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Original Research Article

Optimizing Lactic Acid Production Through Dynamic Simulation in Repeated-Batch Fermentation System

Mohd Noriznan Mokhtar*, Rabitah Zakaria, Mohd Afandi P. Mohammed

Department of Process and Food Engineering, Faculty of Engineering, Universiti Putra Malaysia, 43400, Serdang, Selangor, Malaysia; noriznan@upm.edu.my; rabitah@upm.edu.my; afandi@upm.edu.my

*Corresponding author: Mohd Noriznan Mokhtar, Department of Process and Food Engineering, Faculty of Engineering, Universiti Putra Malaysia, 43400, Serdang, Selangor, Malaysia; noriznan@upm.edu.my

Abstract: In this study, we explored the lactic acid production from molasses through a repeated-batch fermentation process using dynamic simulation. Our investigation revealed that sugar concentrations ranging from 50 to 150 g/l significantly impacted the dynamic profiles of sugar consumption concentration, microbial *Enterococcus faecalis* RKY1 growth, and lactic acid production. Through dynamic simulation, we identified the optimal inlet sugar concentration as 68 g/l (equivalent to 130 g/l molasses), resulting in a lactic acid average productivity of 3.9 g/l h (3-repeated batch). The simulation demonstrated the viability of a sustainable lactic acid production process using *Enterococcus faecalis* RKY1, enabling the repeated-batch fermentation process for multiple cycles. Comparative analysis with continuous fermentation indicated that repeated-batch fermentation offers a superior alternative, characterized by higher substrate conversion to lactic acid. This study contributes valuable insights into optimizing lactic acid production processes, emphasizing the efficiency and sustainability of the repeated-batch fermentation approach.

Keywords: Dynamic simulation; *Enterococcus faecalis* RKY1; lactic acid; molasse; repeated-batch

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1. Introduction

Lactic acid serves as a crucial raw material with diverse applications in industries such as food, pharmaceuticals, and chemicals, including its use in the production of biodegradable poly (lactic acid) (PLA), as highlighted by Ojo and Smidt (2023). Lactic acid production can be achieved through chemical and biological synthesis. However, chemically synthesized lactic acid yields a racemic mixture comprising L(+)-lactic acid and D(-)-lactic

acid. Notably, the D(-)-lactic acid component has been identified as potentially harmful to human metabolism, as Pohanka (2020) reported. As the demand for lactic acid continues to grow across industries, understanding and optimizing the biological synthesis processes become paramount for ensuring product quality and human health considerations.

The choice of substrate significantly impacts the overall bio-manufacturing cost of lactic acid. Lactose, while a potential substrate, is deemed less economical due to its higher market price than alternatives like glucose and molasses. In a study by Daful *et al.* (2023), the utilization of diverse renewable resources, including crude oil and lignocellulosic materials, for lactic acid production was explored. The investigation involved a thorough comparison of various parameters influencing the fermentation process. Using lignocellulosic biomass sourced from agricultural waste as a raw material highlighted the need for additional costs for introducing physical pre-treatment and saccharification processes to release fermentative carbohydrates.

On the other hand, molasses emerged as a cost-effective substrate capable of reducing the production cost of lactic acid, as highlighted by Vidra *et al.* (2017). Molasses, a by-product of sugar manufacturing, comprises approximately 50% (w/w) of total sugar and is predominantly employed as animal feed. The utilization of readily available and cost-effective substrates, such as molasses, not only contributes to lowering production costs but also aligns with sustainability goals by repurposing by-products from other industries. As exploring alternative substrates continues, a balance between cost-effectiveness and sustainability remains critical in optimizing lactic acid bio-manufacturing processes.

