

Bioproduction of succinic acid from potato waste. Kinetic modeling

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ABSTRACT

Succinic acid is a key chemical platform for the implementation of biorefineries and, therefore, for the development of a sustainable bioeconomy. In this work, succinic acid anaerobic production by *Actinobacillus succinogenes* from potato waste and glucose was carried out. Firstly, a simple kinetic model was developed to accurately predict the evolution of succinic acid, substrate, by-products, and biomass based on bottle experiments performed at different initial glucose concentrations. Secondly, experiments with an acid hydrolysate of potato waste as carbon source were performed in orbitally shaken bottles and bioreactor to compare the fermentation performance of the potato waste with the pure glucose. Using this hydrolysate, 32.2 g·L⁻¹ of succinic acid were obtained with a productivity of 0.64 g·L⁻¹·h⁻¹, improving SA yield by 37% compared to results with pure glucose. This is one of the highest values to date for succinic acid bioproduction from food residues (0.92 g·g⁻¹). Finally, the proposed kinetic model was successfully applied to the fermentation data obtained from potato waste fermentation, being this type of models a highly valuable tool for future techno-economic analysis of the bioprocess.

1. Introduction

The world population has been increasing since the middle of the last century from an estimated 2.5 billion people in 1950–8 billion in mid-November 2022 (Nations, 2023). In the last 20 years, while the world population grew linearly by 20%, crop production grew with the same trend, increasing by a factor of 1.43 (FAO, 2023a). A report from the Food and Agriculture Organization (FAO) concludes that around 1300 million tons of food are being destroyed annually, while it is necessary to use around 28% of the available agricultural area to obtain this food amount (FAO, 2023a; Sharma et al., 2021).

Thus, in 2015, the United Nations (UN) approved a set of global goals to eradicate poverty, protect the planet and ensure prosperity: the 17 Sustainable Development Goals (SDGs) (Leal Filho et al., 2022). To reach several of these goals, to avoid or reuse food waste is a must. In fact, SDG 12.3 calls for a 50% reduction in global per capita food waste at retail and consumer levels as well as a decrease in food loss along production and supply chains by 2030 (FAO, 2023b). Furthermore, the amount of food wasted per year is projected to grow by a third by 2030, which involves 66 tons of food wasted per second (Esben et al., 2018). Therefore, considering these trends and the consequences of climate change, it is imperative to build a sustainable economy through the valorization of wastes by diverse processes, including those of

biotechnological nature. In this sense, circular economy need to be further developed and the development and implication of biorefinery processes is a part of the solution.

In the context of the biorefineries, succinic acid (SA) is a key compound. It is considered one of the 12 main platform chemicals according to the United States Department of Energy (US DOE) (Dienst and Onderzoek, 2015). This acid has a wide variety of applications in the food and pharmaceutical industry. In addition, it is the raw material for several basic chemical products and there are great prospects for it in the generation of biodegradable plastics in the bioeconomy era (Mancini et al., 2022; Oreoluwa Jokodola et al., 2022). As an intermediate compound in the tricarboxylic acid cycle, SA can be produced from sugars through fermentation processes. Although many bacteria are capable of generating this acid, it is only the main product in the metabolism of some of them, as in the case of *Actinobacillus succinogenes* (Escanciano et al., 2022a; Xu et al., 2022). Fig. 1 shows the metabolic pathway of *A. succinogenes* from glucose as a carbon source. After sugar has been transformed into glyceraldehyde, bacteria convert phosphoenolpyruvate (the last product of glycolysis) into oxalacetic acid, requiring CO₂ to activate this metabolic pathway. The oxalacetate is reduced until generating SA. As by-products, formic, acetic, lactic and ethanol acids can be obtained. So, theoretically, to obtain 1 mol of SA, two moles of reduced nicotinamide adenine dinucleotide (NADH), 1 mol of CO₂, and

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0.5 mol of glucose would be necessary (Almqvist et al., 2016; Mancini et al., 2019).

