Modification of the Luedeking and Piret Model with a Delay Time Parameter for Biotechnological Lactic Acid Production

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Modification of the Luedeking and Piret Model with a delay time parameter for biotechnological lactic acid production.

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Abstract

Objectives To obtain a mathematical model that adequately describes the time lag between biomass generation and lactic acid production of lactic fermentations.

Methods Seven experimental kinetics from other research works were studied to validate our proposal: four studies of Fungal Submerged Fermentation and three cases of Bacterial Submerged Fermentation, including the data recollected by Luedeking and Piret.

Results We introduce a modification to the Luedeking and Piret model that consist in the introduction of a time delay parameter in the model, this parameter would account for the lag time that exists between the production of biomass and lactic acid. It is possible to determine this time delay in a simple way by approximating the biomass and product formation considering that they behave as a first order plus dead time system. The duration of this phenomenon, which is not described with the classical Luedeking and Piret model, is a function of microorganism physiology (ease of biomass growth), environment (nutrients) and type of inoculum.

Conclusion The Luedeking and Piret with delay model applications reveal an increase of the R^2 in all cases, evidencing the quality of fit and the simplicity of the method proposed. These model would improve the accuracy of bioprocess scaling up.

Keywords: Luedeking and Piret; First Order Plus Dead Time Model; lactic acid; fermentation.

Introduction

The concept of bioeconomy has emerged several years ago as an eco-friendly alternative to stop using petrochemicals as precursors in chemical synthesis, and having the goal to use and revalue biomass, including lignocellulosic waste from agroindustry, as a solid substrate for obtaining a wide range of biosubstances. The objectives of bioeconomy are: sustainable development and circular economy (Bugge et al. 2016). One type of bioproduct that has been intensively studied in recent years is the lactic acid (LA). Lactic acid is a natural occurring organic acid, it has applications in pharmaceutical, cosmetic, chemical and food industry (Gündüz 2005). Lactic acid has also received attention for its use as a monomer in the production of polylactic acid, a completely biodegradable polymer (Herryman Munilla and Blanco Carracedo 2005; Inkinen et al. 2011). Lactic Acid had a world demand in 2016 of 1,220 kilo-ton (Grand View Research 2019). Bacterial and fungal strains are utilized to produce LA. Depending on the culture medium used, they can be classified into submerged liquid fermentations (homogeneous medium) and solid substrate fermentations (heterogeneous medium), basically differentiated by the liquid water content of 100 % and 60-70 %, respectively (Webb and Manan 2017).

For an industrial-scale Bioprocess to become a reality, an appropriate scale-up of the system must be carried out. Mathematical modeling is a very useful tool to achieve an adequate bioprocess scaling up and predicting bioreactor performance (Crater and Lievense 2018; Gonzalez et al. 2016). In order to develop a mathematical model for the process under consideration is necessary to take into account submodels that describe the kinetics, mass and energy transfers. The submodels that describes the kinetics equations involved in the biomass growth and LA production are the first and more important to be developed (Mitchel et al. 2006). The Logistic Model (L model) is the most widely used to describe the microbial growth, as it shows a good fitting in most of the microbial growth cases (Germec and Turhan 2020; Mohsin et al. 2019; Saat et al. 2014). And for the production of LA, the mathematical model with highest application rate is the Luedeking and Piret Model (LP model), which relates metabolite production to biomass concentration and microbial growth rate and is independent of substrate concentration (Luedeking and Piret 1959; Mitchel et al. 2006). It is also possible to apply First Order Plus Dead Time model (FOPDT model), which is widely used in the field of process control due to its simplicity and reproducibility (Arino et al. 2006; Sardella et al. 2020), but which have not been studied thus far in lactic fermentations either homogeneous or heterogeneous.

Generally, the metabolite generation starts after a certain amount of biomass has been produced, observing a time lag between both kinetic curves. This lag time depends on the microbial metabolism that is being developed under certain experimental conditions. On one hand, the knowledge of this delay time would allow a simpler study of microbial metabolism, on another hand it would permit to perform a time optimization to obtain the desire metabolite by varying different factors (nutrients, temperature, pH, among others). This phenomenon is omitted in the LP model, as there is no parameter that takes into account this delay time. Other researchers have proposed modifications to the LP model. (Amrane 2001) added an empirical term that considers the residual content of the limiting carbon substrate (lactose) in LA production using *Lactobacillus helveticus*. (Bouguettoucha et al. 2007) instead of using the residual lactose content, used a term with the limiting lactose concentration using the same microorganism to generate LA. Balannec et al. (2007) incorporated a term that takes into account the inhibitory effect of the undissociated form of LA and the inhibitory concentration of LA in fermentations with *Lactobacillus helveticus*. Rosero-chasoy et al. (2020) observed that the fermentation of complex substrates (agro-industrial waste) generates a delay time in the generation of *Lactobacillus casei* biomass due to an acclimatization time, so they incorporated a time delay term in the fermentation kinetics into the model. This lag time is not precisely the same as the one we propose in the present work, since the lag time we observe is between the time when the biomass starts to be generated and the time when the LA starts to be produced.

In this article, we analyzed the kinetic information available in literature of microbial growth and LA production from other research works. The experimental data extracted from those articles were mathematically modeled. In all cases, a delay time was observed between the microbial kinetic curves and the LA production, which motivated our proposal to modify the LP equation, improving the fitting of the LA production curves in all cases.

Material and Methods

Compilation of experimental information from other research work

Other research was used as a basis for presenting graphs of the kinetics of biomass growth and LA production of different fermentation systems using different carbonaceous substrates, whether amylaceous and lignocellulosic residues or simply glucose. We have differentiated between works using LAB as inoculum and those using fungi of the genus *R. oryzae*. The methodologies applied in each work are described in the following paragraphs.

