South African Journal of Chemical Engineering 41 (2022) 176–181

Contents lists available at ScienceDirect

South African Journal of Chemical Engineering

journal homepage: www.elsevier.com/locate/sajce

Kinetic modeling of biosurfactant production from crude oil using Bacillus subtilis cells

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ARTICLE INFO

Keywords:
Biosurfactant
Bioreactor, Crude oil
Optimizing
Modelling

ABSTRACT

Crude oil and its derivatives have high application in different industries, and unforeseen spills or over-exploitation generate a significant threat in ecosystems, causing negative impacts on soil, water, and air. There are microorganisms capable of metabolizing hydrocarbons through the bioremediation process with biosurfactant production, but large-scale culturing and technification are still a significant challenge due to their high costs and optimization stage requirement. An unstructured kinetic model provides crucial information regarding improvements and process optimization at the first stages. Thereof prediction of bioprocess kinetic behavior is expected from mathematical expressions. Considering the above, biosurfactants' bioprocess modeling tends to be an essential tool to increasingly focus on the efficiency and profitability of oil industries. That is why biosurfactant kinetics production from *Bacillus subtilis* is investigated in this research, implementing a mathematical model. Previous studies refereed experimental data to integrate into Monod, Contois, Haldane, Moser, Powell, Tessier, Aiba-Edward, Luong, Yano-Koga, and Chen-Hashimoto equations. Therefore, a nonlinear regression parameterization procedure is applied using the Matlab Fmincon Function. The best accuracy found between experimental and simulated data was achieved using the Chen-Hashimoto kinetic model with μ_{max}, k_d and k_s values of 2.3239 d^{-1}, 0.3748 d^{-1} and 1.1619 g/L, respectively. This research suggests that biosurfactant production occurs under anaerobic conditions where hydrolysis controls microbial growth. These research results are a promising aim related to industrial biotechnology since computational modeling is essential for process efficiency from a technical and economic perspective.

1. Introduction

Crude oil and its derivatives have high application in different industries. However, an unforeseen spill or over-exploitation generates a significant threat to ecosystems, causing negative impacts on soil, water, and air. Hydrocarbons' exposure to the environment causes carcinogenic and mutagenic effects, endangering humans and animals (Devianto et al., 2020; Ray et al., 2020; Sharma and Pandey, 2020). More than 18.000 crude oil barrels were spilled in Colombia due to illegal oil extraction from 2000 to 2017, causing severe environmental damage (Guerrero, 2018). In Specific regions such as the North of Santander, these problems have caused a shortage of water sources of 80%, exceeding the capacity of ecosystems to recover (Velásquez, 2016).

Microorganisms can metabolize hydrocarbons through the bioremediation process (Xue et al., 2019). The latter is reached by using them to elaborate organic amphiphilic molecules produced as a survival response during their growth in ecosystems contaminated with hydrocarbons (Borges et al., 2019). Biosurfactants are metabolites associated with growth, allowing the hydrophobic solubilization by emulsifying oil chains, generating their degradation in an environmentally friendly way (Câmara et al., 2020; Sharma and Pandey, 2020). In adverse environmental conditions, various microorganisms produce them as an adaptation and survival mechanism. They have low toxicity, high biodegradability, and specificity, characteristics for which they are considered the most economically sought-after biotechnological compounds in the 21st century (Singh et al., 2018). Due to their ability to act in hydrocarbon-contaminated environments, has broad applicability in bioremediation (Ahmad et al., 2021).

According to their different hydrophilic groups, they can classify Biosurfactants into glycolipids, lipopeptides and polymeric surfactants. Among them, lipopeptides and glycolipids are the most commonly used in microbial oil recovery (MEOR) (Liu et al., 2021). The main strains responsible for producing them are Paenibacillus sp., Aeribacillus sp., Bacillus sp., Agrobacterium sp., and Pseudomonas sp. (Ray et al., 2020)

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https://doi.org/10.1016/j.sajce.2022.06.009
Received 3 June 2021; Received in revised form 7 May 2022; Accepted 21 June 2022
Available online 23 June 2022

Based on the above, biosurfactants can be obtained from crude oil microbial degradation. However, its technification and large-scale production are still a significant challenge due to its high costs and the optimization stage requirement. Nevertheless, kinetic models provide valuable information for improving and optimizing processes since their knowledge builds the basis for performing a bio-product's kinetic behavior predictions.