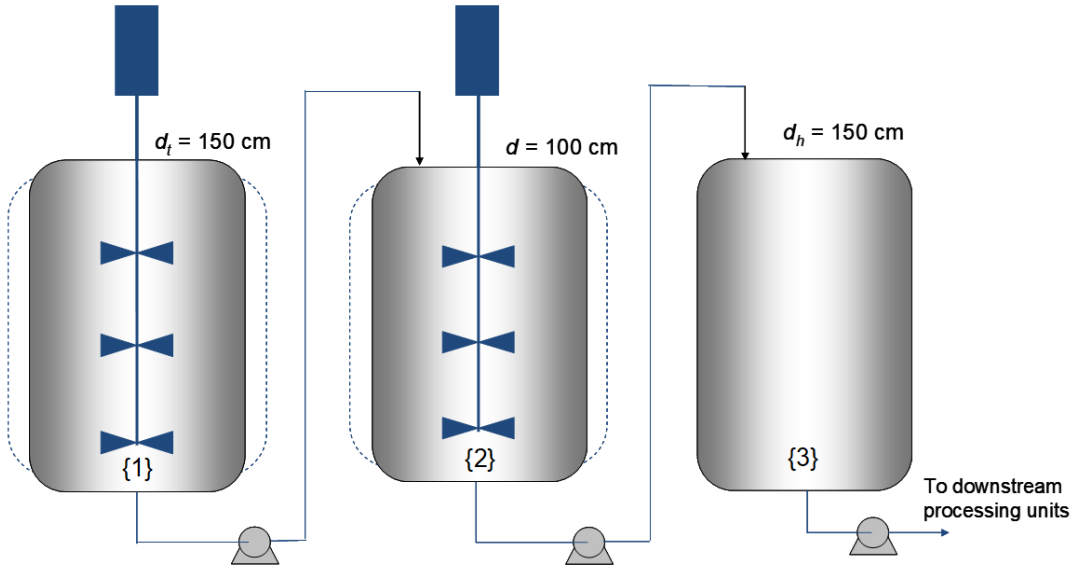
Through a biological fermentation process, lactic acid production can be achieved by utilizing various strains of bacteria, such as *Lactobacillus*, *Lactococcus*, and *Streptococcus*, as detailed by Hofvendahl and Haegerdal (2000). In addition to the conventional fermentation approach, a repeated-batch fermentation process has been successfully employed for lactic acid production, as outlined by Wang *et al.* (2017). The repeated-batch fermentation operation involves the iterative cycles of fermentation, wherein a portion or the entire microbial cells from a previous batch are inoculated into the subsequent batch. This strategy offers several advantages over traditional batch fermentation methods. These advantages encompass reduced operation time for washing and sterilizing, elimination of seed preparation time, accelerated growth rates, and shortened primary culture time facilitated by the high initial inoculation levels, as elucidated by Dashti and Abdeshahian (2016). The efficiency gains from repeated-batch fermentation translate into substantial savings in time, labor, and overall production costs. This approach enhances operational productivity and

underscores its potential as a cost-effective and time-saving alternative in industrial-scale lactic acid production.

Given the heightened demand for lactic acid (Precedence, 2023), determining an efficient fermentation process is essential, often achieved by manipulating the operational mode of the fermenter system. However, optimizing a full-scale fermenter system can incur substantial costs. To address this challenge, researchers frequently resort to dynamic simulation software to reduce expenses and predict the behaviour of the fermentation process under diverse operational conditions without the need for physical implementation. While dynamic simulation is a well-established tool in fermentation studies, its application to repeated-batch bioreactor operations has been relatively limited. Dynamic simulation plays a pivotal role in predicting the behaviour of the entire biological process, encompassing substrate preparation, start-up procedures, sequential processes involving inflow and outflow streams during fermentation, and system shutdown procedures (Calleja *et al.*, 2015). This study endeavours to fill the gap in knowledge by theoretically exploring repeated-batch fermentation. The objective is to assess the feasibility and optimize the fermentation process. By employing dynamic simulation in this context, we aim to enhance understanding, streamline operations, and contribute to the development of more efficient and cost-effective strategies for lactic acid production.

2. Materials and Methods

The dynamic simulation setup comprised a substrate feeding tank, a fermenter, and a holding tank, as illustrated in Figure 1. Sugarcane molasses served as the substrate, and yeast extract (15 g/l) was incorporated into the medium as a nitrogen source. A comprehensive guide to medium preparation can be found in Wee *et al.* (2004). The molasses, constituting 52.3% of total sugar, was utilized without pre-treatment. *Enterococcus faecalis* RKY1 metabolized the sugar to produce lactic acid. The resultant crude lactic acid was temporarily stored in the holding tank before being transferred to downstream processing units. For a detailed listing of nomenclature and symbols about the mathematical models employed in this study, refer to Table S1.



$$Sugar \xrightarrow{microb} Lactic\ acid$$

$$Sugar\ concentration : c_{i=1}$$

$$Microbial\ concentration : c_{i=2}$$

$$Lactic\ acid\ concentration : c_{i=3}$$

Figure 1. The schematic of feeding tank {1}, fermenter {2} and holding tank {3} involved in the dynamic simulation.

2.1 Feeding Tank Model

The substrate was prepared in a tank with a pH of 7.0 and stabilized at the recommended temperature of 38°C, as Wee *et al.* (2004) outlined. The tank had a set diameter of 150 cm. The mathematical models associated with the feeding tank are as follows:

$$m_i = c_i \cdot V_t \quad [g] \tag{1}$$

$$V_t = \frac{\pi (d_t / 2)^2 h}{1000} \quad [l] \tag{2}$$

$$\frac{dm_{i,out}}{dt} = c_i \cdot \dot{V}_{out} \quad [g/h] \tag{3}$$

$$\frac{dV_t}{dt} = -\dot{V}_{out} \quad [l/h] \tag{4}$$

Where i is the number of components. The initial conditions were set as:

$$h = 200\,[\mathrm{cm}]$$

$$m_{i=1,2,3,out} = 0\,[g]$$

2.2 Bioreactor Model

The fermentation process was conducted under isothermal conditions at 38°C, obviating the need for an energy balance. The fermenter's diameter was fixed at 100 cm. In this instance, given the minimal volume of the inoculum, it was presumed that adding the culture had an insignificant impact on the fermenter's working volume. Consequently, the culture's volume was excluded from the model, allowing for the simplification of the volume balance as follows:

$$V_f = \frac{\pi(d/2)^2 h_f}{1000} \quad [l] \tag{5}$$

$$\frac{dV_f}{dt} = \dot{V}_{in} - \dot{V}_{out} \quad [l/h] \tag{6}$$

The component balance for each substance inside the bioreactor is as follows:

$$m_i = c_i V_f \quad [g] \tag{7}$$

$$\frac{dm_i}{dt} = \dot{V}_{in} c_{i0} - \dot{V}_{out} c_i + V_f \nu_i r_i \quad [g/h] \tag{8}$$

where,

$$\nu_i = \begin{pmatrix} -1 \\ 1 \\ 1 \end{pmatrix} \tag{9}$$

Where i is the number of components. The substrate conversion is defined as:

$$X = \frac{c_{i0=1} - c_{i=1}}{c_{i0=1}} \quad [-] \tag{10}$$

The initial conditions were set as:

$h_f = 0 [cm]$

$c_{i=1,2,3} = 0 [g/l]$

The rate at which lactic acid is produced, often referred to as productivity, can be expressed through the following formulation:

$$\text{Productivity} = \frac{c_{i=3,t_{cycle}}}{t_{cycle}} \quad [\text{g/h}] \tag{11}$$

Whereas average productivity for repeated-batch can be simplified as:

$$\text{Average productivity} = \frac{\sum_{n} \frac{c_{i=3,t_{cycle},n}}{t_{cycle,n}}}{n} \ [\text{g/h}] \tag{12}$$

Where n is the number of batch. t_{cycle} is defined as below:

$$t_{cycle} = t_{feed} + t_{reaction} + t_{drainingout} \tag{13}$$

Lactic acid production underwent neutralization through self-regulated base titration to regulate the pH throughout the fermentation process. Wee *et al*. (2004) outlined that 10 M of NaOH proved adequate for neutralizing the lactic acid produced. Given the relatively small volume added by the base, there was negligible impact on the overall working volume of the batch; therefore, this volume from the base titration was excluded from the model. It is worth noting that, compared to scenarios without pH control, it led to a lower yield of lactic acid, as observed in the study by Hetényi *et al.* (2011).

2.3 Kinetic Model

Boonmee *et al.* (2004) conducted extensive kinetic studies on lactic acid production, exploring both batch and continuous anaerobic fermentation processes. Subsequently, Hetényi *et al.* (2011) delved into the impact of pH on the parameters of the kinetic model, revealing a substantial influence on biomass growth and lactic acid production. Their findings indicated that an optimum pH of 7 could be achieved when utilizing the microbial strain *Enterococcus faecalis* RKY1. The strain emerges as a particularly efficient lactic acid bacterium for non-treated molasses, boasting a remarkable yield ranging between 93.3% and 98% (Wee *et al.*, 2004). This underscores its efficacy as a preferred strain for high-yield lactic acid production. A modified kinetic model is proposed to capture the dynamics of lactic acid production by *Enterococcus faecalis* RKY1. This model aims to elucidate the intricate relationship between critical parameters and the dynamics of lactic acid production, offering valuable insights for optimizing and enhancing the efficiency of the fermentation process.

The rate of sugar consumption is proposed as:

$$r_{i=1} = q_{s,\max} \cdot \frac{c_{i=1}}{\left(K_{SS} + c_{i=1}\right)} \cdot e^{-c_{i=3}/K_{PS}} \cdot c_{i=2} \quad [g/lh] \tag{14}$$

The model consists of substrate limitation and exponential product inhibition to substrate utilization.

The specific growth rate, which was adapted from Nandasana & Kumar (2008), is:

$$\mu = \frac{\mu_{\max} c_{i=1}}{\left(K_{SX} + c_{i=1}\right)} \cdot \frac{K_{IX}}{\left(K_{IX} + c_{i=1}\right)} \cdot e^{-c_{i=3}/K_{PX}} \quad \left[h^{-1}\right] \tag{15}$$

The model consists of substrate limitation, substrate inhibition, and exponential product inhibition to biomass growth.

The rate of biomass production is considered as follows:

$$r_{i=2} = \left(\mu - K_d\right) \cdot c_{i=2} \quad \left[g/lh\right] \tag{16}$$

The model consists of a microbial decay rate constant.

The modified lactic acid production rate is formulated as:

$$r_{i=3} = \alpha \cdot r_{i=2} + q_{p,\max} \cdot \frac{c_{i=1}}{\left(K_{SP} + c_{i=1}\right)} \cdot e^{-c_{i=3}/K_{PP}} \cdot c_{i=2} \quad \left[g/lh\right] \tag{17}$$

The model consists of substrate limitation and exponential inhibition of lactic acid production.

2.4 Holding Tank Model

The diameter of the tank was set as 150 cm. The volume and component balances are as follows:

$$m_{i,tot} = c_i V_h \quad \left[g\right] \tag{18}$$

$$V_h = \frac{\pi\left(d_h/2\right)^2 h_h}{1000} \quad \left[l\right] \tag{19}$$

$$\frac{dm_{i,tot}}{dt} = c_{i0}\dot{V}_{in} \quad \left[g/h\right] \tag{20}$$

$$\frac{dV_h}{dt} = \dot{V}_{in} \quad \left[l/h\right] \tag{21}$$

Where *i* is the number of components.