The global SA market was valued at USD 160.8 million in 2022 and it is expected to expand at a compound annual growth rate (CAGR) of 6.5% between 2022 and 2032, reaching a value of USD 301.4 million ("Succinic acid market, ", 2023). The growing environmental awareness on the part of the governments of developed countries is strongly driving the increase in demand for bio-based succinic acid. The bio-succinic acid market is expected to account for a market share of 36.8% and revenue of USD 110.8 million by the end of 2032 ("Succinic acid market, ", 2023). On the other hand, it should be noted that, in recent years, the cost of bio-based succinic acid has continued to decrease and even to reach the low price of the fossil-based acid. In 2015, the market price of this acid produced by the biological route was 2.86 USD·kg⁻¹, while that obtained by the traditional petrochemical route was 2.50 USD·kg⁻¹ (Dienst and Onderzoek, 2015). Currently, some economically competitive processes have been developed and even implemented at an industrial level, producing SA with a market price between USD 2.00 and 2.50 kg⁻¹ (Mancini et al., 2022).

Based on the relevant literature, an important challenge is the production of bio-succinic from wastes at a competitive yield. A residue with great potential for this use is potato waste. According to the Food and Agriculture Organization of the United Nations (FAO), the production of this tuber worldwide reached 370,436,581 tons in 2019 (FAO, 2023a), being the fourth most important worldwide agricultural product after wheat, rice, and corn (Benkeblia, 2020). As potato processing, either domestic or industrial, generates a waste whose mass is 15–40% of potato weight, potato waste global yearly production is estimated to be 55–140 million tons (Ebrahimian et al., 2022). The main by-products of potato processing are: potato peels, discarded potatoes (old potatoes and low quality ones), potato leaves and starch processing wastewater, all of which are rich in carbohydrates, proteins and antioxidants (Torres and Domínguez, 2020).

This work showcases for the first time the production of SA from potato waste as a carbon source using *A. succinogenes* as biocatalyst. Herein, the yield and productivity of the fermentation process were compared in relation to its equivalent with pure commercial glucose, carrying out several batch runs in bottle and in bioreactor. Finally, a kinetic model that allows predicting the evolution of biomass, substrate, and products in an easy way was developed. This mathematical model could serve as a tool for process scale-up, design of control systems, and/or for performing techno-economic analyses.

2. Materials and methods

2.1. Raw materials and hydrolysis procedure

The raw material used in this study were discarded potato pieces (pieces of potatoes that are black in color or that are in poor condition) kindly provided by ESPAFRIMA S.L (Getafe, Madrid). The potato pieces were washed, peeled, and crushed until a puree was obtained. Subsequently, an acid hydrolysis was carried out (Gunnarsson et al., 2014; Huang et al., 2019; Tasić et al., 2009). A 0.5 L round bottom flask with a reflux condenser was used. The process temperature was controlled with a heating mantle. 150 mL of 1 M HCl was poured into the flask and heated to 100 °C; then 150 g of mashed potato were added and 3 mL samples were taken every 10 min for two h. Samples were neutralized with 5 M KOH and centrifuged at 9000 rpm for 10 min. Finally, the concentration of sugars present in the supernatant was determined, as well as furfural and hydroxymethylfurfural (HMF).

2.2. Microorganism, culture medium and fermentation procedure

Actinobacillus succinogenes DSM 22257 was supplied by the German Collection of Microorganisms and Cell Cultures GmbH. The strain was maintained at − 80 °C in Tryptic Soy Broth (TSB) /glycerol 50% v·v⁻¹. TSB composition was (in g per L): 17 Tryptone, 3 Soytone, 2.5 Glucose, 5 NaCl, 2.5 K₂HPO₄.

For its activation, the stored cells were added to bottles with 60 mL of TSB. These bottles have been previously purged with N₂ for 2 min at a 1 L min⁻¹ flow rate. For pH control and as a CO₂ source, NaHCO₃ was added in the same concentration as the carbon source. The cells were incubated at 37 °C and 200 rpm for 24 h. After adding 5% (v·v⁻¹) of reactivated cells, the adaptation of the microorganism to the carbon source was carried out by means of successive growth cycles in bottles under the same conditions of the reactivation stage, but with increasing concentrations of sugar and using the production medium. The production medium was (in g per L): 3 K₂HPO₄, 0.43 MgCl₂·6 H₂O, 0.2 CaCl₂, 1 NaCl, 10 yeast extract. Finally, for the growth of the inoculum, 5% (v·v⁻¹) of adapted cells were injected into bottles prepared and incubated under the same conditions of the adaptation stage, with 40 g·L⁻¹ of glucose. When the inoculum was used in a fermentation process with potato hydrolysate, a progressive adaptation process using the hydrolysate in the bottle was added, increasing the hydrolysate/pure glucose ratio in successive steps until the complete replacement of the commercial sugar.