The latency phase plays a fundamental role in the growth curve of a microorganism. In this adaptation phase, the enzymes necessary for the synthesis of essential metabolites are produced so that the microorganisms can grow and adapt to a new environment (Buckley et al., 2015). Thus, the latency phase plays a fundamental role in microbial growth by supplying the cell's needs against the toxic substrate. Likewise, the growth phase is a crucial parameter for biosurfactant production, and the maximum growth rate measures it u_{max} (Gupta et al., 2020). The latter is typically determined by implementing different kinetic models such as the Monod, Haldane, Moser, Contois, Powell, and Teissier models (Mahsa et al., 2015). However, kinetic parameter estimation for crude oil applications requires extensive experimentation and time to determine its influence on mathematical modeling. For these reasons, biosurfactant production modeling could be an essential tool to achieve the basis for bioprocess optimization's efficiency and profitability. Motivated by the latter, this work's main objective consisted of the design of a mathematical model that would allow predicting the evolution of cell growth, biosurfactant, and crude oil degradation from previous experimental data (Sharma and Pandey, 2020). Particular emphasis is on a procedure incorporating different kinetic models to reduce the error between simulated data and experimental values, considering a nonlinearly constrained optimization technique using the Matlab Fmincon function.

2. Biosurfactant production modeling framework

Experimental data for biosurfactant production from crude oil is referred from (Sharma and Pandey, 2020) in this research. In mentioned work, Bacillus subtilis RSL-2 was isolated from crude oil-contaminated sludge and was chosen due to its excellent surface-active properties and the lowest surface tension reduction. Based on the latter, Bacillus subtilis RSL-2 can generate a lipopeptide-type biosurfactant from a culturing medium composed of 1.5 g/L crude oil as a carbon source and 1.5% yeast extract.

The proposed mathematical expressions for building the framework modeling are based on a batch-type reactor. First, the dynamic growth of the X cells of Bacillus subtilis RSL-2 is modeled considering Eq. (1):

$$\frac{dX}{dt} = \mu X w - k_d X d \tag{1}$$

μ represents the microbial growth rate, k_d is the specific cell death velocity, w and d are constants with initial conditional values of 1 and 0, respectively. However, when the fermentation time is more significant than three days ($t >= 3$), cell growth is negligible and governed by cell death (based on the reported experimental data). Therefore $w = 0$ and $d = 1$. These constants were added to the mathematical model based on Biomass (X) experimental data that decreased significantly due to the rapid cell death.

The impact of the crude oil concentration on microbial growth and biosurfactant formation is simulated by Eq. (2):

$$\frac{dS}{dt} = -(q_s + m_s)X \tag{2}$$

The oil uptake rate is governed by the dynamic Bacillus subtilis concentration and the oil degradation rate. The latter includes the sequential biodegradation of aliphatic, mono-aromatic hydrocarbons, cycloalkanes, asphaltenes, and resins (Ritesh et al., 2021). Thus q_s represents the specific oil degradation rate and is determined by Eq. (3):

$$q_s = \frac{\mu}{Y_{xs}} \tag{3}$$

Y_{xs} is the biomass yield from the substrate, m_s is a maintenance constant that considers intracellular reactions with a proposed value of 0.02. Finally, the biosurfactant concentration produced by Bacillus subtilis at dynamic state is modeled by Eq. (4):

$$\frac{dP}{dt} = q_p X \tag{4}$$

Where $q_p$represents the specific product formation rate and is calculated by the expression:

$$q_p = Y_{xp}\mu + m_p \tag{5}$$

Here Y_{xp} means the biomass yield to the biosurfactant, and m_p is the specific product formation rate due to maintenance with a proposed value of 0.0011. The crude oil biodegradation is modeled using first-order kinetics (Bhattacharya et al., 2018). However, different kinetic models (Table 1) describe the conversion process at the microbial level. Therefore, different kinetic expressions were evaluated in this research to carry out an exhaustive investigation that allows predicting the biosurfactant production from crude oil satisfactorily. The applicability and essential characteristics of each model are described in the results section.

