



Review Article

Elucidation of Kinetic Studies in Biosurfactant Fermentative Production and Their Behaviour: A Mini-Review

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ABSTRACT

Bioprocess engineering, which include kinetic behaviour, is a fundamental form of developing effective product performance and functionality. Kinetic studies are one of the most important steps in any bioprocess and bioproduct development to elucidate the production phase and product behaviour. The lack of comprehensive understanding and limited knowledge of the kinetic behaviour of biosurfactant production, especially in the complex fermentation process involving microorganisms, substrate, and product formation, is major challenge and hinder the development of optimized biosurfactant in the industrial scale. Kinetic studies in the field of biosurfactants need to be emphasized to better understand the mechanisms of biosurfactant formation through biomass growth and substrate consumption. In this mini-review, various kinetic models used in biosurfactant work were critically discussed. The objective of this study is to analyze the kinetic studies of biomass growth, biosurfactant formation and substrate consumption focusing on the biosurfactant itself. This review revealed the various kinetic models used in fermentative biosurfactant production and how the different models were used and interpreted based on different substrates and biosurfactant-producing microorganisms. A key feature of this review is its focus on the biosurfactant, which provides valuable insights into the factors that maximize productivity and improve scale-up. Based on previous works, the logistic model is preferred to represent biomass growth, biosurfactant formation and substrate consumption due to its simplicity and rationale. Throughout this study, the kinetic profile of the biosurfactant able to be established and serve as a foundation for the prediction of the biosurfactant behaviour.

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INTRODUCTION

Biosurfactant is a bio-amphipathic molecule comprised of hydrophobic and hydrophilic moieties. The main property of biosurfactants is to solubilize different immiscible phases such as water and oil (Kong et al., 2017). Biosurfactants are biological compounds produced from a variety of microorganisms such as viruses, bacteria and fungi. The uniqueness of the biosurfactant in terms of low toxicity, high biodegradability, environmentally friendly and high

specificity gained special interest from researchers all over the world as the substitution of chemically synthesized surfactant (Bertrand et al., 2018; Karlapudi et al., 2018; Phulpoto et al., 2023). In this review, the kinetic modelling of the biosurfactant is discussed and reviewed to understand deeply behaviour of the biosurfactant formation.

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This mini review is focused on major parts of kinetic production which are biomass growth, biosurfactant formation and substrate consumption. This review is targeted at the biosurfactant formation through the fermentation process which comprised of the biosurfactant-producing microorganisms (biomass growth kinetic) and substrate medium utilized for the biosurfactant-producing microorganisms to synthesize biosurfactant (substrate consumption kinetic).

In the fermentation culture, a mass transfer process occurs, and metabolic pathways of microorganisms produce amphipathic molecules (Alvarado et al., 2022; Amodu et al., 2016). Biosurfactants, which have amphipathic molecules, undergo lipogenic pathways to produce hydrophobic parts and glycolytic pathways to produce hydrophilic parts (Santos et al., 2016). This biological process is fundamental to be investigated as part of bioprocess engineering, which is able to assist in the various aspects of the establishment products such as optimization, rate limiting factor and kinetic behaviour of the surfactant itself. The objective of this mini review is to assess the kinetic studies of biomass growth, substrate consumption and product formation, focussing on the biosurfactant itself. In the context of biosurfactants, this mini review may be useful to researchers in identifying the best kinetic model for their studies. In biosurfactant fermentation, kinetic studies offer researchers and industry professionals valuable insights into the underlying processes that allow them to maximize productivity and improve scale-up levels, which are ultimately applicable in making decisions for commercialization purposes.

2.0 Kinetic behaviours

Kinetic behaviour is one of the biotechnologies that have been implemented in various industrial processes and that serve as a theoretical framework for the simulation and analysis dynamic behaviour of the system. The mathematical model known as kinetic studies provides insight into the mechanism that explains the rate of reactions in a system over time. The establishment of the kinetic model in term of biosurfactant formation is fundamental and serve as a core to explore the production phase and growth biomass, designation of the scaling-up purpose and prediction of biosurfactant behaviour. In addition, throughout the kinetic behaviour, optimization of the variables is able to be determined and leads to the development of a high yield with high productivity (Câmara et al., 2020). A kinetic model comprised of the microorganisms, substrate and product formation is desirable to be investigated due to the complexity of the fermentation process itself. The ability of unique creatures such as microorganisms to synthesize metabolite (biosurfactant) in fermentation is still indefinite, thus seeking knowledge through kinetic modelling has been prospected as one of the effective strategies. To date, knowledge of kinetic behaviour is still limited and conducting kinetic studies especially in the field of biosurfactants, is essential to comprehend the behaviour of these valuable products. Kinetic behaviour is entitled to be studied, especially in bioprocess engineering, as it is able to grasp the knowledge of the process involved in biological reactions and elucidate the reaction mechanisms. Through the kinetic model, the optimized process is predicted through the identification of reaction rates, which is significant for large-scale industrial applications. Câmara et