The initial conditions were set as follows:

$h_h = 0\left[cm\right]$

$c_{i=1,2,3} = 0\left[g/l\right]$

2.5 Parameter Estimation

All pertinent experimental data, encompassing dynamic profiles of biomass, sugar, and lactic acid, were sourced from Wee *et al.* (2004). The experimental setup involved batch fermentation, maintaining a temperature of 38°C and a pH of 7, with molasses concentrations ranging from 130 to 333 g/l (equivalent to 68–170 g/l of sugar). To integrate these experimental findings into the gPROMS simulation, data from varied sugar inlet concentrations (68, 102, 136, 170 g/l sugar content) were inputted into the 'Experiments' entity. In the 'Parameter Estimations' entity, all unknown parameters such as α, μ_{max}, K_d, K_{SS}, K_{PS}, $q_{s,max}$, $q_{p,max}$, K_{SX}, K_{PX}, K_{IX}, K_{SP}, and K_{PP} were initialized with initial guesses, lower bounds, and upper bounds. The gPROMS simulation, equipped with robust parameter estimation capabilities, systematically evaluated the experimental data to deduce the optimal solution. Table 1 shows the 12 estimated parameters, providing the values derived from the simulation.

2.6 Dynamic Simulation

Dynamic simulation utilized gPROMS - ModelBuilder from Process System Enterprise, London, UK, enabling comprehensive system simulation under varied operational forces. Users can schedule the process with customizable operation models. Models for the feeding tank, bioreactor with kinetic processes, and holding tank (labelled as 'Model 1', 'Model 2', and 'Model 3') were defined in separate entities. A 'Flow sheet model' was declared a distinct entity, serving as a centralized platform for these three models.

2.6.1 Fermentation with 3-repeated batch operation

In the 'Schedule' section of the 'Process' entity, the bioreactor received a flow from the feeding tank at 500 l/h. The feeding tank's sugar concentration was 50 g/l (95.6 g/l molasses). When the fermenter reached a level of 150 cm (h_f = 150), the inlet flow ceased, and 10 g ($m_{i=2}$ = 10) of culture was promptly added. The fermentation process operated for 24 hours. After completion, the product was transferred to the holding tank at a flow rate of 500 l/h, leaving only 1 cm (h_f = 1) of medium in the fermenter. The residual culture was reused in the subsequent cycle. For the second cycle, a new substrate from the feeding tank was fed at 500 l/h until the medium reached 150 cm (h_f = 150), and fermentation continued for an additional 24 h. The process repeated for the third cycle. Dynamic simulation iterations occurred at varying sugar concentrations of 75, 100, 125, and 150 g/l (equivalent to 143.4, 191.2, 239.0, and 286.8 g/l of molasses).

2.6.2 Optimization of sugar concentration

In the 'Schedule' of the 'Process' entity, the fermenter was fed from the tank at 500 l/h with a sugar concentration of 25 g/l (47.8 g/l molasses). When the fermenter reached 150 cm (h_f = 150), the inlet flow ceased, and 10 g ($m_{i=2}$ = 10) of culture was added. The fermentation ran until the conversion reached 95% (X = 0.95). Subsequently, the product was pumped into the holding tank at 500 l/h until the product inside the fermenter reached a balance level of 1 cm (h_f = 1). The residual culture was reused in the second and third cycles, employing the same procedure. Dynamic simulations were repeated at different sugar concentrations: 50, 75, 100, and 125 g/l (equivalent to 95.6, 143.4, 191.2, and 239.0 g/l molasses). Average lactic acid productivity and total fermentation time for each sugar concentration were graphically plotted using Equations 11–13.

2.6.3 Comparison study between repeated-batch and continuous fermentation.

Continuous Fermentation: In the 'Schedule' section of the 'Process' entity, the fermenter was fed from the tank at 500 l/h, with a sugar concentration of 68 g/l (130 g/l molasses). When the fermenter reached 150 cm (h_f = 150), the inlet flow ceased, and 10 g ($m_{i=2}$ = 10) of culture was added. The fermentation process ran until the conversion reached 95% (X = 0.95). Subsequently, the fermenter's inlet and outlet flow rates were simultaneously set at 30 l/h for 240 h. Dynamic simulation iterations were repeated at various flow rates: 60, 90, 120, 150, and 180 l/h. For comparison, 10-repeated-batch fermentation was simulated using the same procedure as in Section 2.6.2. The batch procedure was repeated using the remaining culture for ten cycles. The concentration of sugar, microbial cells, and lactic acid versus time were graphically plotted for continuous and repeated batch fermentation.