The simulations were carried out with Matlab R2017b software. The Runge-Kutta 45 method was used to numerically solve the proposed mathematical model to simulate biosurfactants' production from crude oil. The parametrization through nonlinear restricted optimization was determined by its implementation in the Matlab software using the Fmincon function to minimize the error between the experimental data obtained from the references mentioned above and the data simulated by the mathematical modeling proposed in this research.

In this way, the function to minimize J is expressed by Eq. (16):

$$J = \int_0^{t_f} (y_{msd}(t) - y(p,t)^T W(t)(y_{msd}(t) - y(p,t))dt \tag{16}$$

Restricted to:

$$f\left(\frac{dX}{dt}, x,\ y,\ p,\ v,\ t\right) = 0 \tag{17}$$

$$x(t_0) = x_0 \tag{18}$$

Table 1
Kinetic models used for biosurfactant simulations (Manheim et al., 2019).

Kinetic models	
Contois Model	$\mu = \mu_{max}\frac{S}{k_s X + S}$ (6)
Haldane Model	$\mu = \mu_{max}\frac{S}{k_s + S + K_1 S^2}$ (7)
Monod Model	$\mu = \mu_{max}\frac{S}{k_s + S}$ (8)
Moser Model	$\mu = \mu_{max}\frac{S^n}{k_s + S^n}$ (9)
Powell Model	$\mu = \mu_{max}\frac{S}{(k_s + L) + S}$ (10)
Teissier Model	$\mu = \mu_{max}1 - exp\left(\frac{-S}{k_s}\right)$ (11)
Luong Model	$\mu = \frac{S\mu_{max}}{k_s + S}1 - \left(\frac{S}{S_{max}}\right)^n$ (12)
Aiba-Edward Model	$\mu = \mu_{max}\frac{S}{k_s + S}exp\left(\frac{-S}{K_i}\right)$ (13)
Yano-Koga Model	$\mu = \mu_{max}\left[\frac{S}{S + k_s + \left(\frac{S^2}{k_{si}}\right)\left(1 + \frac{S}{K}\right)}\right]$ (14)
Chen-Hashimoto Model	$\mu = \frac{\mu_{max}\, S}{KS_0 + (1-K)S} - b$ (15)

$$h(x, y, p, v) = 0 \tag{19}$$

$$g(x, y, p, v) \leq 0 \tag{20}$$

$$p^L \leq p \leq p^U \tag{21}$$

Where p is the vector of kinetic constants for the mathematical model that simulates microbial growth, substrate uptake, and biosurfactant production, y_{msd} are the experimental data of the process, and $y(p,t)$ are the simulated results, W is a weighting diagonal matrix, x are the state variables (in this case, biomass, substrate, and product), x_0 are the state variables' initial conditionals, v is a vector of non-estimated parameters, f is the equality constraints of the algebraic and differential equations that describe the system dynamics, h, and g are the possible equality and inequality constraints that express the additional requirements for system performance. Finally, $p^L$and p^U act as kinetic parameter limiters (Moles et al., 2013).

3. Results and discussions

The bioprocess modeling is a critical stage during industrialization and technification as a starting point for optimizing efficiency and profitability. Based on the above, this research is focused on a framework modeling for predicting the evolution of cell growth, biosurfactant, and crude oil degradation from previous experimental data (Sharma and Pandey, 2020). Particular emphasis is concentrated on a procedure incorporating different kinetic models to reduce the error between simulated data and experimental values, considering a nonlinear constrained optimization.

According to the results based on the experimental and simulated data, it takes about three days (exponential phase) to reach the maximum concentration of Bacillus subtilis cells with an average growth rate of 19 d^{-1}. After this stage of cell growth, biomass begins its phase of cell death. In this research, restrictions are proposed for cell growth in the framework modeling shown in Eq. (1) with conditions w and d as explained before (initial conditional values of 1 and 0, respectively). However, when the fermentation time is longer than three days ($t >= 3$), cell growth is negligible ($w = 0$) and governed by maintenance reactions and cell death ($d = 1$). Therefore, according to the results obtained, the biosurfactant production from crude oil is mainly achieved during the exponential growth phase with average values of 3.1 g/L at three days of fermentation.