al., (2020) also stressed the importance of identifying the association between biomass growth, biosurfactant formation and substrate consumption. To understand microbial biology, it is vital to select the best kinetic studies as an overview of the mechanisms of internal control (Câmara et al., 2020). Throughout the kinetic model, the prediction profile of the products is able to be established and the designation of the products is advantageous for the evaluation of the economic analysis (Heryani & Putra, 2017). Each model has their own objective and kinetic coefficient parameters that are essential in each condition of the fermentation. The researcher needs to identify the objective of the research design and able to select the kinetic model accordingly.

Most of the research emphasized kinetic studies to enable their understanding towards the type of growth cycle microorganisms, identifying whether it is growth-associated or non-growth-associated and the relationship of the biomass and biosurfactant. In addition, the prediction of the kinetic coefficient parameter is able to give perspective for the biosurfactant profile, and compatibility of the substrate and microorganisms to synthesize metabolites. Table 1 shows various previous studies on biomass growth and substrate consumption related to biosurfactant formation. Three main categories can be studied in biosurfactant formation, namely biomass growth, biosurfactant production and substrate consumption. Kinetic modelling is able to elucidate the fermentation process in terms of maximum biomass and biosurfactant production, coefficient of the yield and initial specific growth rate by predicting and forecasting product formation and microbial growth (Zhu et al., 2014). Table 1 shows that studies involving kinetic modelling are evolving from 1996 to 2022, but the number is still small when compared with the existence of biosurfactant production every year. Most of the studies investigated the product formation, i.e., biosurfactant and microorganisms-produced biosurfactant (biomass growth model) and substrate utilized in the fermentation process (substrate consumption).

Table 1: Selected previous studies of the kinetic modelling of biomass growth, biosurfactant production and substrate utilization.

Microorganisms	Substrate medium	BGM	BFM	SUM	References
<i>Rhodococcus erythropolis</i> AQ5-07	Waste canola oil	/			(Ibrahim et al., 2020)
<i>Bacillus amyloliquefaciens</i> XZ-173	Soybean and rice straw	/	/	/	(Zhu et al., 2014)
<i>Pseudomonas aeruginosa</i>	Glucose	/	/	/	(Câmara et al., 2020)
<i>Bacillus subtilis</i>	Crude oil		/		(Alvarado et al., 2022)
<i>Pseudomonas aeruginosa</i> BS2	Crude oil		/		(Babu et al., 1996)

<i>Bacillus</i> sp	Glucose	/	/	(Heryani & Putra, 2017)
<i>Pseudomonas</i> sp	waxes	/	/	(Sakthipriya et al., 2015)
<i>Lactobacillus</i>	Lactose, glucose	/	/	(Montoya Vallejo et al., 2021)
<i>Brevundimonas</i> sp	Crude oil	/	/	(Ray et al., 2021)
<i>Bacillus licheniformis</i>	Beta vulgaris	/	/	(Amodu et al., 2016)
<i>Lactobacillus</i> strain	MRS broth	/	/	(Rodrigues et al., 2006)

BGM: biomass growth model, BFM: biosurfactant formation model and SUM: substrate consumption model.

In particular, the kinetic studies of biosurfactant from agro-waste substrates still poorly understood, as it involved the conversion of the soluble substrates into biosurfactant metabolites (Amodu et al., 2016; Babu et al., 1996; Câmara et al., 2020; Heryani & Putra, 2017; Montoya Vallejo et al., 2021; Zhu et al., 2014). The kinetic studies from the agro-waste substrate are worthy to be investigated due to the complexity and heterogenous composition itself. Other than that, the reaction and mechanism pathway for the agro-waste through glycolysis is unique to be studied with the bioconversion of the substrate towards metabolites such as biosurfactant. The most preferred model implemented for these three categories is a Logistic model, which is suitable for most of the substrates and biosurfactant producing microorganisms. The kinetic model discussed in this review comprised of the Monod, Modified Gompertz, Leudeking-Piret, Chen-Hashimoto, Haldane, Haldane-Andrew and Logistic model as follows:

3.0 Kinetic model

3.1 Monod model

The Monod model is a model that was first formulated by Jacques Monod in 1942 and highlighted the relationship between specific growth rate (μ) of microorganisms and rate of the substrate consumption (Muloiwa et al., 2020). Monod model can be classified into two categories, which are based on both biomass and substrate concentration or substrate concentration only (Muloiwa et al., 2020). The Monod model is also one of the models that is widely used to represent the biomass growth model. The coefficient kinetic parameters in the models are displayed below in (Equation 1) such as μ is a specific growth rate (h^{-1}), substrate (S), maximum specific growth rate (μ_{\max}) and half saturation constant (K_s).

$$\mu = \frac{\mu_{\max} \cdot S}{K_s + S} \quad (1)$$

Câmara et al., (2020) analysed the biomass growth of the *P. aeruginosa* through four unstructured models (Monod, Andrew, Alba and Luong) and revealed that Monod is the best fitted for the experimental data. The result revealed that the Monod model with the values of μ_{\max} , K_s , $Y_{p/x}$ and $Y_{x/s}$ are 0.06 h^{-1} , 50.8 g/L , 0.017 g.g^{-1} and 0.43 g.g^{-1} . The Monod model assumes that adaptations of microorganisms towards the substrate are not necessary

and microorganisms start to multiply at the exponential growth phase (Câmara et al., 2020). It can be indicated that the lag phase is not included in the Monod model due to the efficient use of inoculum that permits the transfer of microorganisms to the culture medium in its higher growth velocity phase (Câmara et al., 2020). Monod proposed that all the elements in the culture medium are present with the high concentration, and the substrate is only the limiting compound. With that, the changes in the concentrations of the substances do not impact cell growth (Câmara et al., 2020). K_s is described as a substrate concentration where the growth rate is half that of the maximal rate. The high value of the K_s indicated that the strain was utilized for a longer time in the exponential phase, and the high difference between the value of μ_{\max} in this study, explained by the shorter period in the exponential phase and the value of the growth velocity was distant from its maximum velocity (μ_{\max}) (Câmara et al., 2020; Viggor et al., 2019).

Even though the Monod model is extensively represented in the biomass growth model, several limitations might interfere with the experimental data to fit with the Monod model. The limitations are listed as follows (Muloiwa et al., 2020):

- Incapable of predicting the growth rate when toxic substrate is present. The substrate consumption and microorganism growth are obstructed by the existence of toxic substrate.
- The microorganisms might require substrate for the maintenance during the death phase which is not included in the Monod model.
- The lag and death phase in the bacteria cycle does not account for the Monod model.
- The maximum specific growth rate does not depend on the substrate concentration at a high concentration of substrate.
- The maximum specific growth rate relies on the concentration of substrate when at low concentration.

These limitations do not obstruct many studies from utilizing the Monod kinetic model and remain extensively used in the present studies, as it is suited to be used accordingly to the microorganisms.

3.2 Modified Gompertz model

The Modified Gompertz model is classified under non-substrate inhibition kinetic models. It was also reported that the Modified Gompertz model was employed in the biosurfactant formation (Heryani & Putra, 2017; Zhu et al., 2014). Heryani & Putra (2017) also applied Modified Gompertz in the prediction of biomass profile. The equation for the Modified Gompertz is displayed in Equation 2.

$$P = P_{\max} \cdot \exp\left(-\exp\left[\frac{R_{\max} \cdot e}{P_{\max}} \cdot (t_L - t) + 1\right]\right) \quad (2)$$

Whereas P is a biosurfactant concentration at time (t) (g/L), P_{\max} is a maximum biosurfactant concentration (g/L), R_{\max} is a maximum rate of the biosurfactant formed [$\text{g}/(\text{L}\cdot\text{h})$], t_L is a lag to the exponential time to the product formation (h) and t is a time of fermentation.

The study conducted by Zhu et al., (2014) investigated the biosurfactant model using Modified Gompertz through

two types of temperature fermentation (isothermal and non-isothermal) and the result showed that the prediction profile of the P_{max} was the highest in the isothermal flask. [Zhu et al., \(2014\)](#) also added that a high concentration of lipopeptide was reported along with the low value of the biomass after a period of fermentation, which might occur because the microorganisms lysed the membrane and caused the degradation of the cells. Microorganisms also build up resistance towards accumulation of the lipopeptides as a self-resistance ([Zhu et al., 2014](#)). Even though the studies conducted by [Zhu et al., \(2014\)](#) had a stirring and temperature control device, it also found trouble with the heat transfer and limitation of nutrients. Thus, leading to the low productivity of the lipopeptide formation ([Zhu et al., 2014](#)).

