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MODELING OF REACTION KINETICS FOR THE PRODUCTION OF MICROBIAL POLYHYDROXYALKANOATES BY BACILLUS MEGATERIUM

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Summary. The production of poly(3-hydroxybutyrate) PHA by *Bacillus. megaterium* depends exclusively on the concentration of the carbon source (glucose), so it is proposed to use mathematical simulation models for the production of poly(3-hydroxybutyrate) PHA mathematical simulation models are proposed for the kinetics the kinetics applied in the production of microbial polyhydroxyalkanoates. Bacteria can be isolated from California redworm humus, and a logistic model including an inhibition factor and a constant associated with cell maintenance is used for biomass growth. In the kinetics of product formation, the Leudeking-Piret model is proposed, where the product formation coefficient depends on cell growth and the constant associated with cell maintenance, both of which are determined by the fermentation pH, and correspond to associated and non-associated growth, respectively. The model for substrate consumption considers that cells metabolize substrate for growth, product synthesis and energy generation, as well as for internal pH control activities and exchange of cellular components. Kinetic equations are proposed to estimate experimental results of this case study based on the logistic, Leudeking-Piret and substrate consumption models, to determine the values of biomass and product yields, depending on the substrate used in the stoichiometry of PHA production. The next stage involves the application of UV-Vis spectrophotometry to estimate cell growth in Colony Forming Units (CFU) and its comparison with the McFarland scale to equivalently quantify the number of bacterial cells.

Key words: Kinetic models, Bacillus. megaterium, biopolymer.

MODELACIÓN DE LA CINÉTICA DE REACCIÓN PARA LA PRODUCCIÓN DE POLIHIDROXIALCANOATOS MICROBIANOS MEDIANTE BACILLUS MEGATERIUM

Resumen. La producción de poli-(3-hidroxibutirato) PHA por *Bacillus. megaterium* depende exclusivamente de la concentración de la fuente de carbono (glucosa), por lo que se propone utilizar modelos matemáticos de simulación para la cinética aplicada en la producción de polihidroxialcanoatos microbianos. Las bacterias pueden aislarse del humus de la lombriz roja californiana, y para el crecimiento de biomasa se utiliza un modelo logístico que incluye un factor de inhibición y una constante asociada al mantenimiento celular. En la cinética de formación de producto se propone el modelo de Leudeking-Piret, donde el coeficiente de formación del producto depende del crecimiento celular y la constante asociada al mantenimiento celular, ambas están determinadas por el pH de la fermentación, y corresponden al crecimiento asociado y no asociado respectivamente. El modelo para consumo de sustrato considera que las células lo metabolizan para crecimiento, síntesis de producto y generación de energía, así como para actividades de control de pH interno e intercambio de componentes celulares. Se plantea ecuaciones de cinética para estimar resultados experimentales de este caso de estudio basadas en los modelos logístico, de Leudeking-Piret y de consumo de sustrato, para determinar los valores de los rendimientos de biomasa y de producto, en función al sustrato utilizado en la estequiometria de la producción de PHA. La siguiente etapa contempla la aplicación de la espectrofotometría UV-Vis para estimar el crecimiento celular en Unidades Formadoras de Colonias (UFC) y su comparación con la escala de McFarland para cuantificar de forma equivalente el número de células bacterianas.

Palabras clave: Modelos cinéticos, Bacillus. megaterium, biopolímero.

Introduction

Animals and plants possess reserve substances of different chemical nature that provide them with matter and energy for the realization of numerous functions, this type of reserves also exist in microorganisms and provide them endogenously with energy and carbon for specific processes such as the maintenance of the internal pH, osmotic regulation and sporulation (Anderson, 1990; Dawes, 1973). One form of intracellular accumulation of reserve substances among many bacterial genera of the most diverse types is the formation of polymers composed of hydroxy acid units: Polyhydroxyalkanoates (PHA's). In most bacterial species the PHA's are located in intracellular granules that can be visualized under the light microscope by staining with the specific dyes Sudan Black (Smibert, 1981) and Nile Blue (Ostle, 1982).

The *Bacillus megaterium* is a Gram-positive bacillus-like bacterium, mainly aerobic in metabolism, which forms spores and is found in a wide variety of habitats, reaching 1.5 to 3 μm in diameter and up to 5 μm in length (Holt, 1994) it is one of the largest known bacteria. Its cells usually occur in pairs or chains, it grows at temperatures between 3°C and 45°C, with an optimum around 30°C, in some isolates from an Antarctic hydrothermal lake they grow at temperatures of 63°C.