Interestingly, the biosurfactant production is still appreciated after this time, suggesting that the mentioned metabolite could be indirectly associated with cell growth metabolism. Considering these findings, the term $m_p$was included in Eq. (5) to incorporate the biosurfactant production due to maintenance. Based on the above, it is evident that crude oil biodegradation rate is also influenced by the substrate used for cell maintenance. In such a way, the term "m_s" was added in Eq. (2) to simulate cell maintenance effects during crude oil uptake by Bacillus subtilis cells. Finally, the cells reach a crude oil biodegradation rate of 55%, according to reported simulated data obtained through simulation. All simulations were parameterized using the nonlinear restricted optimization technique with the Matlab Fmincon function. Besides, ten kinetic models were proposed to obtain the maximum growth velocity (μ_{max}) and reduce the error (F_{val}) between the experimental and simulated data. The results can be seen in Table 2.

The Monod kinetic model (Manheim et al., 2019; Monod, 1949) simulated in Fig. 1(a) is one of the most widely used expressions in bioprocess modeling due to its versatility and simplicity regarding computational implementation. In this study, the specific microbial growth rate μ_{max} and the saturation constant k_s using the Monod model, respectively, determined at 19.99 d^{-1} and 9.995 g/L. Likewise, the error was minimized to a final value of 5.919 using the Fmincon function.

The latter indicates that error was obtained almost four times higher than the best estimates (Chen-Hashimoto Model). Discrepancies reached can be explained considering the physical meaning of the model since it is characterized by relating microbial growth and the limiting substrate with the process's global kinetics without including any additional effect during the fermentation process (Mahsa et al., 2015). Therefore, during crude oil biodegradation, the participation of chemical species affecting microbial growth speed is evident. Unfortunately, the Monod model does not capture these aspects.

There are other variations to the Monod equation that have been used in bioprocesses. Thus, the Moser model (Manheim et al., 2019) considers the potential interactions between the enzyme-substrate binding sites by integrating a parameter n in the Monod model. Additionally, the mentioned model has been used in microbial mutants, where the intracellular composition is independent of time (Mahsa et al., 2015). In this research, a value of 19.99 d^{-1} was obtained for μ_{max} and a constant n of 5.631, with a minimized error of 2.18. The latter results are close to the Contois model's estimates (see Fig. 1(b)).

Previous studies (Bhattacharya et al., 2018) have shown the importance of the enzymatic mechanisms of saturation, affinity, and interaction in biodegradation reactions. In this way, the Moser model captures the effects above, and probably the global production of biosurfactants is regulated by enzymatic interaction and affinity phenomena. Furthermore, the above is supported by the simulated data found in this research.

In addition to the different enzymatic mechanisms studied in the Moser model, mass transfer in a crude oil biodegradation process can be crucial in determining the degree of bio-conversion. Based on the latter, the rheology of crude oil, biofilm formation, and material transport to the cell's interior could limit the microbial growth kinetics. Considering the above, Powell's model (Fig. 1(c)) was included in this research to identify the possible effects of mass transfer on biosurfactant production kinetics. According to the results obtained, a value of 1.3372 d^{-1} was calculated for μ_{max} and 0.6686 g/L for k_s with a constant L of 0.3084.

Powell's model does not consider inhibition effects (Mahsa et al., 2015; Muloiwa et al., 2020), and its degree of prediction in biosurfactants' formation could be limited. Alternatively, the Teissier Model (Manheim et al., 2019; Tessier, 1942) shown in Fig. 1(d) describes the microbial growth kinetics as an exponential substrate concentration function. It has been used in applications involving growth deficiency due to incorporating hormones in microbial cultures (Mahsa et al., 2015; Muloiwa et al., 2020). The value of μ_{max} and k_s determined in this investigation presented 19.99 d^{-1} and 9.995 g/L, respectively.