In the studies conducted by [Heryani & Putra, \(2017\)](#) for the kinetic study of *Bacillus* sp, they proposed a Modified Gompertz model for the biomass growth and biosurfactant production and revealed that it was well predicted for both types. In addition, [Heryani & Putra, \(2017\)](#) also applied extended Modified Gompertz to enable the prediction of the surface tension in broth media and found that the R^2 of 0.99 and well fitted with the experimental data.

3.3 Leudeking-Piret model

The Leudeking-Piret model is one of the models that is frequently applied in the biotechnology field, as it is concerned with product formation and microbial growth. [Zhu et al., \(2014\)](#) used Leudeking-Piret in their studies and mentioned that consumption of substrate might be able to be interpreted as the conversion of substrate to the lipopeptide and maintenance. The equation for the Leudeking-Piret is as follows (Equation 3):

$$-\frac{dS}{dt} = \frac{1}{Y_x^s} \cdot \left(\frac{dX}{dt}\right) + \frac{1}{Y_p^s} \cdot \left(\frac{dP}{dt}\right) + mX \quad (3)$$

In linear form:

$$S = S_0 - \frac{1}{Y_x^s} \cdot (X - X_0) - \frac{1}{Y_p^s} \cdot (P - P_0) - m \cdot \frac{X_{max}}{\mu_{max}} \cdot \ln\left(1 - \frac{X_0}{X_{max}} \cdot (1 - \exp(\mu_{max} \cdot t))\right) \quad (4)$$

Whereas, S is the substrate concentration at time (t) (g/L), S_0 is the initial substrate concentration (g/L), $Y_{x/s}$ is the yield coefficient of the biomass to the substrate (g/g), $Y_{p/s}$ is the yield coefficient of biosurfactant to the substrate (g/g), X is the biomass concentration at time (t) (g/L), X_0 is the initial biomass concentration (g/L), X_{max} is the maximum biomass concentration (g/L) and m is the maintenance.

According to the studies conducted by [Zhu et al., \(2014\)](#), a reduction of the substrate (total sugar) concentration occurred after 24 hrs of the fermentation. It is also reported that cell growth and product formation are concerning substrate consumption ([Zhu et al., 2014](#)). In a comparison between non-isothermal and isothermal processes, high consumption of the substrates was required resulting in a greater reduction of the total sugar concentration in the culture in the isothermal process. The larger the consumption of substrates acting as an indicator, the smaller the concentration of sugar in the flask associated with the higher yield of lipopeptides and biomass ([Zhu et al., 2014](#)). Other than that, [Zhu et al., \(2014\)](#) also stressed that temperature also influenced the consumption of substrate,

biomass and lipopeptide formation. The microbial activity of the microorganisms could be improved in higher temperatures, which caused the non-isothermal process to expedite the substrate transformation. As a consequence, it enhanced the product formation, carbon in the substrate utilized for cell formation as well as maintenance purposes ([Zhu et al., 2014](#)). [Amodu et al., \(2016\)](#) also investigated Logistic incorporated Leudeking-Piret model in their studies and found that it gave a good-fitted model with correlative coefficient (R^2) of 0.9855.

3.4 Chen-Hashimoto model

Chen-Hashimoto model is extensively used in the anaerobic digestion process with the assumption that substrate concentrations (S) are in association with the initial substrate concentration (S_0). In addition, this model considers that substrate concentrations influenced the organic matter degradation ([Alvarado et al., 2022](#)). [Alvarado et al., \(2022\)](#) assessed various kinetic models of biosurfactants from *B. subtilis* such as Powell, Monod, Haldane, Moser, Teissier, Contois, Luong and Aiba-Edward model and found that Chen-Hashimoto is the best-represented model. The remarkable of the studies compared with other studies was through numerical computational modelling, Runge-Kutta 45 method (Fmincon Matlab R2117b). The biosurfactant modelled by Chen-Hashimoto is calculated by the expression below (Equation 5) with kinetic coefficient parameters maximum specific growth rate (μ_{max}), S is a substrate concentration, S_0 is an initial substrate concentration and K_s is a half saturation constant, K_d is a specific cell death velocity:

$$\mu = \frac{\mu_{max}S}{K_{S_0} + (1-K)S} - b \quad (5)$$

Through this model, the result revealed that μ_{max} , K_d , K_s and S_0 values of 2.3239 d⁻¹, 0.3748 d⁻¹, 1.1619 g/L and 1.1286 g/L ([Alvarado et al., 2022](#)). It was concluded that kinetic modelling by *B. subtilis* to degrade the crude oil occurred during anaerobic conditions in which the biomass growth was governed by hydrolysis ([Alvarado et al., 2022](#)). To date, only one research article reported the kinetic modelling with the Chen-Hashimoto mathematical model of a biosurfactant ([Alvarado et al., 2022](#)). However, Chen-Hashimoto model has been applied in various studies such as biomethane production and methane production ([Husain, 1998](#); [Ketsub et al., 2021](#)).