It is considered an endophyte (lives as an endosymbiont of plants without causing damage) and is a potential agent for plant disease control. It has been shown that certain strains can be used to fix nitrogen. It produces the penicillin amidase enzyme used to produce synthetic penicillin, several amylases used in the baking industry and glucose dehydrogenase (enzyme used in blood glucose testing). In addition, it is used for the production of pyruvate , vitamin$_{B12}$, drugs with fungicidal and antiviral properties , etc.. It produces enzymes to modify corticosteroids and several amino acid dehydrogenases. It produces poly-γ-glutamic acid, the accumulation of the polymer increases considerably in saline environments (2-10% NaCl), in which the polymer is largely formed by L-glutamate (the content of the L-isomer is up to 95%).

At least one strain of *B. megaterium* can be considered halophilic, as it grows in concentrations of up to 15% NaCl. This species can be found in soils, seawater and its sediments, rice husks, dried foods and milk, throughout all latitudes of the planet.

This bacterium is very versatile because, in addition to its arsenal of enzymes usual for all Gram-positive microorganisms, it possesses other types of enzymes uncommon for its genus; this gives it great metabolic plasticity, which translates into a remarkable ability to survive in different environments and ecological niches (Rao, 2019). Its ability to metabolize different compounds rivals that of the genus Pseudomonas. (Vary, 1992). The most important reserve compound in this species is poly(3-hydroxybutyrate) (PHB), which accumulates under limiting conditions of nitrogen, phosphates, sulfur or potassium (Slepecky, 1961).

Worldwide, more than 30% of waste corresponds to petroleum-derived plastics that generate toxins and are not biodegradable. Polyhydroxyalkanoates (PHA's) are biopolymers that represent a solution to the environmental pollution caused by synthetic plastics, since they come from a biological organic synthesis and therefore their degradation is fast, it can be in a moderate time of up to 9 months (Bailey, 1986; Verlinden, 2007).

In this proposal it is assumed that PHB production by *B. megaterium* depends exclusively on glucose concentration. Since the specific growth rate variable depends on the biomass of the microorganism involved, it is essential to evaluate its reproduction rate for stoichiometric product generation.

The glucose fermentation system in microbial metabolism is integrated by three different reactions that occur simultaneously, for this work we propose a kinetic model for substrate consumption (glucose), one more for biomass growth (*Bacillus megaterium*bacterial cells) and finally another one for PHA's production. The feasibility of biopolymer synthesis under controlled parameters such as pH, dissolved oxygen and temperature is theoretically estimated. The objective of the present work is to propose three mathematical simulation models for reaction kinetics.

The growth of cells is obtained by the product of interactions between biochemical reactions and the phenomena of matter and energy transfer through various stages comprised in systems. The mixture of new and old cells undergoes constant changes during the development process represented by a growth curve, while adapting to an environment with permanent variations in physical and chemical conditions. Because of the difficulty in accurately modeling growth kinetics, some assumptions must be made to obtain simple models for the design, operation and prediction of fermentation process behavior. The unstructured model is the simplest and most commonly employed, and is based on the following implications: the cells can be represented by a single component such as cell number, cell mass or the concentration of proteins, deoxyribonucleic acid (DNA) or ribonucleic acid (RNA); the bacterial population has a uniform distribution; the cell suspension is homogeneous; the heterogeneous nature of the cells is not considered and the cell concentration is expressed as mass of cells on a dry basis per unit volume; the medium is formulated so that only one component is rate limiting; the other components are present in sufficiently high concentrations to avoid the effect of small changes on the reaction rate; the biological reactor is controlled to ensure a constant level of suitable environmental conditions (González, 2013).

Microbial kinetics is analyzed in relation to the formulation of unstructured growth models. The simplest models of bacterial growth, also called unstructured models, are stated in terms of abstract units of life, i.e., they are oriented to the term biomass, leaving aside the organelles that make up the cells that in turn make up the biomass; in practical terms, the microbial population is considered as a homogeneous unit. Although these models propose simple equations by virtue of the fact that the microorganism is estimated as an elementary

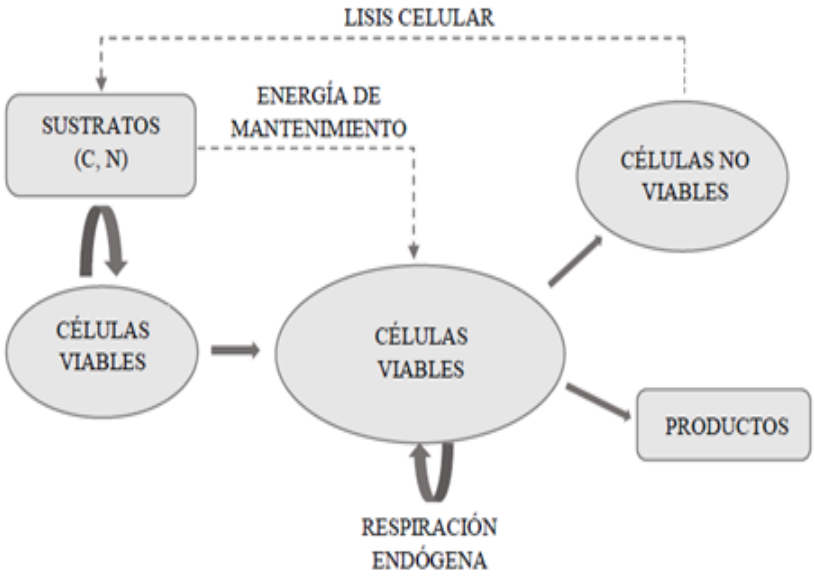
reactant species, they are valid for applications with technological objectives.