Luedeking and Piret (1959) used *Lactobacillus delbrueckii (L. delbruecki)* as inoculum, a thermophilic and homofermentative LAB, carrying out the fermentation at 45°C and keeping the pH constant at 6.0 (with sodium carbonate). The liquid culture medium was an aqueous solution with 5% anhydrous dextrose, 3% dehydrated yeast extract and mineral salts. They used a two liter closed fermenter with temperature, pH, and CO_2 controllers. To exclude atmospheric O_2, the surface was covered with CO_2.

Jin et al. (2005) used different agroindustrial wastes, including corn, pineapple, potato and wheat waste streams, each containing approximately 20 g/l of starch or sugars, supplemented with peptone, yeast extract, KH_2PO_4, and $MgSO_4.7H_2O$. Fermentations were carried out at 30°C in 250 ml Erlenmeyer flasks with shaking (150 rpm), using 100 ml of culture medium, inoculated with 5 ml of a spore suspension of *R. oryzae 2062* (10^5 spores/ml).

Palaniraj and Nagarajan (2012) used *Lactobacillus casei MTCC 1423* (*L. casei*) as inoculum (6 x 10^9 cfu/100ml), which is a homofermentative and anaerobic acid tolerant LAB. The medium was a water solution with 100 g/l potato waste, 12 g/l of yeast extract, 3 g/l of ammonium chloride (for burring) and 10 ml/l of enzymes (α-amylase and glucoamylase). The fermentation was carried out in flasks at 37 ºC, pH 6.5 and for 60 h.

Berry et al. (1999) used *Lactobacillus rhamnosus ATCC 10863* (homofermentative) to produce LA. The fermentation was carried out in fermenters of 2 liter working volume (Applikon BV, Netherlands). Five milliliters of inoculum (concentration was not mentioned in the paper) was added in 200 ml of culture medium. The culture medium contained glucose enriched with a wide variety of minerals salts, vitamins and aminoacids. The fermentation was carried out at 40 ºC, with agitation at 110 rpm, without aeration and maintaining the pH constant at 5.5.

Mathematical modeling

Microbial growth

In this work, we propose the use of an alternative mathematical model to fit de kinetic growth of different fermentative process. Microorganisms have an adaptation time in their first metabolic phase of development, showing a lag phase, where the cells are adapting to their environment and may also involve the synthesis of adaptive enzymes. The duration of the lag time is very important to be determined in fermentation processes. The length of this phase depends on the physiology of the microorganism, the environmental condition, the type and amount of inoculum, even the conditions under which the

propagation of the micro-organism was developed before fermentation and also on the kind of fermentation that is being performed (Rodrıíguez León et al. 2017). Another mathematical model that can responds to this phenomenon is the FOPDT model also largely used in process control (Arino et al. 2006; Sardella et al. 2020) :

$$\frac{dX}{dt} + \frac{X}{T_p} = \frac{1}{T_p}.X_{max}(t - T_0) \quad , \quad X(0) = X_0 \quad (1)$$

Where the function X_{max} is defined by:

$$X_{max}(t - T_0) = \begin{cases} X_{max} & for \quad t \geq T_0 \\ 0 & for \quad t < T_0 \end{cases} \quad (2)$$

The solution to the equation (1) it is shown in equation (3):

$$X(t) = \begin{cases} C.exp^{-(t-T_0)/T_p} + X_{max} & for\ t \geq T_0 \\ X = X_0 & for\ t < T_0 \end{cases} \quad (3)$$

In order to find the integration constant C, for t = T_0:

$$X(0) = X_0 = C + X_{max} \quad \rightarrow \quad C = X_0 - X_{max} \quad (4)$$

Replacing (4) in (3):

$$X(t) = \begin{cases} (X_0 - X_{max}).exp^{-(t-T_0)/T_p} + X_{max} & for\ t \geq T_0 \\ X = X_0 & for\ t < T_0 \end{cases} \quad (5)$$

Rearranging:

$$X(t) = \begin{cases} X_0.exp^{-(t-T_0)/T_p} + X_{max}\left(1 - exp^{-(t-T_0)/T_p}\right) & for\ t \geq T_0 \\ X = X_0 & for\ t < T_0 \end{cases} \quad (6)$$

Where dX/dt: Biomass growth rate [$g.l^{-1}.h^{-1}$]; X(t): Fungal Biomass concentration obtained for a specific amount of time [$g.l^{-1}$]; X_{max}: Maximum biomass concentration [$g.l^{-1}$]; X_0: Initial biomass concentration or inoculum [$g.l^{-1}$], t: Time [h]; T_0 [h] is the parameter which provides a quick and easy way to find out the approximate duration of the latency phase, and T_p [h] is a parameter of the process which provides information on the speed of growth up to X_{max}. In this FOPDT model, the microbial growth profile

144 does not show an acceleration stage between the lag phase and the exponential phase, as described by the
145 L model, so the T_0 parameter would include both stages, the adaptation phase and the cell acceleration
146 phase.

147 In the FOPDT model, when $T_0 = 0$, the model takes the form of the First Order model (FO) but as
148 microbial growth kinetics rarely occur without a lag phase, this is why $T_0 > 0$ generally, and therefore the
149 system can be modelled with the FOPDT model. In the case of microbial kinetic the stationary state is
150 reached when X_{max} = constant.

151 In the FOPDT model the parameters T_0 and T_p are obtained with the equations 7 and 8 (Sardella et
152 al. 2020):

153 $$T_p = 1.5\ (t_2 - t_1) \qquad (7)$$

154 $$T_0 = t_2 - T_p \qquad (8)$$

155 Where t_1 is the time [h] in which 28.3 % of the X_{max} is reached and t_2 is the time [h] in which 63.2
156 % of the X_{max} is reached. According to the exposed, a first approximation of the parameter T_p is to consider
157 the time in which a change is produced from the initial conditions (63.2 % of ($X_{max} - X_0$)). As X_0 is generally
158 much less than X_{max}, it can be neglected. Then, T_p can be estimated as the time in which 63.2 % of X_{max} is
159 reached.