3.5 Haldane model

Haldane model was created by Haldane in 1930 ([Muloiwa et al., 2020](#)). [Muloiwa et al., \(2020\)](#) also stated that the Haldane model is an extension of the Monod model, with the third constant which is K_i . K_i is an inhibition constant that is applicable when the substrate is toxic and leads to specific growth rate inhibition at high or low concentrations. The derived equation of the Haldane model is as follows (Equation 6):

$$\mu = \frac{\mu_{max}S}{K_s + S + \frac{S^2}{k_i}} \quad (6)$$

In a medium where the substrate concentration is high, there is a possibility that the specific growth rate of the organisms may be inhibited by the existence of a toxic substrate. K_i is substantial at addressing the specific growth rate inhibition at high and low substrate concentrations. K_i

is the inhibition constant equal to the highest substrate Concentration at which the specific growth rate is equal to half the maximum growth rate without inhibition (Muloiwa et al., 2020). This model is also reasonable to be applied to investigate whether the substrate is toxic or non-toxic. The Haldane model has been hardly found waiting when being fitted to any experimental data (Muloiwa et al., 2020). In a study conducted by Ibrahim et al., (2020), with waste canola oil-degrading bacteria, it was found that the value of K_i was 0.399%, K_s was 7.74% and μ_{max} was 0.142 h^{-1} . The study of *Rhodococcus erythropolis* AQ5-07 used canola oil as a substrate medium showed that a high concentration of the canola oil had an inhibitory effect as it impacted the lag phase of the bacteria growth (Ibrahim et al., 2020). In the Haldane model, high substrate caused the point of inhibition in the single continuous fermentation as the growth rate reached the exponential phase (X_{max}) and the growth rate dropped down until zero (asymptote) (Ibrahim et al., 2020). Despite that, Haldane has major drawbacks as it cannot be applied when the growth rate becomes zero at a very high substrate concentration and it is unable to predict inhibition constant (S_m) (Ibrahim et al., 2020). Substrate inhibition model is a mathematical model in which the substrate has a tendency to inhibit the formation of metabolites. High concentration of the substrate might cause the inhibition of the reaction thus leading to lower rate of reaction.

Haldane was selected due to its simplicity and capability to represent the growth substrate inhibition kinetics by the integration of the growth-inhibition constant and substrate (Ibrahim et al., 2020). Even though the Haldane model is suited for the situation where inhibition of substrate presence, this model faces the limitation of the inability to describe certain conditions where the growth rate reaches zero at very high substrate concentration (Ibrahim et al., 2020). The advantage of applying the Haldane model is the suitability to formulate the experimental data at all growth phases, such as lag, exponential, stationary and death phases (Muloiwa et al., 2020).

3.6 Haldane-Andrew Model

Other than the Haldane model, the Haldane-Andrew model is also one of the models formulated with the conditions of the inhibition constant. The Haldane Andrew model is used to determine the growth kinetics with the inhibition constant as follows (Equation 7):

$$\mu = \mu_{max} \frac{S}{K_s + S + \frac{S^2}{K_i}} \quad (7)$$

The Haldane-Andrew model was applied by Ray et al., (2021) for the investigation growth kinetic of different types of bacterial strains (*Brevundimonas* sp. IITISM 11, *Pseudomonas* sp. IITISM 19 and *Pseudomonas* sp. IITISM 24) and substrate (anthracene and fluorene). The studies found that the Haldane-Andrew model is best fitted for the studies compared with the Monod model (Ray et al., 2021). Haldane-Andrew model applied at the high substrate concentration and portrayed the inhibition of the microbial growth (Priyadarshini et al., 2021). Furthermore, Monod model is only applied on the kinetics of the single substrate, while Haldane-Andrew model able to be implemented at the many substrates reaction (Ray et al., 2021).