For the comparative study of the fermentation in bottles, with a working volume of 60 mL, with commercial glucose or from potato

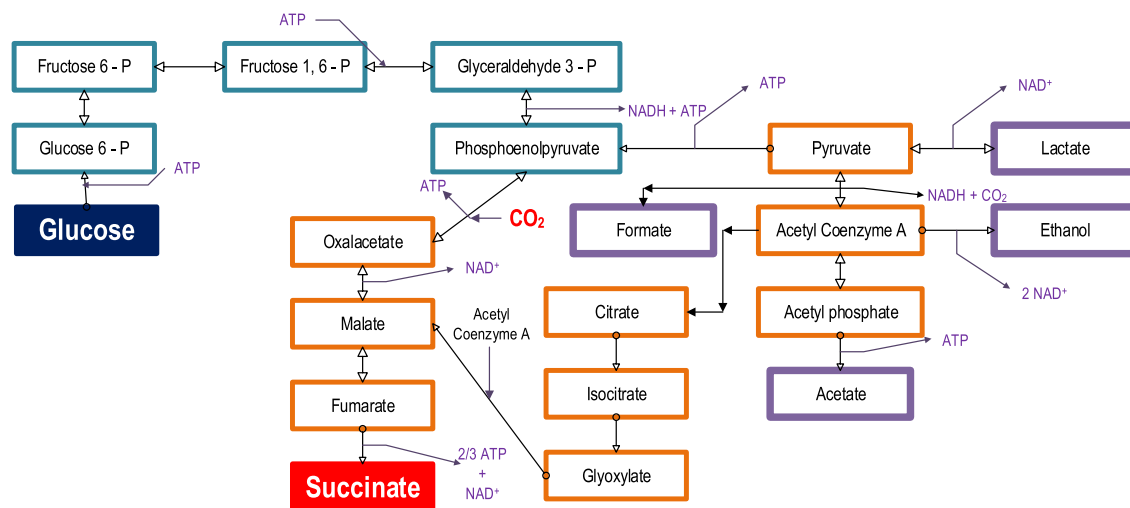


Fig. 1. Metabolic pathway of *A. succinogenes* for SA production from glucose.

residues, incubations were carried out at 37 °C and 200 rpm using the production medium and 40 g·L⁻¹ of pure glucose or potato hydrolysate in the amount enough to reach the same concentration (around 600 mL). After measuring the cell concentration in the inoculum, a sufficient amount of biomass was injected to start fermentation from 0.05 g·L⁻¹ of biomass.

The runs in reactor were performed in duplicate in a 2 L stirred tank BIOSTAT B-Plus (Sartorius AG, Germany) with a working volume of 1 L. The previously described production medium was used. The carbon source was 40 g·L⁻¹ of commercial glucose or potato hydrolysate in sufficient quantity to reach the same sugar concentration. Fermentation was carried out at 37 °C, with a stirring speed of 300 rpm, pH 6.8 (controlled by automatic addition of 5 M NaOH), and a CO₂ flow rate of 0.1 vvm. The initial biomass concentration was 0.05 g·L⁻¹.

2.3. Analytical methods

To determine suspended biomass concentration, a spectrophotometer (Shimadzu UV-Vis spectrophotometer UV-1603) was used to measure the cell optical density at 600 nm of the samples.

The concentration of sugars, furfurals and carboxylic acids was calculated from the measurements made by a refractive index detector (RID) at 55°C of a high-performance liquid chromatography (HPLC) equipment (Agilent Technologies 100 series). A REZEX ROA-Monosaccharide H⁺ (8%) column (300 × 7.8 mm, Phenomenex, USA) working at 80 °C was employed, pumping 0.5 mL min⁻¹ of a 5 mM H₂SO₄ solution as mobile phase. A BP-800 Pb column (8%, 300 × 7.8 mm, Benson) was also used, pumping 0.5 mL min⁻¹ of filtered Milli-Q water as mobile phase.

2.4. Mathematical methods

Based on the evolution of the concentrations of glucose, succinic acid and by-products (acetic and formic acids), a kinetic model has been proposed whose parameters have been adjusted to the experimental data thanks to the computer software Aspen Custom Modeler v11 (AspenTech, USA). In order to integrate the differential equations and estimate the parameters applying the least square method, an implicit Euler method coupled to an adaptative non-linear least-square solver method (NL2SOL) has been used.