160 We compared the FOPDT model with the L Model. Equations 9 and 10 show the L model in its
161 differentiated and integrated form (Mitchel et al. 2006), respectively:

162 $$\frac{dX}{dt} = \mu_{max}\left(1 - \frac{X}{X_{max}}\right)X \quad ; \quad X(0) = X_0 \qquad (9)$$

163 $$X(t) = \frac{X_{max}}{1+\left(\frac{X_{max}}{X_0}-1\right)exp^{-\mu_{max}.t}} \qquad (10)$$

164 Where μ_{max}: Maximum Specific growth rate [h^{-1}]. This model presents three important parameters:
165 X_0, X_{max} and μ_{max}, where X_0 is the inoculum used in the experiment therefore it is a known value, whereas
166 X_{max} and μ_{max} have to be calculated by regression.

167 ***Lactic acid production***

Lactic acid is a primary metabolite, since it is formed directly in the cell main metabolic pathways, so LA generation speed has a direct relationship with the microbial growth rate. For the production of LA, the most applied mathematical model is the LP Model (Luedeking and Piret 1959; Mitchel et al. 2006):

$$\frac{dP}{dt} = Y_{p/x}\frac{dX}{dt} + m_p X \qquad P(0) = 0 \quad (11)$$

Where: dP/dt: Lactic Acid formation rate [$g.l^{-1}.h^{-1}$]; $Y_{p/x}$: Lactic Acid yield [g LA . g $biomass^{-1}$]; and m_p: Coefficient for LA production related to maintenance metabolism [g LA . g $biomass^{-1}$. hour]. The parameters $Y_{p/x}$ and m_p will be known by regression, since the microbial growth rate could be adjusted to the proposed models. Equation 11 is rearranged to express P as a function of time, P (t), applying integral:

$$\int_0^P dP = Y_{p/x}\int_{X_0}^{X} dX + m_p \int_0^t X(t)dt \qquad (12)$$

The kinetics of metabolite generation can be classified into three categories according to the values of the $Y_{p/x}$ and m_p coefficients. When $Y_{p/x} \neq 0$ and $m_p = 0$, the metabolite has a direct relationship with growth, being Type 1; When $Y_{p/x} \neq 0$ and $m_p \neq 0$, the metabolite has a mixed relationship with microbial growth and concentration of microorganism, being Type 2; and when $Y_{p/x} = 0$ and $m_p \neq 0$, the metabolite has no relationship to microbial growth, being Type 3 (Gaden 2000). In the present work we applied the Type 1, since LA production is stabilized at a constant value, without presenting an increasing production in a linear way indefinitely, a situation that would occur in the case of $m_p \neq 0$. So, the production of LA when $Y_{p/x} \neq 0$ and $m_p = 0$ is expressed as:

$$\int_0^P dP = Y_{p/x}\int_{X_0}^{X} dX \qquad (13)$$

In the case of the L model, replacing equation 10 into equation 13 and solving results:

$$P(t) = Y_{p/x}\left[\frac{X_{max}}{1+\left(\frac{X_{max}}{X_0}-1\right)exp^{-\mu_{max}\cdot t}} - X_0\right] \quad (14)$$

$$P(0) = 0$$

The parameters $Y_{p/x}$ and m_p can vary with fermentation conditions. This coefficients have been widely studied in fermentative processes with bacteria of the genus Lactobacillus (Altıok et al. 2006), but not using fungi to obtain LA.

1.1.1. *Data analysis.*

193 Matlab R2015a was used to test the fitting mathematical models for the kinetic growth and for the
194 LA production in all cases.

195 ## Results and discussion

196 ### Application and description of the proposed model

197 The procedure applied to arrive at our proposed modification of the LP equation is described
198 below:

199 1. We took an example of Fungal Submerged Fermentation (FSF) of corn waste stream using *R.*
200 *oryzae* to obtain LA from the work of Jin et al. (2005) and obtained the experimental points of
201 biomass and LA generation.
202 2. We fitted the L model, widely used and known in microbial growth kinetics, and then the L model
203 combined with the LP model (L-LP model) for LA production, as traditionally done. We obtained
204 Figure 1 and the parameters shown in Table 1.

205 **Figure 1 about here**

206 **Table 1 about here**

207 Table 1 shows that the model applied in biomass growth fits adequately according to the
208 R^2 value of 91.37%, when combining the L model with the LP model for the production of LA,
209 there is a clear delay time that can be observed in Figure 1 b and also the greatly reduced value of
210 R^2, showed in Table 1.
211 3. Based on the observations made in step 2, we decided to apply the FOPDT model on biomass and
212 LA production, in order to know the time evolution characteristics of the experimental responses
213 T_p and T_0 (equations 7 and 8), so as to be able to study the observed lag between biomass and LA.
214 The results are shown in Figure 2 and Table 2. The FOPDT equation for the LA production used
215 was:

216 $$P(t) = \begin{cases} P_0 . exp^{-(t-T_0)/T_p} + P_{max}\left(1 - exp^{-(t-T_0)/T_p}\right) & for\ t \geq T_0 \\ P = P_0 & for\ t < T_0 \end{cases} \quad (15)$$

217 Where P(t) is the LA concentration obtained for a specific amount of time [$g.l^{-1}$]; P_{max}:
218 Maximum LA concentration [$g.l^{-1}$]; P_0: Initial LA concentration [$g.l^{-1}$], t: Time [h]; T_0 [h] is the

delay time for LA production and T_p [h] is the time constant of the process which provides information on the speed of growth up to P_{max}. In the case of LA production $P_0 = 0$, since at the time t = 0 of the inoculation of the microorganism, lactic acid has not yet been produced. It should be noted that $Y_{p/x} = P_{max} / (X_{max} - X_0)$, so that by applying the FOPDT model, the value of LA formation yield with respect to cell biomass could be obtained.