4.0 Logistic model

4.1 Biomass growth

The logistic model is widely used in studies involving biosurfactant formation, biomass growth and substrate consumption (Amodu et al., 2016; Montoya Vallejo et al., 2021; Rodrigues et al., 2006; Sakthipriya et al., 2015; Zhu et al., 2014). The logistic model was employed to portray the microbial growth, inactivation or inhibition of the microorganisms (Wachenheim et al., 2003). Theoretically, the logistic model targeted the exponential growth of the growth cycle until it reached the saturation point (Wachenheim et al., 2003; Zhu et al., 2014). The logistic model is strongly linked with the growth cycle of the microorganisms, which takes into consideration exponential growth and measures the fixed limit of the growth (Zhu et al., 2014). It became the top choice of the microbial growth model as it does not involve substrate term and is appropriate for microbial growth, due to the constant value of inoculation volume and initial substrate concentration (Zhu et al., 2014). Muloiwa et al., (2020) also added that the Logistic model is a substrate-independent model, and dependent on the biomass concentration only. This model is also suitable for the inhibition of substrate and based on the assumption that the growth rate of an organism is proportional to the current population, and the unutilized resources in a closed habitat (Muloiwa et al., 2020).

In 1838, Verhulst gave insight to Pearl and Reed (1920) to formulate their first logistic model (Wachenheim et al., 2003). It was stated that the graph of the population density against time has a sigmoidal shape for numerous organisms (Wachenheim et al., 2003). The mathematical model proposed by Verhulst is as follows (Bac  er, 2011) (Equations 8 and 9):

$$\frac{dX}{dt} = \mu_0 X \left(1 - \frac{X}{X_{max}}\right) \quad (8)$$

In linear form:

$$X(t) = \frac{X_0 X_{max} \exp(\mu_0 t)}{X_{max} - X_0 + X_0 \exp(\mu_0 t)} \quad (9)$$

The kinetic coefficient parameters identified in Equation 9 are X_0 , X_{max} , and μ_0 . Equation 9, proposed by Verhulst, is the development of the Malthusian exponential model, with the μ_0 as kinetic coefficient parameters (Amodu et al., 2016). Equation 9 is suitable for the experimental data focusing on the early exponential growth phase (S-shape), which is the instantaneous growth response towards environmental conditions. Even though Equation 9 is desirable to be applied for the early exponential phase, prediction of the μ_{max} is not able to be obtained or the impacts of the nutrient's availability at a higher population density. Amodu et al., (2016) and Rodrigues et al., (2006) applied Equation 9 in their studies for biomass growth, biosurfactant and substrate consumption.

The evolution of the logistic models toward different kinetic parameters occurred due to the suitability of the study, parameters have an unreasonable value or the models unable to simulate the microbial growth (Mercier et al., 1992). Each coefficient parameter has been modified to have its biological meaning that able to describe microbial growth in empirical (Wachenheim et al., 2003). Next, Wachenheim et al., (2003) re-investigated the logistic model to facilitate the growth cycle of the microorganisms and

proposed the equation below (Equation 10 and 11). Some studies reported the logistic model from [Wachenheim et al., \(2003\)](#) and found the factors that inhibit microbial growth. Logistic form in the derived and linear model is presented as follows (Equation 10 and 11):

$$\frac{dX}{dt} = \mu_{max} \cdot \left(1 - \frac{X}{X_{max}}\right) \cdot X \quad (10)$$

In linear form:

$$X = \frac{X_0 \cdot \exp(\mu_{max} \cdot t)}{1 - \left(\frac{X_0}{X_m}\right) \cdot (1 - \exp(\mu_{max} \cdot t))} \quad (11)$$

[Zhu et al., \(2014\)](#) used Equation 11 to investigate the kinetic of microbial growth of *B. amyloliquefaciens* XZ-173 in isothermal and non-isothermal conditions. The isothermal condition for the *B. amyloliquefaciens* has a low value of X_{max} and a high level of μ_{max} when compared to the non-isothermal, and validated that the variation temperature caused the decreasing specific growth rate (μ) of apparent biomass leading to the positive influence on the accumulation of the biomass ([Zhu et al., 2014](#)).

In addition, a new derivation of the logistic model was mathematically modelled by [Mercier et al., \(1992\)](#) for the kinetics of the lactic acid fermentation. According to [Mercier et al., \(1992\)](#), many models have been successfully reported for lactic acid fermentation, however, none of them are able to represent their kinetic experimental data process. Thus, [Mercier et al., \(1992\)](#) attempted to formulate a mathematical model which comprised the biomass concentrations, lactic acid formation and substrate consumption. As a result, many researchers adapted the Mercier equation to represent the kinetic modelling of biosurfactant production. It has stated that the logistic model reasonably represented the product accumulation, biomass growth and substrate consumption kinetic pattern ([Rodrigues et al., 2006](#)). [Rodrigues et al., \(2006\)](#) added that logistic models fairly predict the biosurfactant production with adjustment of a statistical significance of the parameters determined. The mathematical model proposed by Mercier is as follows (Equations 12 and 13):

$$\mu = \frac{1}{X} \frac{dX}{dt} = \mu_{max} \left(1 - \frac{X}{X_{max}}\right) \quad (12)$$