These types of models respond to the representation shown in Figure 1, which represents the consumption of substrate by microorganisms, both for their reproduction and for the generation of products, and at the same time explains the concepts of endogenous respiration and maintenance energy (Kim D., 2007).

Unstructured models consider the parameters involved in the microbial kinetic pathway, such as biomass growth, substrate consumption and product formation.

Figure 1

Simplified feedback for the unstructured model



Note. Taken from (Kim D., 2007)

Method

Unstructured models are adequate when microorganisms have a cellular composition close to steady state; when there are changes in cellular composition, they provide in most cases a poor approximation to reality. If unstructured models are not able to reflect the influence of certain variables such as the composition of the medium, changes in the internal structure of the microorganism must be considered and in very complex cases only these will be able to explain the evolution of the culture.

For the study of the kinetic behavior of biomass, substrate and product in the production of biopolymers, we considered the case study reported in Mathematical modeling and optimization of the production process of polyhydroxyalkanoates using the bacterium *Burkholderia cepacia* B27 from fatty acids, where tests were carried out for 77 hours of reaction (Suriyamongkol P., 2007)where tests were carried out during 77 hours of reaction.

Table 1 shows the concentration data for biomass (X), sucrose (S) and product (P)obtained during the kinetic monitoring of this case.

Table 1

Biomass (X), sucrose (S) and product (P) concentrations

t (h)	Concentration X (g/L)	Concentration S (g/L)	Concentration P (g/L)
0	1.46	20	0.22
6	1.83	19.56	0.58
11	3.19	17.94	1.47
23	5.7	14.94	4.34
30	7.17	13.19	6.02
35	11.23	8.34	8.68
47	13.54	5.59	13.08
53	15.91	2.75	14.88
59	15.08	3.74	14.19
71	15.09	3.73	14.32
77	15.4	3.36	13.2

Note. Taken from (Khalseh, 2016)

The most commonly used empirical formula for the organic fraction of cells is$C_5H_7O_2N$. The formulation$C_{600}H_{87}O_{23}N_{12}P$can be used when phosphorus is also considered. Both formulations are approximations and may vary.

To support microbial growth in biological systems, appropriate nutrients must be available. Prokaryotic cells are composed of approximately 80% water and 20% dry material, of the latter 90% is organic and 10% is inorganic.

Considering that the composition of prokaryotic cells consists of approximately 80% water and 20% dry material, of which 90% is organic and 10% is inorganic, the formula C10H21O3N2 is obtained (Almudena, 1999)the formula $C_{10}H_{21}O_3N_2$is obtained; for biomass, this is shown in Table 2.

Table 2

Typical percentage composition of a bacterium

Cellular elements	Dry weight percentage (%)
Carbon	50
Oxygen	22
Nitrogen	12
Hydrogen	9
Phosphorus	2
Sulfur	1
Potassium	1
Sodium	1
Calcium	0.5
Magnesium	0.5
Chlorine	0.5
Fierro	0.2
Other trace elements	0.3

Note. Taken from (Metcalf & Eddy, 2005).

Then for one mole of substrate and in the particular case glucose($C_6H_{12}O_6$), the stoichiometric reaction involved in the production of the PHB biomonomer as well as that of polymerization are shown in Equations 1 and 2 respectively.

$$C_6H_{12}O_6 + \tfrac{2}{3}NH_3 + \tfrac{5}{12}O_2 \longrightarrow \tfrac{1}{3}C_{10}H_{21}O_3N_2 + \tfrac{1}{3}C_4H_6O_2 + \tfrac{4}{3}CO_2 + \tfrac{5}{2}H_2O + \Delta H_r \quad (1)$$

$$nC_4H_6O_2 \rightarrow (C_4H_6O_2)_n \quad (2)$$

The generation of the PHB biopolymer $(C_4H_6O_2)_n$ depends on the growth or development of the biomass ($C_{10}H_{21}O_3N_2$), which in turn is a function of the substrate concentration ($C_6H_{12}O_6$), as shown in equation 3.

$$C_6H_{12}O_6 + aNH_3 + b\,O_2 \longrightarrow Y_{X/S}\,C_{10}H_{21}O_3N_2 + Y_{P/S}\,C_4H_6O_2 + Y_{CO_2/S}\,CO_2 + wH_2O + \Delta H_r \quad (3)$$

where:

a = Mass ratio of the amount of nitrogen source consumed/substrate consumed.

b = Mass mass ratio of the amount of oxygen consumed/substrate consumed.