Figure 2 about here

Table 2 about here

By applying the FOPDT model to the data, the fitting improved significantly reflecting an R^2 for biomass data that went from 91.37% to 98.37% and for LA production went from 38.72% to 97.72%. In addition to this improvement, the implementation of this model made it possible to obtain the parameters T_0 and T_p, where T_0 is indicative of when the biomass growth or LA production phenomenon begins, and T_p is indicative of the growth rate once the phenomenon has already begun. These parameters permit an analysis of what is happening in this type of fermentation, such as how long it will take for the biomass to start growing, what conditions can increase or shorten this time, how long it will take to obtain the metabolite of interest, among other questions. These results allowed us to conclude that the evolution of biomass and LA did not satisfy the LP equation. We observed that the experimental data reflect a clear delay time between the time when biomass production started ($(T_0)_B$) = 4 h) and the time when LA started to be produced ($(T_0)_{LA}$ = 12 h), a time difference that we call T_d [h], which was 8 h ($T_d = (T_0)_{LA} - (T_0)_B$). The parameter T_0, both for biomass growth and LA production, allowed us to study this delay time and to achieve an improvement in the R^2 of the kinetic models of LA production. With this procedure we were able to observe that both biomass generation and LA production have the same T_p value (6.62 h), while they differ in the T_0 value. In this case, this is evidence that once biomass and LA growth start, both will follow a variation with the same time constant.

4. Based on the observations in item 3, we propose a modification of the LP model, as shown in the next item.

Proposed Model: Luedeking and Piret equation with a delay time

We suggest a modification to the LP model, when $m_p = 0$, to obtain a better description of the phenomena associated with LA production and also to obtain an increase in the R^2 value, which leads to a better representation of the reality of fermentations. The proposed modification is expressed by:

$$\frac{dP}{dt} = Y_{p/x} \frac{dX}{dt} (t - T_d) \quad for\ T_d \geq 0 \quad (16)$$

Where T_d [h] is the time difference between the time when LA production and the biomass generation starts. This modification is based on the better fit observed in the LP model when applying the T_d between the biomass and LA adjustments. In addition, the LP model, by omitting this delay time, omits biological phenomena that directly affect the time to obtain LA. The proposed modification probably also functions when $m_p \neq 0$, but has not been tested in the present work, as LA production has a direct relationship with microbial growth.

The L Model has the parameter μ_{max}, but it must be kept constant in the microbial and LA production kinetics, otherwise it would not adequately represent the phenomenon under study. Therefore, by incorporating the parameter T_d in the L-LP model (equation 14), the system can be represented more faithfully, achieving an increase in the R^2, resulting in:

$$P(t) = \begin{cases} Y_{p/x}\left[\frac{X_{max}}{1+\left(\frac{X_{max}}{X_0}-1\right)exp^{-\mu max.(t-T_d)}} - X_0\right] & for\ t \geq T_0 \\ P\ (0) = P_0 = 0 & for\ t < T_0 \end{cases} \quad (17)$$

On the other hand, the FOPDT model, which already incorporates the T_0 parameter, offers the possibility of easily modifying and studying the time in which neither biomass nor LA is produced, without the need to modify or add parameters. Replacing equation 6 into equation 16 and solving, results:

$$\boldsymbol{P(t) = \begin{cases} Y_{p/x}\left[\left(X_0.exp^{-[t-(T_0+T_d)]/T_p} + X_{max}\left(1 - exp^{-[t-(T_0+T_d)]/T_p}\right)\right) - X_0\right] & for\ t \geq T_0 \\ m_p X_0 t & for\ t < T_0 \end{cases}} \quad (18)$$

The equation 18 is the LA formation rate equation applying the FOPDT Model combined with LP model incorporating the parameter T_d (FOPDT-LP with delay).

As it is known, $T_0 = f$ (microorganism physiology, environment and type of inoculum), so using the L-LP with delay model or FOPDT-LP with delay model could decrease the T_d, modifying the fermentation conditions, and thus optimize the fermentation.

The settings of equation 17 (L-LP with delay) and equation 18 (FOPDT-LP with delay) to our example of item 3.1 are shown below:

Figure 3 about here

Table 1 about here

It can be seen that fitting the L-LP with delay model improves the fitting from 38.72 % (see Table 1) to 94.57 % (see Table 3). This shows that by adding the parameter T_d to the LP equation, the approximation of the model with the experimental points is significantly improved. In the case of the FOPDT-LP with delay model the fit is the same as if we use the FOPDT model alone, so from here on we will show the FOPDT fit without combining it with LP to make it easier to identify the models.

To further demonstrate the validity of our proposal, the same steps proposed were followed with the other works chosen. Table 4 shows the parameters obtained for biomass generation using the L model and the FOPDT model, and the parameters obtained for LA production, using on the one hand the traditional L-LP model, and on the other hand the FOPDT model, with which we obtain the value of the parameter T_d, which is used in the logistic model combined with LP (equation 17), a model that we call L-LP with delay. It can be observed that in all cases what we have been describing is fulfilled, which clearly affirms and validates our proposal.

Table 4 about here

Analysis of models adjustments

To make clear the advantages of our proposal, bar charts were generated showing comparative R^2 results for each model in the case of biomass generation and LA production. Figure 4a clearly shows that in biomass generation, the FOPDT model in the first four examples improves the fit with respect to the logistic model, while in the last three examples it is observed that the logistic model has a better fit, although the difference is minimal, so we can say that the FOPDT model is very useful and simple for modeling biomass generation.