Linear form:

$$X = \frac{X_0 X_{max} \exp \mu_{max} t}{X_{max} - X_0 + X_0 \exp \mu_{max} t} \quad (13)$$

Whereas, X_0 = initial biomass concentration (g/L), X_{max} is a maximum biomass concentration (g/L), μ_{max} is a maximum specific growth rate (h^{-1}), t is a time and X is a biomass concentration (g/L).

The mathematical model proposed by [Mercier et al., \(1992\)](#) was followed by [Rodrigues et al., \(2006\)](#) and [Sakthipriya et al., \(2015\)](#) for biosurfactant production. [Sakthipriya et al., \(2015\)](#) revealed the absence of the relationship between biosurfactant production and growth of the microorganisms. It has been mentioned that production of the biosurfactant is considered as non-growth associated with the reason of the rhamnolipid production consistent even at the stationary growth phase ([Sakthipriya et al., 2015](#)). While ([Rodrigues et al., 2006](#)) found that the

formation biosurfactant was observed at the early phase of the cell growth (first four hours) which can be regarded that substrate consumption in the low level and biomass growth is nearly absence. The specific growth rate (μ) is defined as the growth rate per individual concerning time which can be described as the rate at which the microorganisms are able to multiply in time. Each coefficient parameter has been modified to have its biological meaning that able to describe microbial growth in empirical ([Wachenheim et al., 2003](#)). Equations 4 and 6 employed the μ_{max} as the coefficient kinetic parameter with the measurement of the maximum microbial growth rate in response to substrate concentrations. It assumed that the growth rate solely depended on the availability of nutrients until it reached saturation point. Other factors such as initial population size, environmental conditions and competition do not influence the rate of microbial growth. With the prediction of the X_{max} , it is able to predict the level of the microorganisms to reach saturation value, in which the value is important for designation optimum condition. There are many factors influenced the value of X_{max} and μ_{max} in each medium, such as type of microorganisms, type of substrate medium, temperature, incubation period and agitation.

4.2 Biosurfactant formation

The logistic model is not only applied to the biomass growth kinetic, but it is also preferable to be used in the biosurfactant formation. The logistic model for biosurfactant formation was mathematically modelled by analogy of the biomass growth model. The logistic models proposed by ([Mercier et al., 1992](#)) been applied in the biosurfactant formation are written as follows (Equation 14 and 15):

$$\frac{dP}{dt} = P_r \left(1 - \frac{P}{P_{max}}\right) P \quad (14)$$

Equation 14 was integrated to obtain Equation 15 as follows:

$$P = \frac{P_0 P_{max} \exp P_r t}{P_{max} - P_0 + P_0 \exp P_r t} \quad (15)$$

Whereas, P is a biosurfactant concentration, P_0 is an initial biomass concentration, P_{max} is maximum biomass concentration, P_r is ratio between the volumetric rate of product formation and product concentration (h^{-1}).

Equation 15 was applied in the biosurfactant formation and comprised of the kinetic parameters such as P_0 , P_{max} and P_r ([Montoya Vallejo et al., 2021](#); [Rodrigues et al., 2006](#); [Sakthipriya et al., 2015](#)). The Logistic equation used in the biomass model is similar to the biosurfactant model due to the close relationship between microorganisms' growth and product formation. Even though [Sakthipriya et al., \(2015\)](#) employed the logistic model for biosurfactant production, the value of the P_{max} is not stated in their studies. The kinetic modelling of *L. plantarum* produced biosurfactant with a P_{max} value of 0.199 g/L and X_{max} of 4.15 g/L in the duration of 24 hrs and presumed that biosurfactant produced at the exponential phase, been identified as growth-associated metabolites ([Montoya Vallejo et al., 2021](#)). While [Rodrigues et al., \(2006\)](#) revealed that the biosurfactant production started at the beginning of the cell growth (lag phase), mainly in the first 4 hours, in which low substrate consumption and absence of cell growth. P_0 for the *L. pentosus* obtained in the studies with

the value of 0.4 g/L and predicted to reach a P_{max} of 1.4 g/L (Rodrigues et al., 2006). Even though biosurfactant production started in an early phase and continued up until all 72 hrs of fermentation with a slow production rate (Rodrigues et al., 2006). The slow production rate can be assumed as a consequence of the pH reduction due to the lactic acid and accountable for the product inhibition (Rodrigues et al., 2006). It can be observed the maximum production of the biosurfactant occurred at the early of the exponential phase.