3. Results and Discussions

The gPROMS simulation employed in this study showcases robust and state-of-the-art parameter estimation capabilities, successfully addressing the challenges presented by our specific problem. As illustrated in Figure 2, a comparative analysis between experimental data and simulation results reveals an excellent fit. The simulation results closely align with the four experimental data sets, each corresponding to different sugar inlet concentrations. The estimated parameters in Table 1 exhibit high reliability, with R-squared values consistently exceeding 0.88. Our results closely align with those reported by Nandasana and Kumar (2008), with remarkable similarity, except for variations observed in the substrate limitation constant for sugar consumption (K_{SS}), the substrate limitation constant for the growth of biomass (K_{SX}), and the growth-associated constant (α). Our study did not consider

substrate inhibition effects on sugar utilization and lactic acid production, given the exceptional fit observed between experimental data and the simulation models. Consequently, these parameters are deemed insignificant or exert minimal influence on sugar utilization and lactic acid production rates in our investigation compared to work done by Nandasana and Kumar (2008).

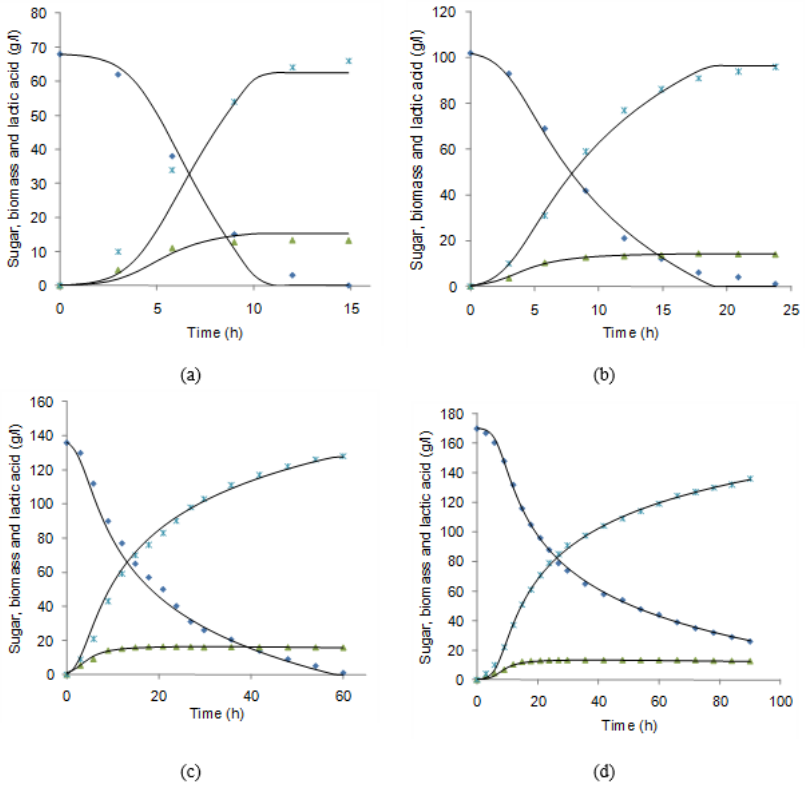


Figure 2. Experimental data (points) and simulation line batch culture of *Enterococcus faecalis* RKY1 with a) 68 g/l, b) 102 g/l, c) 136 g/l, and d) 170 g/l of inlet sugar concentration. (♦) sugar (▲) biomass (ж) lactic acid

Table 1. Kinetic model parameters using *Enterococcus faecalis* RKY1

Kinetic parameters	Value	
	This study	Nandasana and Kumar (2008)
Sugar utilization model		
K_{SS}	0.0015	0.1
K_{PS}	35.77	29.17
$q_{s,max}$ (g/(gh))	1.54	3.33
Biomass production model		
μ_{max} (h^{-1})	1.56	1.6
K_{SX} (g/l)	4.61	0.89
K_{IX} (g/l)	122.52	167.46
K_{PX} (g/l)	16.34	17.07
K_d (h^{-1})	0.0022	0.00318
Lactic acid production model		
α (g/g)	0.043	0.26
K_{SP} (g/l)	0.12	0.1
K_{PP} (g/l)	36.15	29.17
$q_{p,max}$ (g/(gh))	1.42	3.00

Refer to Table S1: Nomenclature and symbols

Figure 3 presents the dynamic simulation results, illustrating the profiles of sugar, microbial cells, and lactic acid at various inlet sugar concentrations and the working volume profiles of the feeding tank, fermenter, and holding tank. At lower sugar concentrations of 50 g/l and 75 g/l, complete sugar consumption was achieved at 14 hours and 20 hours, respectively. Lactic acid production remained steady as the reaction time extended, while microbial cells gradually declined due to product poisoning or inhibition. The harvested crude lactic acid product was transferred to the holding tank, leaving a 1 cm medium height as inoculum for the subsequent cycle. Under a new substrate introduced during the second feeding, the residual microbial cells resumed growth and initiated fresh lactic acid production. The simulation results highlight the feasibility of reusing the remaining culture for repeated-batch fermentation, significantly reducing the preparation time and costs associated with cultivating a new culture.