$Y_{X/S}$ = Coefficient mass coefficient of biomass yield/substrate consumed.

$Y_{P/S}$ = Coefficient mass coefficient of product/substrate yield consumed.

$Y_{CO_2/S}$ = Mass ratio of the amount of$_{CO2}$ released/substrate consumed.

w = Mass coefficient of the amount of water produced/substrate consumed.

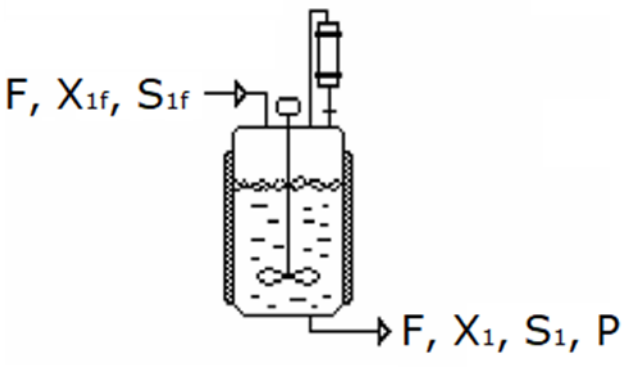
The coefficient $Y_{X/S}$ is a function of the initial amount of substrate available, as well as the nutrients and operating conditions or environmental parameters.

The coefficient $Y_{P/S}$ is a function of the amount of biomass generated and the capacity of the bacteria for the production and accumulation of the biopolymer, which depends proportionally on the degree of stress to which the bacteria can be subjected, due to the change in the concentration of the nutrient selected as limiting reagent, as long as the bacteria are not in the endogenous phase.

The mathematical model proposed to describe the process takes into account an input and an output in the bioreactor, behaving as a continuous stirring system (CSTR). The process scheme, as well as the nomenclature of the streams, is shown in Figure 2.

Figure 2

Schematic of a CSTR for a fermentative process



Note. Taken from (Khalseh, 2016).

The reaction carried out in the reactor can be expressed in the form 4:

$$S + X \rightarrow P + X \qquad (4)$$

where:
S=Substrate.
X=Biomass.
P=Product concentration.

The overall mass balance for the system in Figure 2 is given by 5 (Khalseh, 2016):

$$Entrada - Salida + Generación = Acumulación \qquad (5)$$

Consider the system presented in Figure 2 where there is only one input and one output.

For the process in a batch reactor, it must be considered that there are no inputs or outputs, therefore, the behavior for the constant reaction volume in the balance for biomass takes the form of equation 6.

$$Generación = Acumulación \qquad (6)$$

Fermentative production of PHA is normally operated as a two-stage fed-batch process (Metcalf & Eddy, 2005). An initial growth phase in a nutritionally enriched medium produces sufficient biomass, followed by a product formation phase in a nitrogen-poor medium. Single fed-batch fermentations that are nitrogen-limited lead to low amounts of polymer, because there is insufficient biomass accumulation (Katırcıoğlu H., 2003). PHA production in pure cultures is limited by an external nutrient. When cells are exposed to a medium with very low amounts of nutrients for a long time, the bacteria are physiologically altered (Daigger, 1982). The sudden increase in carbon substrate concentrations causes the cell to change its physiology again. As PHA synthesis requires less adaptation than growth, the culture begins to produce polymer, this type of fermentation is known as endogenous decay. (Días J., 2005; Lemos P., 2006).

The kinetics of microbial growth, substrate consumption and product formation are routinely formulated in terms of equations that lead to coupling between the associated cups as for example in the case of the equations:

$$\frac{dX}{dt} = f(X\ P, S) \qquad (7)$$

$$\frac{dP}{dt} = g(X\ P, S) \qquad (8)$$

$$\frac{dS}{dt} = h(X\ P, S) \qquad (9)$$

Consequently, it is often not possible to find analytical solutions to these equations so numerical solution techniques are often usefully employed.

Batch microbial growth is characterized by two regions, the exponential growth region shown in equation 10 and the stationary growth region $\frac{dX}{dt} = 0$. It is not uncommon for the transition region between these two kinetic regions of substrate-dependent growth rates to occupy 10% to 20% or less of the total fermentation time. Consequently, for many batch fermentations, a simpler approach is available as an equation that takes a self-contained growth form, i.e, $\frac{dX}{dt} = f'(X)$.