The error generated (F_{val} = 6.602) showed a behavior similar to that

Table 2
Summary of best performing model parameter values.

Parameter	Model									
	Monod	Moser	Powell	Teissier	Haldane	Contois	Aiba-Edward	Luong	Yano-Koga	Chen-Hashimoto
μ_{max} (d^{-1})	19.999	19.999	1.3372	19.999	18.180	19.999	0.5558	1.4505	19.999	2.3239
k_d (d^{-1})	0.3730	0.3558	0.4111	0.3920	0.3986	0.3712	0.3969	0.3989	0.3845	0.3748
Y_{px} (g/g)	0.4770	0.6195	0.6684	0.6770	0.5533	0.5039	0.6988	0.6904	0.6406	0.5382
Y_{xs} (g/g)	3.3780	11.158	5.3366	3.7930	6.5380	7.9050	0.49824	5.0505	5.6973	15.7913
S_0 (g/L)	1.7090	1.2000	1.2000	1.5530	1.2000	1.4654	1.2000	1.2000	1.2000	1.1286
k_s (g/L)	9.9950	9.9950	0.6686	9.9950	9.0900	9.9990	0.2779	0.7252	0.9950	1.1619
F_{val} (error)	5.919	2.18	6.4532	6.602	11.47	1.977	6.5781	6.9402	11.1204	1.2133