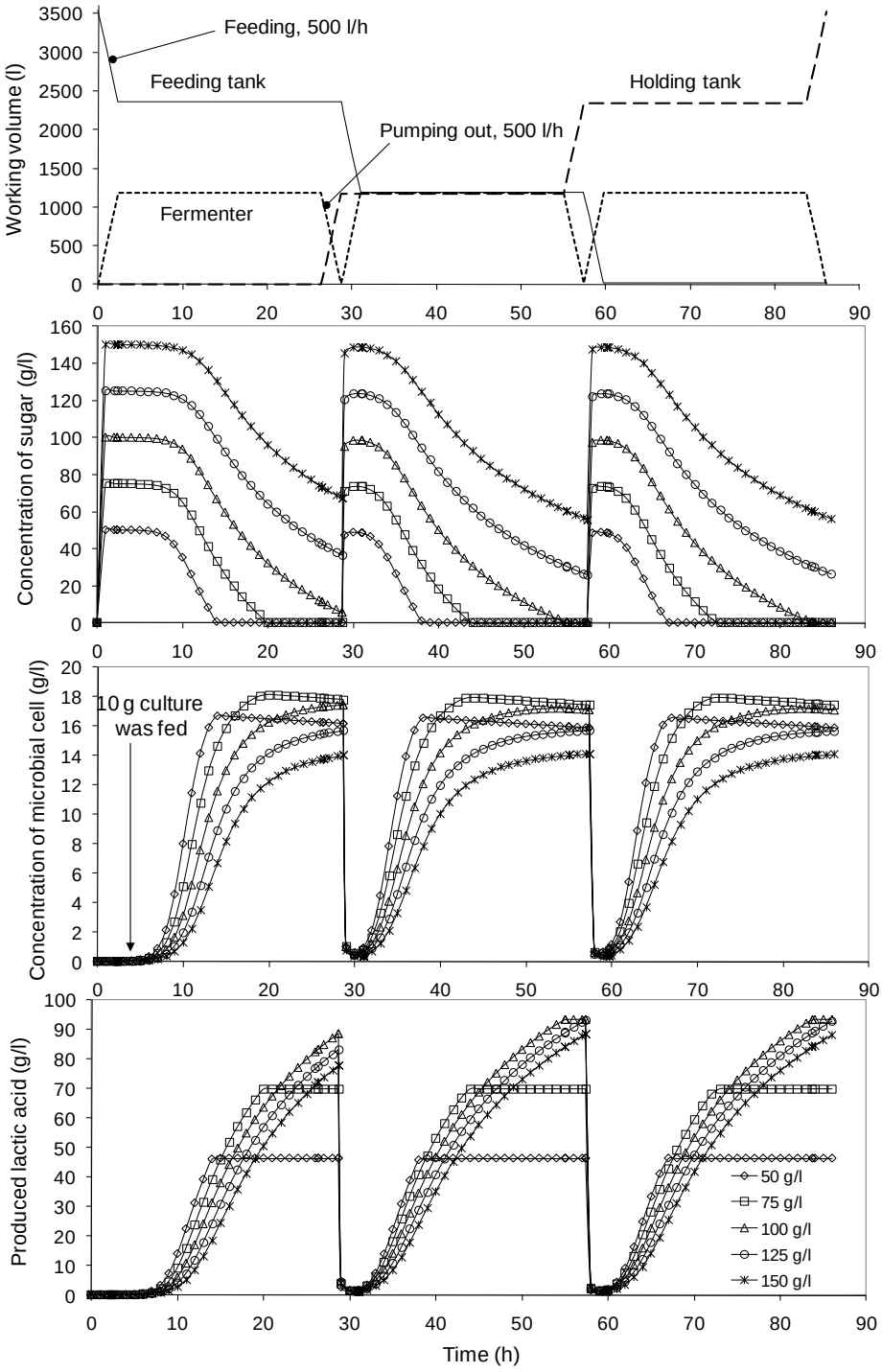


Figure 3. Dynamic profile of sugar, microbial cell and lactic acid during 3-repeated-batch with different inlet sugar concentrations

Numerous experimental studies have verified the successful implementation of repeated-batch fermentation, consistently yielding a stable product profile (Dashti & Abdeshahian, 2016; Bondarenko *et al.*, 2017; Gaber *et al.*, 2021; Liu *et al.*, 2018). This study

revealed that higher inlet sugar concentrations led to lower microbial cell growth rates, attributed to substrate inhibition effects on microbial growth. Consequently, this inhibition reduced lactic acid production and sugar consumption rates. Product inhibition emerged as a critical factor influencing the sustainability of repeated-batch fermentation. In this study, nearly the entire product was harvested, leaving only a minimal volume (1 cm; 99.3% of product volume was removed). Introducing a new substrate further reduced and diluted the lactic acid concentration by 150 times. This strategic approach effectively minimized product inhibition, thereby ensuring the sustained production of lactic acid through repeated-batch fermentation.

The 3-repeated-batch fermentation process was simulated across various inlet sugar concentrations, as illustrated in Figure 4. The graph reveals that the optimal inlet sugar concentration was identified at 68 g/l (equivalent to 130 g/l molasses), producing a lactic acid average productivity of 3.9 g/l h. In a similar study, Wee *et al.* (2004) conducted a single-batch fermentation experiment, obtaining a higher optimal sugar concentration of 104.6 g/l (equivalent to 200 g/l molasses) based on economic considerations regarding final lactic acid concentration and productivity. In contrast, various previous reports (Monteagudo *et al.*, 1994; Göksungur & Güvenç, 1999; Kotzamanidis *et al.*, 2002) indicate that lactic acid productivity in single batch fermentation typically ranges from 2.0 to 4.0 g/l h. Notably, an increase in molasses concentration correlates with an exponential rise in the total time required to achieve 95% conversion. Consequently, excessively long cycle times may result in lower productivity.