Figure 4 about here

295 Figure 4b shows the comparative fits of the Logistic combined with LP model (L-LP), FOPDT
296 model and Logistic combined with LP with delay model (L-LP with delay). In all cases, the L-LP model is
297 the worst fit, even reaching an R^2 of less than 40% in the example where corn was used as fermentation
298 substrate. This shows that an improvement is needed in the model that has been traditionally used and was
299 supposed to give good results. This is evidenced by the better adjustments shown in all cases, both with the
300 FOPDT model and with the L-LP with delay, showing that the proposal made in this work achieves a
301 substantial improvement in the mathematical adjustment with respect to the traditional L-LP model.
302 Online Resource shows the plots of the experimental data and the kinetic model fits, together with
303 the analyses carried out in each case.

304 ## Analysis of T_d parameter

305 Table 5 compares the fermentation conditions of the works studied with the T_d values obtained in
306 each case:

307 **Table 5 about here**

308 In Table 5 can be seen that they are all fermentations in liquid medium, so this would not change
309 the T_d value. The big difference in the T_d value that is observed when comparing the type of microorganism.
310 It is seen that when using a fungus such as *R. oryzae*, the T_d value is 8 h, a much higher value than when
311 using LAB, such as *L. delbruecki* (T_d = 1 h), *L. casei* (T_d = 3 h) and *L. rhamnosus* (T_d = 2 h). This may be
312 due to the fact that fungal growth is in the form of mycelium, which generates an increase in the viscosity
313 of the liquid medium, which hinders the growth of the fungus, and therefore could slow down the generation
314 of LA (Xu et al. 2017). In contrast, bacterial growth in liquid media does not bring about large changes in
315 viscosity, so LA generation and secretion would be faster. In addition, fungi in general are not nutritionally
316 demanding, so nutrients should not limit LA production, although in the experiment each byproduct (which
317 provides the carbon source) was reinforced with nitrogen sources (yeast extract), amino acids (peptone)
318 and minerals (K^+ and Mg^{+2}), so in this case nutrients would not be a limiting factor.

319 ## Study Contributions

320 The present study demonstrates that the Luedeking and Piret model is deficient to represent
321 adequately the LA obtention by biotechnological process. Then, by adding the parameter T_d, is possible to
322 analyze the time delay between biomass and LA production. The kinetic constants for microbial growth

were obtained. The fit of the FOPDT model is comparable and in some cases even superior to the Logistic model. This shows the versatility and usefulness of the FOPDT model, which is widely known and used by process engineers in industrial production plants because of their mathematical simplicity. For the mathematical model of LA production, the T_d parameter added to the Luedeking and Piret model achieved a significant improvement in R^2. Furthermore, the T_d parameter allows the study of the delay phenomenon between the generation of biomass and the metabolite in question, which has not been clearly defined so far. With this proposal, we show the advantages of using the FOPDT model in a complementary way to the Logistic model, since it allows the study of a phenomenon that the Logistic model alone does not allow. Although the T_d was obtained by approximating the experimental biomass and LA data with the FOPDT model, this delay time could also be used in the Logistic combined with Luedeking and Piret model, which is a very novel contribution and implies an advance in the mathematical modeling of LA production. It's important to mention that the kinetic growth and the LA production have the same T_p value, while they differ in the T_0 value, both will follow a variation with the same time constant. This reveals the adequacy of the Luedeking and Piret with delay model that we propose in this paper. In future work we will apply the Luedeking and Piret with delay model to other bioprocesses.

Compliance with Ethical Standards

Funding No funding was received for conducting this study.

Conflict of interest The authors declare no competing interests.

Availability of data and materials All data generated or analyzed during this study are included in this published article and it supplementary information file.

Authors' contributions MCG and GS conceived and designed the study. MCG conducted the literature search. MCG, GS and SEN were involved in the analysis and interpretation of data. MCG and SEN drafted the manuscript. The study was supervised by GS, OAO and SEN. All authors read and approved the final manuscript.

Ethical approval This article does not contain studies with human participants or animals performed by any of the authors.

Informed consent Informed consent was obtained from all individual participants included in the study.

References

Altıok, D., Tokatlı, F., and Sebnem, H. (2006) Kinetic modelling of lactic acid production from whey by Lactobacillus casei (NRRL B-441).J. Chem. Technol. Biotechnol.*81*, 1190–1197.

Amrane, A. (2001) Batch cultures of supplemented whey permeate using Lactobacillus helveticus : unstructured model for biomass formation, substrate consumption and lactic acid production.Enzyme Microb. Technol.*28*, 827–834.

Arino, O., Hbid, M.L., and Dads, E.A. (2006) Some general results and remarks on delay differential equations.In Delay Differential Equations and Applications NATO Science Series,(The Netherlands: Springer Dordrecht),pp.31–40.

Balannec, B., Bouguettoucha, A., and Amrane, A. (2007) Unstructured model for batch cultures without pH control of Lactobacillus helveticus — Inhibitory effect of the undissociated lactic acid.Biochem. Eng. J.*35*, 289–294.

Berry, A.R., Franco, C.M.M., Zhang, W., and Middelberg, A.P.J. (1999) Growth and lactic acid production in batch culture of Lactobacillus rhamnosus in a defined medium.Biotechnol. Lett.*21*, 163–167.

Bouguettoucha, A., Balannec, B., Nacef, S., and Amrane, A. (2007) A generalised unstructured model for batch cultures of Lactobacillus helveticus.Enzyme Microb. Technol.*41*, 377–382.

Bugge, M.M., Hansen, T., and Klitkou, A. (2016) What Is the Bioeconomy ? A Review of the Literature.Sustainability*8*, 691.

Crater, J.S., and Lievense, J.C. (2018) Scale-up of Industrial Microbial Processes.FEMS Microbiol. Lett.*365*, fny138.