Besides that, Amodu et al., (2016) reported the different logistic models for biosurfactant formation. Amodu et al., (2016) mentioned in their studies that this logistic model is also proposed by Mercier (Equations 14 and 15), even though the equation was different from Equations 16 and 17. The logistic model mentioned by Amodu as follows:

$$\frac{dP}{dt} = \frac{P_r P}{P_0} \left(1 - \frac{P}{P_{max}}\right) \quad (16)$$

Which can be integrated to obtain Equation 10 as follows:

$$P(t) = \frac{P_0 P_{max} \exp\left(\frac{P_r t}{P_0}\right)}{P_{max} - P_0 + P_0 \exp\left(\frac{P_r t}{P_0}\right)} \quad (17)$$

Whereas $P(t)$ is a biosurfactant concentration at time, P_0 is an initial biomass concentration, P_{max} is maximum biomass concentration, P_r is ratio between the volumetric rate of product formation and product concentration (h^{-1}).

The association of cell growth and product formation also can be validated with a logistic-incorporated Leudeking and Piret model (Amodu et al., 2016). The above equation also can be applied to predict biosurfactant production (Equation 18):

$$P(t) = P_0 + \alpha X_0 \left[\frac{\exp(\mu_0 t)}{1 - \left(\frac{X_0}{X_m}\right)(1 - \exp(\mu_0 t))} - 1 \right] + b \frac{X_{max}}{\mu_0} \ln \left[1 - \frac{X_0}{X_{max}(1 - \exp(\mu_0 t))} \right] \quad (18)$$

Whereas α is a growth product formation coefficients and b is a non-growth product formation coefficient.

Several authors proposed that the rate of growth influenced the rate of biosurfactant production Amodu et al., (2016) stated the relationship growth of the microorganisms and biosurfactant able to be validated through a Logistic incorporated Leudeking and Piret model (Equation 18) with the value of α (growth) and β (non-growth) product formation coefficient. Amodu et al., (2016) added that the early exponential phase is a time when the biosurfactant production starts and grows until it reaches the stationary growth phase after 40 hours. Mostly biosurfactant production is reported to be growth-dependent, which means that often reached at the onset of the stationary growth phase or the peak of the mid-exponential phase (Amodu et al., 2016).

4.3 Substrate consumption kinetic

The substrate functions as a major carbon source for the microorganisms to synthesize biosurfactants. The importance of the substrate and set-up is fundamental to understanding the complex biological substrate consumption kinetics. It is one of the strategies to comprehend the mechanisms of internal control and allows

an overview of the up-scaling process (Câmara et al., 2020). The logistic equation for the substrate consumption is as follows (Equation 19):

$$S = S_0 - \frac{1}{Y_{P/S}}(P - P_0) - \frac{1}{Y_{X/S}}(X - X_0) \quad (19)$$

Whereas S_0 is an initial substrate concentration (g/L). $Y_{P/S}$ is a yield coefficient of biosurfactant to the substrate (g/g), $Y_{X/S}$ is a yield coefficient of the biomass to the substrate (g/g).

Several previous studies employed the logistic model for substrate consumption kinetic model (Montoya Vallejo et al., 2021; Rodrigues et al., 2006; Sakthipriya et al., 2015). It is vital to identify the value of the substrate yield as an indicator of the distribution of substrate consumption for growth and metabolite synthesis (Montoya Vallejo et al., 2021).

4.0 Conclusion

In conclusion, all types of kinetic models representing biomass growth, formation and substrate consumption have their uses as well as limitations. The selection of the model is essential to establish the strong profile of the biosurfactant produced. One of the most important criteria other than selecting a good kinetic model, the ability to experiment, collect, analysing data also influenced the efficiency of the kinetic behaviour. The kinetic parameters for these models also show the interrelationship between biomass growth, biosurfactant production and substrate consumption. The most reported model that is suitable for biomass growth, biosurfactant formation and substrate consumption is a logistic model. The Monod faces limitations in representing the lag and death phase of the microorganisms, and the Haldane model has the advantage of expressing the whole microorganism cycle. As observed, the biomass growth model has the same analogy as the biosurfactant model and can be used together. While the substrate model is used to investigate either the growth-associated or non-growth associated with the biomass. Using the kinetic model allows bioprocess engineers to develop high-quality biosurfactants and predict parameters that can be used in the designation of the bioprocess.

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