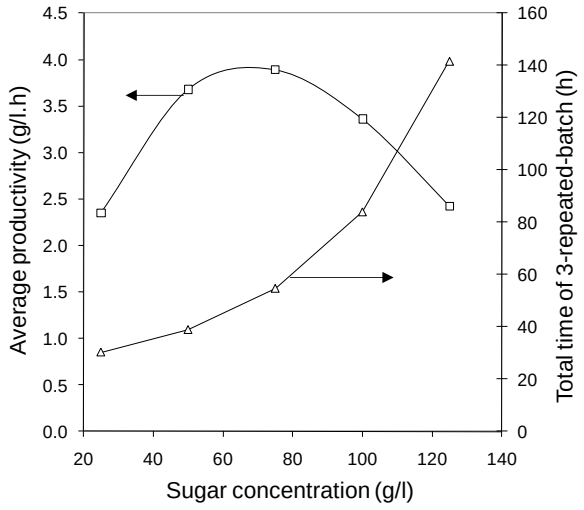


Figure 4. Average lactic acid productivity and total time to reach 95% conversion of 3-repeated-batch fermentation at different inlet sugar concentrations.

Figure 5(a) illustrates the dynamic profiles of sugar in continuous fermentation processes (at different flow rates) and a 10-repeated-batch fermentation process. Continuous feeding commenced when the fermentation reached 95% conversion. The simulation predicted that, following the initiation of continuous feeding, unconverted sugar levels would increase and eventually stabilize at a particular time (steady state). Notably, higher feeding flow rates led to a significant increase in the amount of unconverted sugar. Examining microbial cell growth, the simulation revealed that the lowest concentration was achieved under the lowest flow rate, attributed to substrate limitation.

Conversely, increasing the flow rate resulted in elevated and stable microbial cell growth, likely due to excessive sugar levels or substrate inhibition, as depicted in Figure 5(b). Consequently, a higher flow rate decreased lactic acid concentration, as shown in Figure 5(c). Thus, achieving a higher lactic acid concentration necessitates longer space-time, achievable by applying a lower flow rate.

Diverging from the 10-repeated-batch fermentation process, the simulation outcomes distinctly elucidate periodic fluctuations in the dynamic profiles of sugar, microbial cells, and lactic acid concentration, consistently maintaining comparable magnitudes. A noteworthy observation is the prolonged lag phase exhibited by the inoculum during the initial batch, as visually depicted in Figure 5. This delay in microbial growth can be attributed to the lower initial concentration of microbial cells, a key factor influencing the fermentation kinetics. Notably, the dynamic simulation forecasts a sustainable process for both lactic acid production and microbial growth, providing a foundation for the prolonged extension of repeated-batch fermentation over numerous cycles. The inherent adaptability of the system facilitates this longevity to maintain consistent performance across multiple batches. The congruence between the simulation outcomes and the earlier experimental work by Wee *et al.* (2004), who utilized the same strain of inoculum, underscores the reliability and consistency of the dynamic profiles observed throughout the extended 10-repeated-batch fermentation process. This alignment between simulation and experimental results enhances the credibility of the model. It validates the robustness of the insights gained from both approaches, contributing to a comprehensive understanding of the fermentation dynamics.