Gaden, E.L. (2000) Fermentation process kinetics.Biotechnol. Bioener.*67*, 629–635.

Germec, M., and Turhan, I. (2020) Enhanced production of Aspergillus niger inulinase from sugar beet molasses and its kinetic modeling.Biotechnol. Lett.*42*, 1939–1955.

Gonzalez, K., Tebbani, S., Lopes, F., Thorigné, A., Givry, S., Dumur, D., and Pareau, D. (2016) Modeling the continuous lactic acid production process from wheat flour.Appl. Microbiol. Biotechnol.*100*, 147–159.

Grand View Research (2019) Lactic Acid Market Size Worth $8.77 Billion By 2025 | CAGR: 18.7%.

Gündüz, M. (2005) Lactic acid production by Lactobacillus casei NRRL B-441 immobilized in chitosan stabilized Ca-alginate beads.

Herryman Munilla, M., and Blanco Carracedo, G. (2005) Ácido láctico y poliláctico: Situación actual y tendencias.ICIDCA Sobre Los Deriv. La Caña Azúcar*XXXIX*, 49–59.

Huang, L.P., Jin, B., Lant, P., and Zhou, J. (2005) Simultaneous saccharification and fermentation of

potato starch wastewater to lactic acid by Rhizopus oryzae and Rhizopus arrhizus.Biochem. Eng. J.*23*, 265–276.

Inkinen, S., Hakkarainen, M., Albertsson, A.C., and Södergård, A. (2011) From lactic acid to poly(lactic acid) (PLA): Characterization and analysis of PLA and Its precursors.Biomacromolecules*12*, 523–532.

Jin, B., Yin, P., Ma, Y., and Zhao, L. (2005) Production of lactic acid and fungal biomass by Rhizopus fungi from food processing waste streams.J. Ind. Microbiol. Biotechnol.*32*, 678–686.

Luedeking, R., and Piret, E.L. (1959) A kinetic study of the lactic acid fermentation. Batch process at controlled pH.J. Biochem. Microbiol. Technol. Eng.*1*, 393–412.

Mitchel, D.., Luz, L.F.., Berovič, M., and Krieger, N. (2006) Approaches to Modeling SSF Bioreactors.In Solid-State Fermentation Bioreactors,D.A. Mitchell, M. Berovič, and N. Krieger, eds.(Springer, Berlin, Heidelberg),pp.159–178.

Mohsin, A., Sun, J., Khan, I.M., Hang, H., Tariq, M., Tian, X., Ahmed, W., Niazi, S., Zhuang, Y., Chu, J., et al. (2019) Sustainable biosynthesis of curdlan from orange waste by using Alcaligenes faecalis: A systematically modeled approach.Carbohydr. Polym.*205*, 626–635.

Palaniraj, R., and Nagarajan, P. (2012) Kinetic Studies In Production Of Lactic Acid From Waste Potato Starch Using Lactobacillus casei.Int. J. ChemTech Res.*4*, 1601–1614.

Rodríguez León, J.A., de Carvalho, J.C., Pandey, A., Soccol, C.R., and Rodríguez Fernández, D.E. (2017) Kinetics of the Solid-State Fermentation Process.In Curr. Dev. Biotechnol. Bioeng.,pp.57–82.

Rosero-chasoy, G., Durán-páramo, E., and Chairez, I. (2020) Time-delay mathematical model of lagged lactic acid production using agro-industrial wastes as substrate.Appl. Math. Model.*83*, 136–145.

Saat, M.N., Annuar, M.S.M., Alias, Z., Chuan, L.T., and Chisti, Y. (2014) Modeling of growth and laccase production by Pycnoporus sanguineus.Bioprocess Biosyst. Eng.*37*, 765–775.

Sardella, M.F., Serrano, M.E., Camacho, O., and Scaglia, G.J.E. (2020) Improvement of linear algebra controllers using sliding surface concepts : applications to chemical processes.IEEE Lat. Am. Trans.*100*, 1–8.

Webb, C., and Manan, M.A. (2017) Design Aspects of Solid State Fermentation as Applied to Microbial Bioprocessing.J. Appl. Biotechnol. Bioeng.*4*, 511–532.

Xu, Q., Fu, Y., Li, S., Jiang, L., Rongfeng, G., and Huang, H. (2017) Integrated transcriptomic and metabolomic analysis of Rhizopus oryzae with different morphologies.Process Biochem.

413 **Table 1:** Biomass and LA mathematical model parameters obtained from L model and LP model fitting.

Medium	Kinetic	Model	X_0	X_{max}	μ_{max}	$Y_{p/x}$	R^2
Corn	BIOMASS	L	0.0014	5.37	0.9912	-	91.37
	LA	L-LP				2.7	38.72

414

415 **Table 2:** Biomass and LA mathematical model parameters obtained from FOPDT model fitting.

Medium	Kinetic	Model	X_0	X_{max}	P_{max}	T_p	T_0	T_d	R^2
Corn	BIOMASS	FOPDT	0.0014	5.37	-	6.62	4	8	98.20
	LA			-	14.5		12		97.72

416

417 **Table 3:** Biomass and LA mathematical model parameters obtained from FOPDT and L with LP with

418 delay models.

Medium	Kinetic	Model	X_0	X_{max}	μ_{max}	$Y_{p/x}$	T_p	T_0	T_d	R^2
Corn	LA	FOPDT-LP with delay	0.0014	5.37	0.9912	2.7	6.62	12	8	97.72
		L-LP with delay		-	-			8		94.57

419

420 **Table 4:** Biomass and LA mathematical model parameters obtained from the fitting of the works.