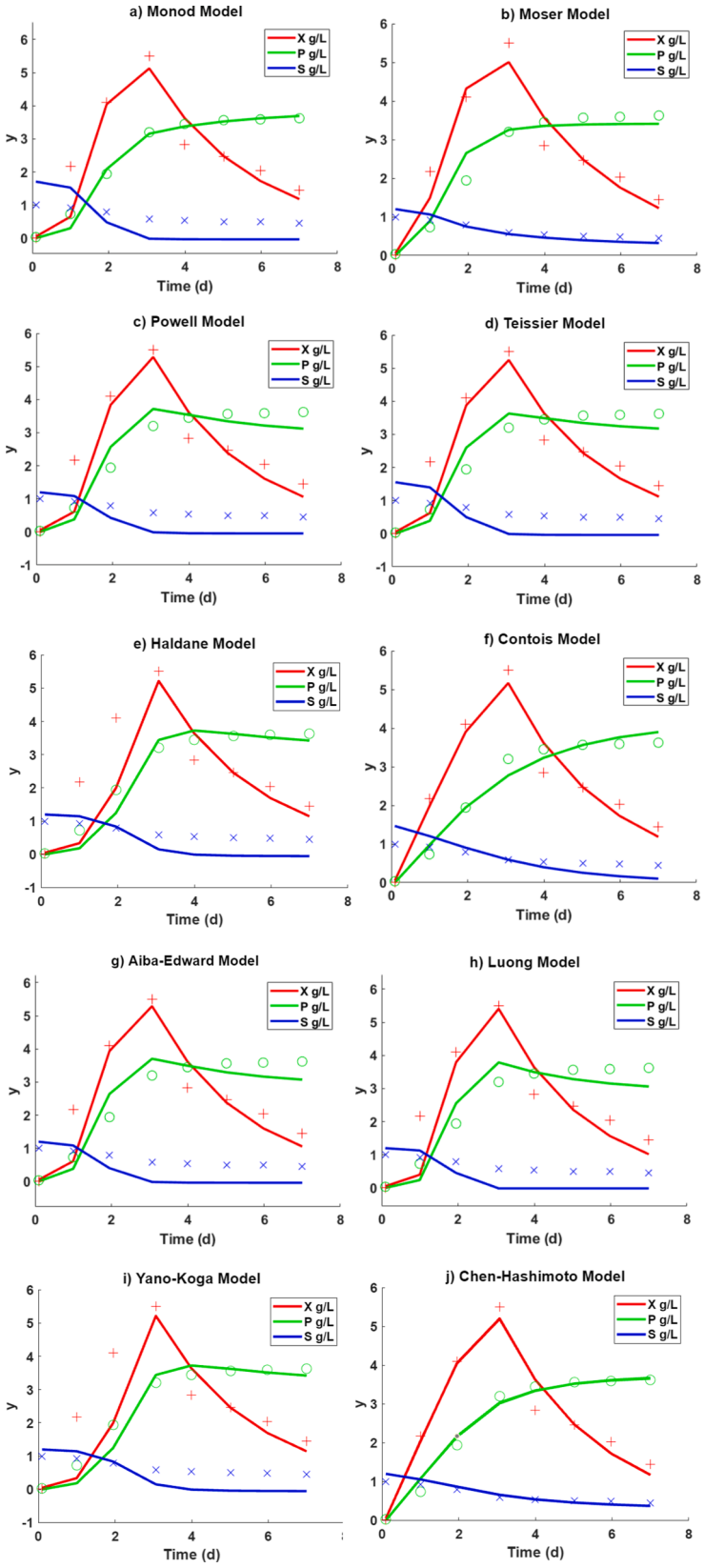

Fig. 1. Comparison of experimental (marks) and simulated data (solid lines) for biosurfactant fermentation. (a) Monod kinetic model; (b) Moser kinetic model; (c) Powell kinetic model; (d) Teissier kinetic model; (e) Haldane kinetic model; (f) Contois kinetic model; (g) Aiba-Edward kinetic model; (h) Luong kinetic model; (i) Yano-Koga kinetic model and (j) Chen-Hashimoto kinetic model.

obtained with the Monod model, Powell model, and Aiba-Edward model, exceeding the degree of discrepancy almost five times obtained using the Chen-Hashimoto model. The differences found could also be attributed to the lack of term that considers inhibition effects. Based on the results at this research stage, the study of modeling with expressions involving the inhibition of the kinetics of biosurfactant production was proposed. For this reason, the Haldane substrate inhibition model, the Contois inhibition model, the Aiba-Edward model, the Luong model, the Yano-Koga model, and the Chen-Hashimoto model by cell concentration were evaluated.

The Haldane model (Manheim et al., 2019) is widely used in batch-type processes to identify the microorganism capacity adaptation and inhibitory substrates' presence in the medium (Ibrahim et al., 2020; Ray et al., 2020). Regarding biosurfactant production from crude oil, it is very likely that various molecules of aromatic compounds may induce an inhibitory effect on the biosurfactant formation from Bacillus subtilis cells. In this research, the values of μ_{max}, k_s and k_1 were estimated at 18.18 d^{-1}, 9.09 g/L, and 0.0134 g/L, respectively. This model obtained the highest error of all the simulations considered, 11.47 (see Fig. 1(e)).

Considering the simulated results, inhibition effects per substrate are insufficient to satisfactorily predict the production kinetics of biosurfactants from crude oil. In addition, the Contois Model (Contois, 1959; Manheim et al., 2019) shown in Fig. 1(f) includes changes in population density that affect the specific microbial growth rate. Thus, it is achieved by including the term cell concentration in a dynamic state.

The Contois model has also been widely studied in organic compounds' anaerobic and biodegradation processes in wastewater, where hydrolysis controls the microbial growth rate (Borges et al., 2019; Velásquez, 2016). According to the results, the second-highest accurate model was obtained from the Contois expression, determining an error minimization with a value of 1.977. Likewise, the values of μ_{max} and k_s were 19.99 d^{-1} and 9.999 g/L, respectively.

An essential characteristic of the Contois model is that the cell growth rate depends on the substrate concentration and biomass generated at a dynamic state. In such a way, the inhibition effects are captured at high biomass concentrations. This phenomenon may be present in the biosurfactant kinetics from crude oil since high Bacillus subtilis concentration can compete with the available surface area for substrate uptake by other cells and the mass transfer limitations through the cell membrane. Results simulated in this research agree with findings reported by (Bahmani et al., 2020). Therefore, it is concluded that the Contois model can accurately describe the biodegradation of oily sludge when using Aneurinibacillus migulanus and Bacillus toyonensis strains.

The Aiba-Edward kinetic model shown in Fig. 1(g) relates product inhibition to the culture's growth rate by introducing an exponential constant; this constant takes into account the presence of the toxic substrate concentration in the bioreactor (Soler and Alcázar, 2020). In the simulation of this model, values of 0.5558 d^{-1}, 0.2779 g/L, and 10.8 were obtained for μ_{max}, k_s and K_i, respectively. In addition, an F_{val} of 6.5781 was evidenced.