A *logistic* model is used for biomass growth, so the equation that adequately describes the biomass growth rate is shown in the Monod equation (10).

$$\frac{dX}{dt} = \mu X \qquad (10)$$

where:

$$\mu = k\left(1 - \frac{X}{X_{máx}}\right) \qquad (11)$$

$\frac{dX}{dt}$ = Cell growth rate (M/L^3t).
μ = Specific growth rate (t^{-1}).

$k =$ Inhibition factor (t^{-1}).
$X =$ Biomass concentration (M/L^3).
$X_{máx} = \frac{1}{\beta} =$ Maximum biomass concentration (M/L^3).

Combining 10 and 11 gives12 , known as the Riccati equation

$$\frac{dX}{dt} = kX(1 - \beta X) \qquad (12)$$

Product formation occurs throughout the cell growth phase and is expressed in equation 13.

$$\frac{dP}{dt} = \alpha \frac{dX}{dt} + \beta X \qquad (13)$$

where:

$\alpha =$ Product formation coefficient associated with cell growth.

$\beta =$ Constant associated with cell maintenance (L^3/M).

The rate of substrate consumption is given by 14.

$$\frac{dS}{dt} = -\frac{1}{Y_{X/S}} \frac{dX}{dt} - \frac{1}{Y_{P/S}} \frac{dP}{dt} - k_e X \qquad (14)$$

where:

Yx/s= actual biomass yield with respect to substrate.

Yp/s= Actual product yield with respect to the substrate.

ke=Maintenance coefficient in [gS / gX h].

The logistic model is used for biomass growth X, which is presented in equation 12. This model includes an inhibition factor k [$^{h-1}$] and a constant associated with cell maintenance β [g P / g X h]. The resolution of the model (equation 15) is used to find the values of the constants in the velocity expressions.

$$ln\left(\frac{X/X_0}{1-X/X_s}\right) = kt - ln\left(\frac{X_s - X_0}{X_0}\right) \qquad (15)$$

where:

Xs = Biomass concentration in stationary phase obtained from a plot of X vs t.

For the kinetics of P product formation, the Leudeking-Piret model is proposed (equation 13), where the constants ∝ y β are determined by the fermentation pH, and correspond to the associated and non-associated growth of this parameter, respectively. By integrating 13, 16 is obtained.

$$P - P_0 = \frac{1}{k} ln\left(1 - \beta X_0 (1 - e^{kt})\right) + \alpha (X - X_0) \qquad (16)$$

Substrate consumption S (equation 14), considers that cells consume substrate for growth, product synthesis and energy generation, internal pH control activities and exchange of cellular components. Integrating 14 yields 17.

$$S - S_0 = \frac{\eta}{k\beta} ln\left(\frac{1}{1 - \beta X_0 (1 - e^{kt})}\right) - \delta (X - X_0) \qquad (17)$$

being:

$$\delta = \frac{1}{Y_{X/S}} + \frac{\alpha}{Y_{P/S}} \qquad (18)$$

$$\eta = \frac{\beta}{Y_{P/S}} + k_e \qquad (19)$$

Equations 18 and 19 are expressed in units of (g S/ g X) and (g S / g X.h) respectively.

The culture of *B. megaterium* bacteria comes from worm humus from the isolation of colonies in nutrient agar, an inoculum of 0.5 g/L is prepared, which is approximately equivalent to 1.0 x^{108} bacteria/mL, and the concentration of colony forming units (CFU) is confirmed on the scale of the Mac Farland Nephelometer and the UV-visible Spectrophotometer (McFarland, 1907)the concentration of colony forming units (CFU) is confirmed on the Mac Farland Nephelometer scale and in the UV-visible Spectrophotometer.

Results

It is assumed that PHA production by *Bacillus megaterium* depends exclusively on glucose concentration. Once the rate equations for biomass formation, product and substrate consumption were established, we proceeded to solve them analytically and graphically, taking as a basis the experimental values reported in the work by (Méndez, 2016). The results of equations 12, 13 and 14 are shown in Table 3 and presented graphically in Figures 3, 4 and 5:

Table 3

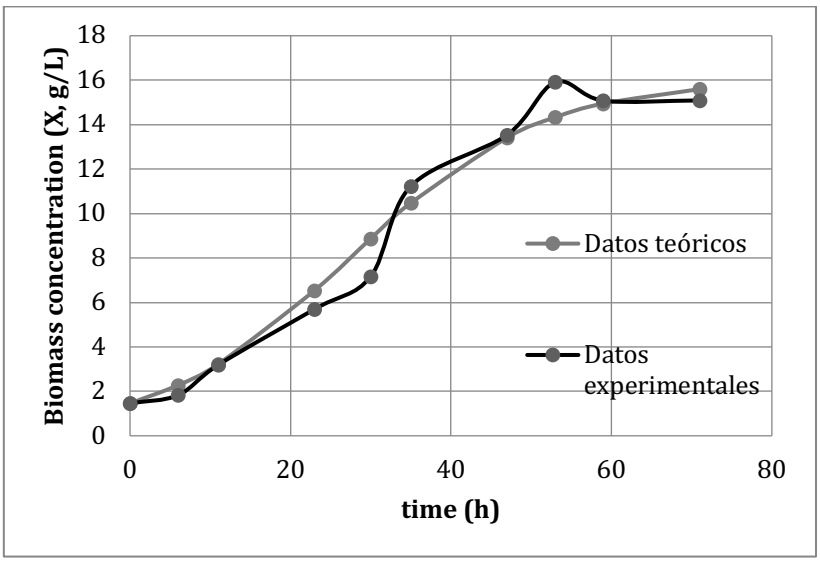
Calculated values for the parameters of the kinetic equations

Parameter	Definition	Value	Units
k	Inhibition factor	0.084	h^{-1}
α	Coefficient of product formation associated with cell growth	1.033	g P / g X
β	Constant associated with cell maintenance	0.0625	g P / g X · h
δ	Parameter associated with biomass and product formation	0.667	g S / g X
η	Parameter associated with cell maintenance	0.014	g S / g X · h

Figure 3 shows a comparison of experimental and theoretical data of the model proposed in equation 12, for the growth kinetics of biomass X.

Figure 3

Biomass growth kinetics X(g/L) vs time(h)



Note. Own elaboration (2022)

Figure 4 shows graphically the experimental and theoretical data of the model proposed in equation 13, for the growth rate of product P.

Figure 4

Kinetics of product concentration P(g/L) vs time(h)

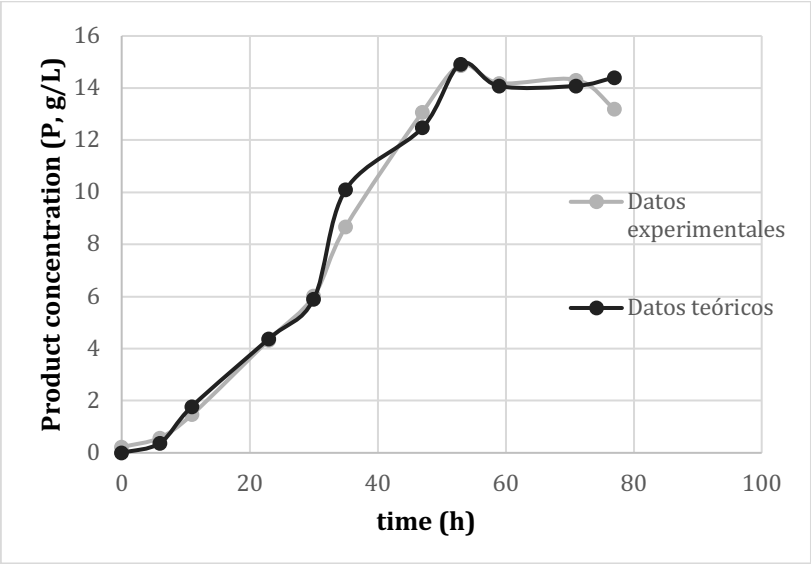
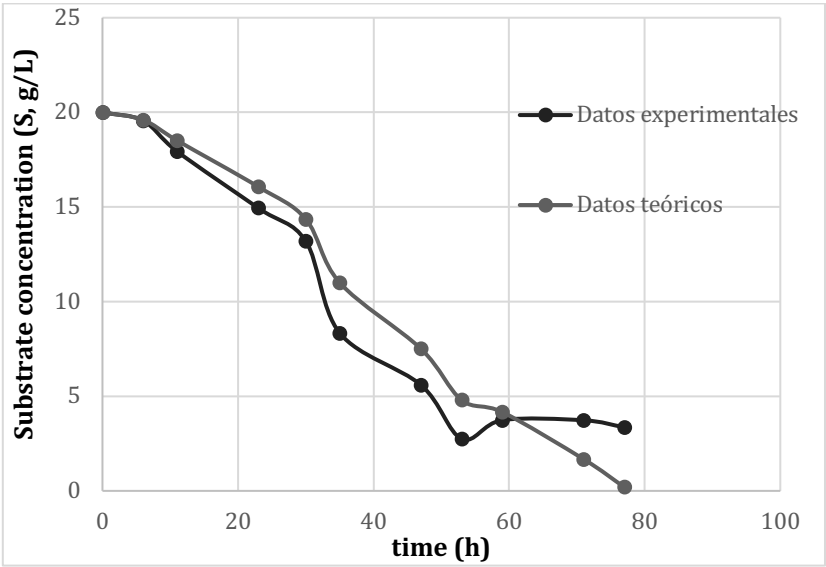


Figure 5 shows graphically the experimental and theoretical data of the model proposed in equation 14, for the rate of substrate consumption S.