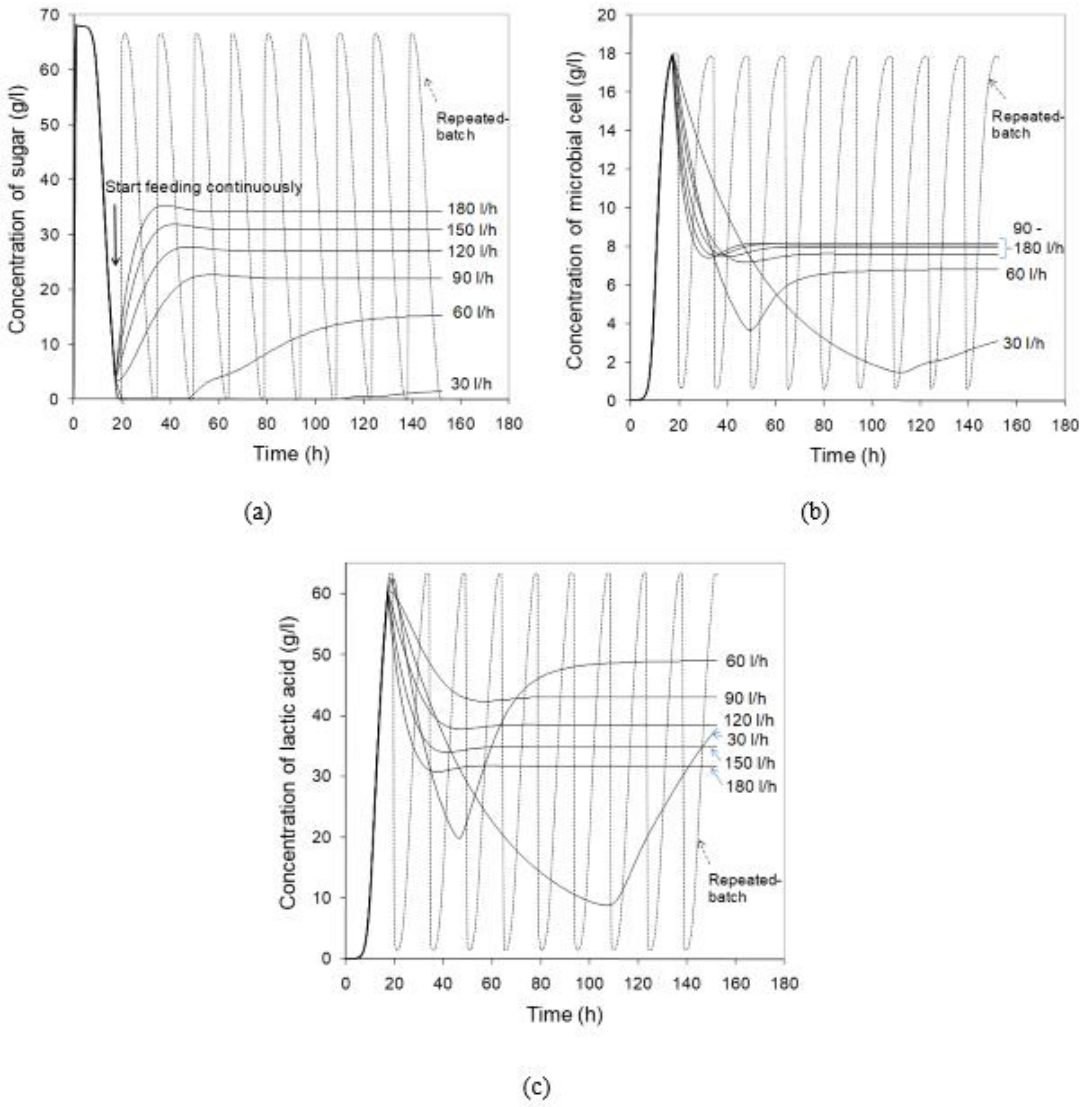


Figure 5. Dynamic profile for continuous fermentation at different flow rates (line) and for 10-repeated-batch fermentation (dot) with inlet concentration of 68 g/l (a) sugar concentration, (b) microbial cell, and (c) lactic acid

When employing higher flow rates for continuous fermentation, specifically within the range of 60 l/h to 180 l/h, the observed conversion decreased from 80% to 50%. This trend indicates that, as the system reaches a steady state, increasing the flow rate correlates with an increase in productivity, as detailed in Table 2. An essential consideration is that excessively high amounts of unconverted molasses are associated with elevated flow rates, posing challenges in downstream processing for obtaining pure lactic acid. The accumulation of unconverted substrate may complicate the purification process, leading to operational difficulties and potentially lower product quality. These findings, high substrate conversion and productivity, and reduced downstream processing challenges make repeated-batch fermentation a more viable alternative. The repeated-batch fermentation approach allows for

better control over the process, mitigating the challenges associated with high flow rates in continuous fermentation. Moreover, the sustained high conversion rates in repeated-batch fermentation and favourable productivity levels contribute to its status as a preferable and more practical alternative to continuous fermentation for lactic acid production.

Table 2. Predicted productivity of repeated-batch and continuous fermentation mode

Mode	Productivity (g/l h)
10-repeated-batch[1]	4.17
60 l/h – continuous[2]	1.87
90 l/h – continuous[2]	2.47
120 l/h – continuous[2]	2.94
150 l/h – continuous[2]	3.32
180 l/h – continuous[2]	3.63

[1]average productivity

[2]based on final product concentration ($c_{i=3}$ × dilution rate)

4. Conclusions

This study has established the efficacy of gPROMS dynamic simulation for predicting repeated-batch fermentation processes at larger industrial scales. The simulation outcomes offer valuable insights into the impact of inlet molasses concentration on lactic acid production, presenting a comprehensive behaviour profile throughout the feeding, reaction, and draining processes. The results indicate that implementing a repeated batch fermentation process enhances the productivity of lactic acid. Consequently, dynamic simulation emerges as an indispensable tool for understanding, investigating, and optimizing lactic acid production, providing crucial process characteristics that are pivotal for larger-scale applications in industrial settings.

Supplementary Materials: Table S1: Nomenclature and symbols.

Author Contributions: Conceptualization, M.N.M., R.Z. and M.A.P.M; methodology, M.N.M.; software, M.N.M.; validation, R.Z. and M.A.P.M; data curation, R.Z. and M.A.P.M.; writing—original draft preparation, M.N.M.; writing—review and editing, R.Z. and M.A.P.M.

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