The model described above is widely used to predict bioaccumulation kinetics in the presence of inhibition (Muloiwa et al., 2020). Due to the high error value, five times higher than the Chen-Hashimoto model, it is inferred that there is no bioaccumulation of toxic substrate in the bioreactor; however, greater accuracy is denoted in the simulated biomass data and experimental data because this model can describe the lag and death phase.

Substrate inhibition on the growth of microorganisms by substrate stimulation at low concentrations as well as substrate inhibition at high concentrations (Dahalan and Hassan, 2019) is mediated by the Luong Model (see Fig. 1(h)). The simulation of this model allowed obtaining values for μ_{max} and k_s of 1.4505 d^{-1} and 0.7252 g/L, respectively. Likewise, this model presents the ability to predict the maximum value of the substrate concentration at which growth is wholly inhibited using the S_{max} parameter of equation (12). In this study S_{max} took the value of 14.86 g/L and an F_{val} of 6.9402. This model is also implemented for the m-cresol degradation by a Batch culture immersed in wastewater with S_{max} of 0.9 g/L and a kinetic rate μ_{max} of 0.64 (Saravanan et al., 2009)

In Fig. 1(i), the simulation of the Yano-Koga model is shown. This model evaluates the dynamic behavior of continuous fermentation, taking into account growth inhibition at high substrate concentration (Saravanan et al., 2012). In the simulation carried out, the values obtained for μ_{max} and k_s were 19.99 d^{-1} and 9.999 g/L, respectively. Likewise, a substrate inhibitory constant of 14.7 g/L was observed. This model obtained the second-highest error of all those used, with F_{val} 11.12.

Finally, the Chen-Hashimoto model observed in Fig. 1(j) is widely used in anaerobic digestion processes and considers that the substrate concentration in the effluent (S) is related to the substrate concentration in the feed (S_0) (Martín et al., 1992). In addition, it evaluates organic matter degradation processes taking into account substrate utilization (Fernández, 2008). The lowest error between simulated and experimental data was obtained employing this kinetic model, achieving values of de 2.32 d^{-1}, 1.16 g/L, 1.13 g/L y 1.21 for μ_{max}, k_s, S_0 and F_{val}.

The Fmincon function minimizes the error between the experimental and the simulated data. The F_{val} term is calculated in each simulation and is responsible for providing information about the optimization process. Therefore, values close to zero are always desired. The Yano-Koga and Haldane models obtained the highest simulated errors in this research work. Due to this, it is inferred that the substrate concentration does not limit the growth rate of the microorganism. At the same time, the models that showed lower Fval values were the Chen-Hashimoto Model, Contois Model, and Moser Model, being considered the best for optimizing the lipopeptide-type biosurfactant produced by Bacillus subtilis RSL-2. The three kinetic models that presented the lowest error have been used in biodegradation processes under anaerobic conditions with the implementation of enzymatic machinery. Therefore, from the error calculated by the Fmincon function, it is inferred that the biosurfactant production by Bacillus subtilis RSL-2 is carried out under anaerobic conditions where hydrolysis controls microbial growth, besides being regulated by interaction phenomena and enzyme affinity.

4. Conclusions

In this research, the kinetics of biosurfactant production from crude oil was modeled and simulated by evaluating different kinetic expressions, and the results were compared with experimental data obtained in previous work. Furthermore, the observed and simulated data error was implemented using a nonlinearly constrained optimization technique. Considering the results found in this research, different scenarios predicted by the kinetic models allowed elucidating the primary basis of the mechanism of action for biosurfactants production using crude oil. That is why enzymatic interactions regulate biosurfactant production under anaerobic conditions where hydrolysis and biomass concentration control microbial growth. Therefore, the results obtained in this study are a reasonable hope for biosurfactant production from bioremediation processes. Furthermore, these research results are a promising target in industrial biotechnology as computational modeling is an essential tool for process efficiency from a technical and economic point of view.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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