Figure 5

Kinetics of substrate consumption S (g/L) vs time (h)



To validate the simulated values in the proposed solution models, (equations 12, 13 and 14) an Analysis of Variance (ANDEVA) was used considering a confidence level of 95%; in the case of biomass (X), product growth (P) and for substrate consumption (S), it is found that the experimental values $_{F0}$ *=0.0807*, $_{F0}$ *=0.09450*,$_{F0}$=0.0714 respectively, which are lower than the$_{Fcritical}$F= 4.4138 in all cases, indicating that the experimental and theoretical data are statistically equal.

Discussion and conclusions

In the contemporary world, the production of microbial biopolymers is a booming alternative, being this a biodegradable product in the final stage of its life cycle that reduces the impact on the environment, although some strains producing bioplastics are pathogenic to humans, others do not present this disadvantage to carry out research work of this profile. In the search for bacteria that do not transmit diseases, but produce biopolymers, the presence of *Bacillus megaterium*is favored, since it is a microorganism capable of producing polyhydroxybutyrate (PHB) from fruit residues, besides being a bacterium widely distributed in soil, and also easily recoverable in humus of red Californian earthworms fed with fruit residues.

The kinetic models developed as a function of time allow the evaluation of the biochemical process of the microorganism, the production of metabolites and the evolution of energy, and its dependence on controllable factors such as pH, temperature, concentration, etc.

The basis for this work is the study of the growth kinetics of the bacterium *Burkholderia cepacia,* which made it possible to identify the performance and productivity indicators for biomass production based on mathematical models (equations 12, 13 and 14); with this information, the kinetic parameters of the fermentation process were obtained: biomass growth, product generation and substrate consumption.

In the modeling of biomass growth X , the *logistic* model of the unstructured type is used, expressed in terms of abstract units of life: microbial population or biomass, completely ignoring the internal structure of the cells that compose the biomass, since they consider the population as a homogeneously distributed unit. Although unstructured models represent a great simplification of the real problem, they are useful for technological purposes, since they provide simple equations that make physical sense, in which the microorganism is treated as a simple reactant species. The most complicated scheme to which this type of models could respond, considers the consumption of substrates by the cells, both to grow and to generate products, as well as the concepts of endogenous respiration and maintenance energy, this is fulfilled for the objective of this work that proposes to quantify in a theoretical way the amount of biopolymer produced, and in a subsequent stage will be tested with the bacterium *B. megaterium*.

In the case of biomass growth X , the *logistic*model is used, which is presented in equation 12. This model includes an inhibition factor k and a constant associated with cell growth β. The solution of the model presented in Equation 15 is used to find the values of the constants in the velocity expressions associated with Figure 3, which shows the kinetics of biomass formation with respect to time.

For *P-product* formation, the Leudeking-Piret model shown in Equation 13 is proposed, where the constants α and β are determined by the fermentation pH, and correspond to associated and non-associated growth, respectively. This implies that the first term corresponds to the proportional production of the metabolite with respect to cell growth, while the second term shows how all microorganisms produce a constant proportion of product regardless of the growth phase (Reynolds, 1996) The kinetic monitoring of the biomass allows observing an exponential growth phase of a maximum of 35 hours of reaction and a stationary phase close to 72 hours. Equation 16 presents the analytical solution of Equation 13, associated with Figure 4, where the kinetics of product formation is shown with respect to time.

The substrate consumption S presented in equation 14 takes into account that cells metabolize the carbon source for growth, product synthesis and generation of energy and new

cellular components. The solution of equation 14 is shown in equation 17, which is represented in Figure 5, where the kinetics of substrate consumption is shown with respect to time.

The experimental data and the theoretical values shown in the three cases: biomass development, product formation and substrate consumption, were statistically equal according to an analysis of variance, which implies that the reliability is acceptable for its application in the calculation of bacterial biological reaction kinetics for the formation of products of industrial interest as substitutes for synthetic plastics.

According to the analysis carried out, the proposed models prove to be effective to establish the evolution of a process where microbiological organisms intervene in the degradation of a substrate with the consequent formation of a degradable biopolymer, therefore, the next stage is the experimental test of the procedure of biotransformation of nutrients to PHA's using a biological reactor type CSTR with a volumetric capacity of 5 liters operated in batches at 80% of its capacity, with a glucose solution of concentration 20 g/l and a bacterial suspension of *Bacillus megaterium* isolated from earthworm humus equivalent to the concentration of scale 0.5 of Mac Farland (McFarland, 1907).