Medium	Kinetic	Model	X_0	X_{max}	μ_{max}	P_{max}	$Y_{p/x}$	T_p	T_0	T_d	R^2
Corn (Huang et al. 2005)	BIOMASS	L	0.0014	5.37	0.9912	-	-	-	-	-	91.37
		FOPDT			-	-	-	6.62	4	-	98.20
	LA	L-LP		5.37	0.9912	-	2.7	-	-	-	38.72
		FOPDT		-	-	14.5	-	6.62	12	8	97.72
		L-LP with Delay		5.37	0.9912	-	2.7	-	8	8	94.57
Pineapple (Huang et al. 2005)	BIOMASS	L	0.0014	5	1.1133	-	-	-	-	-	95.77
		FOPDT		5.17	-	-	-	4.02	4	-	96.87
	LA	L-LP		5	1.1133	-	2.86	-	-	-	47.18
		FOPDT		-	-	14.37	-	4.02	12	8	94.84
		L-LP with Delay		5	1.1133	-	2.86	-	8	8	94.83
Potato (Huang et al. 2005)	BIOMASS	L	0.0014	4.51	0.6538	-	-	-	-	-	95.76
		FOPDT		4.76	-	-	-	9.7	4	-	96.83
	LA	L-LP		4.51	0.6538	-	2.9	-	-	-	63.51
		FOPDT		-	-	13.80	-	9.7	12	8	98.64
		L-LP with Delay		4.51	0.6538	-	2.9	-	8	8	94.74
Wheat (Huang et al. 2005)	BIOMASS	L	0.0014	4.78	0.7286	-	-	-	-	-	95.17
		FOPDT		4.94	-	-	-	8.11	4	-	97.87
	LA	L-LP		4.78	0.7286	-	2.9	-	-	-	63.50
		FOPDT		-	-	14.37	-	8.11	12	8	98.47
		L-LP with Delay		4.78	0.7286	-	2.9	-	8	8	94.25
D+YE+MS [(a)] (Luedeking and Piret 1959)	BIOMASS	L	0.1000	11.74	0.4734	-	-	-	-	-	99.10
		FOPDT		12.54	-	-	-	5.1	6.5	-	98.25
	LA	L-LP		11.74	0.4734	-	4.50	-	-	-	96.49
		FOPDT		-	-	57.68	-	5.1	7.5	1	96.10

		L-LP with Delay		11.74	0.4734	-	4.5	-	1	1	99.39
Waste potato starch (Palaniraj and Nagarajan 2012)	BIOMASS	L		67.60	0.1412	-	-	-	-	-	98.75
		FOPDT		72	-	-	-	16.21	15	-	97.88
	LA	L-LP		67.60	0.1412	-	0.79	-	-	-	89.42
		FOPDT	1.7700	-	-	57.6	-	16.21	18	3	97.88
		L-LP with Delay		67.60	0.1412	-	0.79	-	3	3	97.96
Glucose +Micronutrients [(b)] (Berry et al. 1999)	BIOMASS	L		10	0.3512	-	-	-	-	-	99.12
		FOPDT		10.53	-	-	-	6.74	8	-	98.36
	LA	L-LP		10	0.3512	-	6.6	-	-	-	89.42
		FOPDT	0.1151	-	-	68.43	-	6.74	10	2	97.43
		L-LP with Delay		10	0.3512	-	6.6	-	2	2	97.96

421 [(a)] Dextrose, yeast extract and mineral salts.

422 [(b)] Mineral salts, vitamins and amino acids.

423 **Table 5:** Comparison of fermentation conditions of the works analyzed with the T_d value.

Author	Microorganism		Medium	Nutrients	Environment			T_d
	Type	Inoculum			Tº	pH	O_2	
(Huang et al. 2005)	*R. oryzae*	0.0014 g/l	Liquid	-Corn / Pineapple / Potato or Wheat (10 g/l soluble starch) -Peptone (5 g/l); -Yeast extract (5 g/l); KH_2PO_4 (0.2 g/l), -$MgSO_4$ (0.2 g/l).	30ºC	Not informed.	Aerobic with agitation	8 h
(Luedeking and Piret 1959)	*L. delbruecki*	0.1 UOD/l	Liquid	- 5% dextrose, - 3% yeast extract, - Mineral salts.	45ºC	6.00	Anaerobic, the surface was covered with CO_2.	1 h
(Palaniraj and Nagarajan 2012)	*L. casei*	1.77 g/l	Liquid	-Potato waste (100 g/l); -Yeast extract (12 g/l); -Enzyme mixture (10ml/l); -Ammonium chloride (3 g/l).	37ºC	6.00	Anaerobic	3 h
(Berry et al. 1999)	*L. rhamnosus*	0.1151 g/l	Liquid	-Glucose (80 g/l); -Minerals salts (K^+, Na^+, Mg^+, Ca^{++}, Mn^{++}, Fe^{++}, Co^{++}, Cu^{++}, Ni^{++}, NH_4^+, Zn^{++}); -10 Vitamins types;-20 Aminoacids types.	40 ºC	5.50	Microaerobic with agitation	2 h

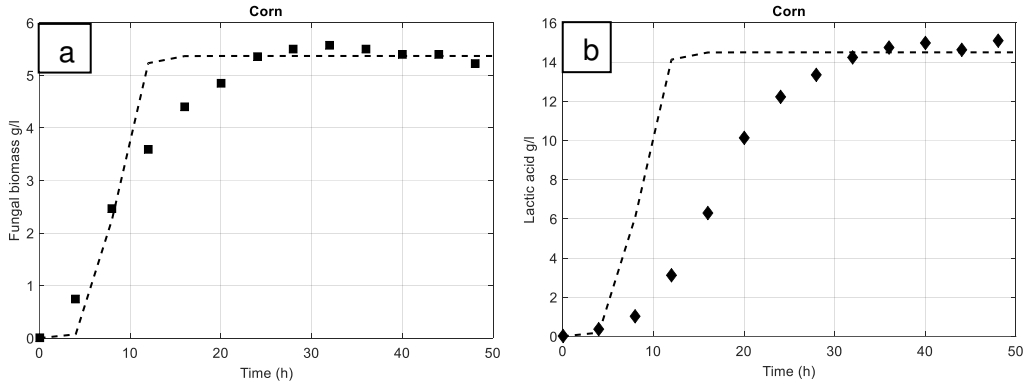
424

Fig. 1: *FSF Mathematical modeling:* a). Experimental points of *R. oryzae* Biomass (■) with L Model (script line) and b). Experimental points of LA production (♦) with L Model combined with LP model (script line).

