Publications.

Santos, B., Ponezi, A., Fileti, A.M.F., 2014, Strategy of Using Waste for Biosurfactant Production Through Fermentation by Bacillus subtilis, Chemical Engineering Transactions, 37, 727-732, DOI:10.3303/CET1437122.

Santos B.F., 2015, Study of biosurfactant production using agro-industrial waste with development of statistical models and soft sensor by artificial neural network. Doctoral Thesis– University of Campinas. Campinas/SP-Brazil.

Secato J., Coelho D., Rosa N., Lima L., Tambourgi E.B., 2016, Biosurfactant Production Using Bacillus subtilis and Industrial Waste as Substrate, Chemical Engineering Transactions, 49, 103-108 DOI: 10.3303/CET1649018