Knowledge of how the cell population expands is useful for the design of methods to control microbial growth. A relevant point is the increase in biomass and the generation of the products that can be obtained. The biological processes carried out in this project are mainly chemical, involving microbiological agents. Thus, such a bioprocess can be represented by a stoichiometric chemical reaction. The growth of microorganisms is related to the number of viable cells present, the amount of substrate and limiting nutrients, as well as other environmental factors such as temperature, humidity, pH, dissolved oxygen, etc. In the cell growth curve four phases can be distinguished, usually referred to as: adaptation, exponential growth, stationary, logarithmic or endogenous death (Méndez, 2016).

The quantification of microorganisms is a fundamental factor in projects involving biological processes, the most commonly used technique is that which considers the plate count of bacteria present in a unit volume by serial dilutions from an initial sample and then seeding a standard amount with a sterile glass support on a plate containing suitable culture medium, and incubating until visible colonies are obtained for counting, a disadvantage of the method is that it leads to a high consumption of nutrients, substrate and reagents, in addition to the time invested. On the other hand, there are visual and optical methods that allow quantifying the number of previously isolated and identified cells in less time, such is the case of the optical method of the McFarland scale, where the bacterial suspensions are compared visually with the standards until the most similar in turbidity is found and the number of cells according to each standard [colony forming unit (CFU)/mL] is related to the number of cells according to each standard [colony forming unit (CFU)/mL].

In addition, a methodology is proposed to compare the inoculum concentration of *Bacillus megaterium*bacteria, through the correlation between spectrophotometric and turbidimetric methods, using the solutions of the test tubes of the concentrations of the McFarland scale. A series of ten tubes are prepared with a fine precipitate solution of barium sulfate obtained by mixing barium chloride dihydrate of concentration 0.048M with 0.18M sodium sulfate, in different proportions, which resembles bacterial suspensions that are instrumentally compared with the standards to correlate with the number of cells, using a Varian UV-vis double beam spectrophotometer model Cary 300 Scan at a wavelength of 600 nanometers**(nm**).

In conclusion, biomass production in a biological reaction tends to be exponential up to a certain time, which depends on the amount of substrate, types and concentration of nutrients and development parameters. The stoichiometry of the biochemical reaction is proposed to

determine the production of PHB by the optimal metabolic process of the bacterium *Bacillus megaterium*. The proposal proposes the application of mathematical models of reaction kinetics to analyze the PHB formation process, biomass and product yields, from the ideal and theoretical points of view. The calculation of the limiting substrate depends on the selected microorganism and the metabolism that characterizes it. The proposed simulation models can determine the values of real biomass yield with respect to the substrate ($Y_{x/s}$), real product yield with respect to the substrate ($Y_{p/s}$), and stoichiometry for PHA production; they also adequately describe the evolution of the process. It should be noted that with the available information it is only possible to estimate the range of energy for cell maintenance, since there is no exact theoretical or real information available on the cell maintenance coefficient(k_e)

This work explores sustainable biotechnological alternatives for the substitution of synthetic polymers. The proposal offers the advantage of a kinetic model of bacterial growth for the sustainable generation of PHB biopolymer from a waste used as a substrate, in this case fruit peels, to generate a biodegradable product.

Bacillus megaterium bacteria will be isolated from California red earthworm humus(*Eisenia foetida*) fed with fruit residues, which will provide added value to the work, since this condition complies with a sustainable production and consumption model, in which the raw material is focused on the use of residues and is part of the productive cycles with inputs that can be used recurrently and thus minimize the generation of residues. A methodology is also proposed to quantify the concentration of the *Bacillus megaterium* bacteria inoculum for the preparation of the bacterial suspension that will be inoculated into the biological reactor at laboratory scale. To know the cell concentration of the inoculum, UV-vis spectrophotometry is used, this is supported by the data of the correlation percentage coefficients (R^2). This proposal allows calculating the bacterial density at the beginning of the process and also during the sampling intervals to know the progress of the reaction and development of the bacteria with respect to the substrate, nutrients and environmental conditions.

The energy that microorganisms absorb from their environment is transformed into chemical energy which is then converted to perform the chemical work involved in the biosynthesis of cellular organelles, the osmotic work necessary for the transport of materials inside the cell (as happens in the synthesis of PHB), or the mechanical work of contraction or locomotion; as well as in the generation of new cells.

The above corroborates that the biotransformation carried out for the generation of the product depends on the type of metabolism developed by each microorganism, the physical and chemical environmental conditions, the type of substrate and the methodological sequence applied.

The work contributes to create an eco-sustainable environment with a technological approach at the service of people and the well-being of the planet, to recover harmony with nature and common resources, where waste is used to generate environmentally friendly products such as bacterial bioplastics, replacing those that are pollutants such as synthetic plastics derived from petroleum.

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