Fig. 2: *FSF Mathematical modeling:* a). Experimental points of *R. oryzae* Biomass (■) with FOPDT Model (solid line) and b). Experimental points of LA production (♦) with FOPDT Model (solid line).

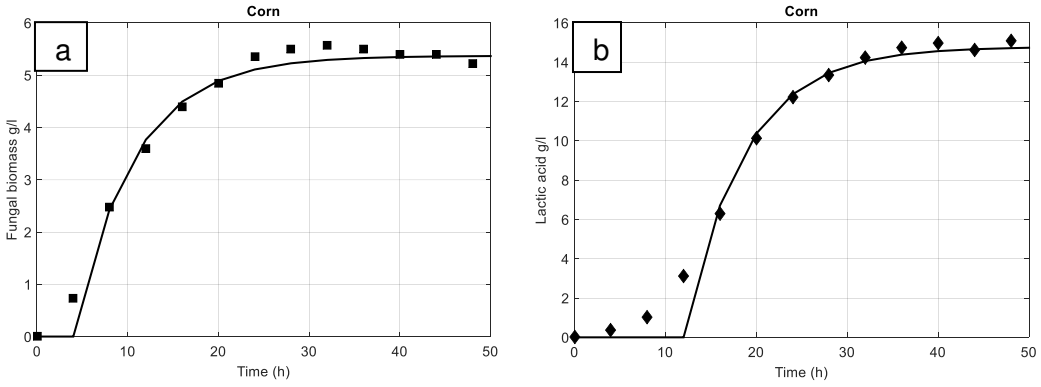
Fig. 3: *FSF Mathematical modeling:* Experimental points of LA production Lactic Acid (♦); FOPDT-LP with delay Model (dotted line) and L-LP Model with delay model (script and dotted line).

Fig. 4: Comparative results of the R^2 for each model applied to: a). Cell biomass generation: Logistic model (■) and FOPDT model (■); and b). LA production: Logistic combined with LP model (■), FOPDT model (■) and Logistic combined with LP with delay model (■).



436

437 **Fig. 1**



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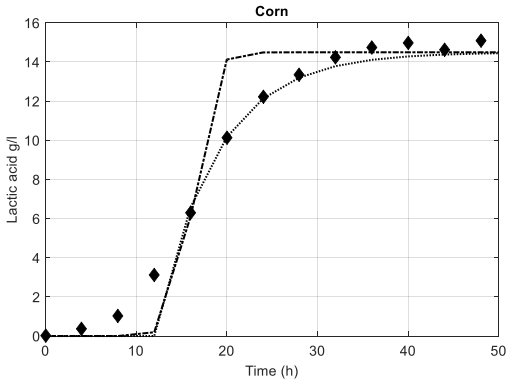
439 **Fig. 2**



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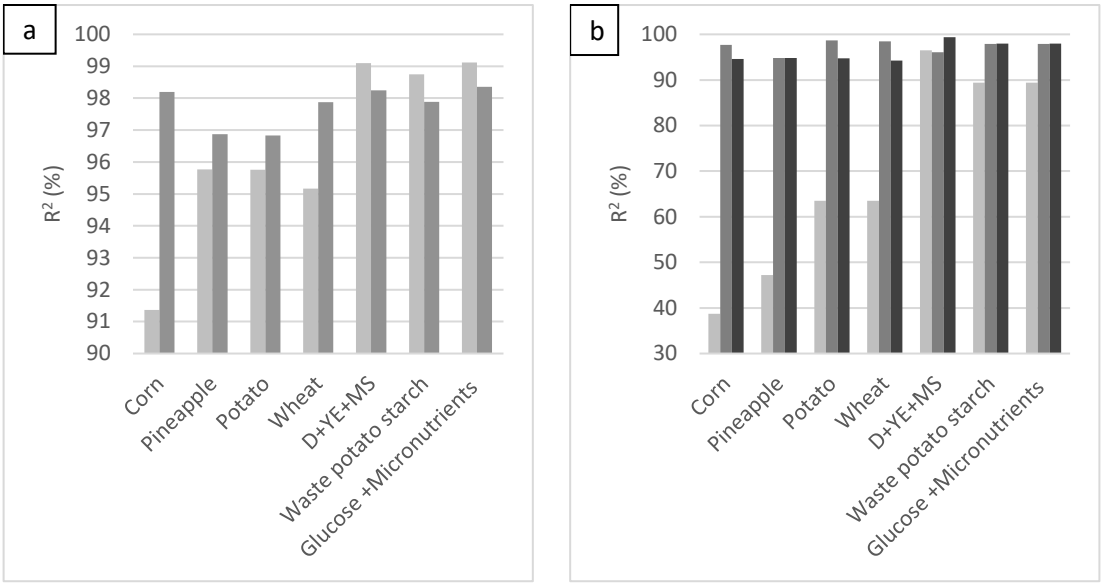
441 **Fig. 3**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R² (%)
Corn
Pineapple
Potato
Wheat
D+YE+MS
Waste potato starch
Glucose +Micronutrients
b
100
90
80
70
60
50
40
30
R² (%)
Corn
Pineapple
Potato
Wheat
D+YE+MS
Waste potato starch
Glucose +Micronutrients

Fig. 4

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