The statistical parameters that have been studied to determine the goodness of fit are calculated using Eqs. (1)–(3). Fisher's F-value (*F*) (Eq. (1)) should be higher than its tabulated value at 95% confidence to overcome the null hypothesis; over this threshold, the higher its value, the better. The Root Mean Square Error (*RMSE*) (Eq. (2)) is based on the variance computed with the experimental values and those values predicted by the kinetic model; when both values are identical in all conditions, variance and this parameter are zero, showing a perfect fit of the model to the experimental data. The variation explained (*VE*) (Eq. (3)) measures the capacity of the kinetic model to explain the variation of the dependent variables with the independent variables (in this case, the process time); its best value is 100%.

$$F = \frac{\sum_{i=1}^N \left(\frac{y_{i,calc}}{K} \right)^2}{\sum_{i=1}^N \left(\frac{SSR}{N-K} \right)} \quad (1)$$

$$RMSE = \sqrt{\frac{SSR}{N-K}} \quad (2)$$

$$VE(\%) = 100 \left(1 - \frac{\sum_{i=1}^L SSQ_i}{\sum_{i=1}^L SSQ_{mean}} \right) \quad (3)$$

$y_{i,calc}$ are the estimated values, *K* is the number of kinetic parameters, *SSR* is the sum of variances or squared residues, *N* is the number of experimental data, SSQ_i is the sum of the quadratic residues, and

SSQ_{mean} is the squared sum of deviations between the experimental and the mean score with respect to the calculated values [10].

To determine the process performance and the effect of the carbon source, the following fermentation parameters have been calculated: the yield of the product (*P*), succinic acid, with respect to the initial concentration of the substrate (*S*), which is glucose ($Y_{S,0}$ – Eq. (4)), the yield of the product with respect to substrate consumed ($Y_{S,cons}$ – Eq. (5)), the substrate conversion ($S_{conv.}$ – Eq. (6)), the succinic acid productivity ($Prod.$ – Eq. (7)), and the succinic acid selectivity ($Sel.$ – Eq. (8)).

$$Y_{S,0} = \frac{C_{P,max}}{C_{S,0}} \quad (4)$$

$$Y_{S,cons} = \frac{C_{P,max}}{C_{S,cons}} \quad (5)$$

$$S_{conv.} = \frac{C_{S,cons}}{C_{S,t,0}} \quad (6)$$

$$Prod. = \frac{C_{P,max}}{time} \quad (7)$$

$$Sel. = \frac{C_{P,max}}{C_{P,max} + C_{BP,max}} \quad (8)$$

$C_{P,max}$ is the concentration of SA at the end of the fermentation, $C_{S,cons}$ is the difference between the glucose concentrations at the beginning and at the end of the fermentation, $C_{S,t,0}$ is the concentration of the substrate at the beginning of the fermentation, and $C_{BP,max}$ is the concentration of the sum of the by-products (BP) (acetic and formic acids) at the final time of fermentation.

3. Results

3.1. Determination of the optimal hydrolysis time

Compared with enzymatic hydrolysis, acid hydrolysis has been reported in the literature as an operation that allows for higher yields, greater reproducibility, and reaction rates. In addition, potato residues have a low hemicellulose content, so the generation of acetic acid and furfural, usually active as enzyme inhibitors, is avoided during the acid treatment as a consequence of the hydrolysis of the acetyl groups attached to the sugar and the dehydration of pentoses (Chen, 2015; Lenihan et al., 2010). Using ion-exclusion HPLC analysis with a Pb²⁺ column of the hydrolysate, glucose was found as the only sugar in significant amounts. Since it was no longer necessary to determine concentrations of other sugars, to monitor glucose and the dehydration product of glucose, 5-hydroxymethylfurfural (HMF), throughout the hydrolysis, an H⁺ column was enough to analyze both species. Nitrogen content was not determined since its amount is low and insignificant for this process in which, in addition, a high amount of commercial yeast extract is used as a nitrogen source. According to literature, in the composition of the potato the amount of starch is between 15% and 18% while protein nitrogen is 0.14–0.16% (Kita, 2002).

During the acid hydrolysis of potato pieces at 100 °C, glucose (substrate, *S*) was produced from amylose and amylopectin at a high rate (1.8 g·L⁻¹·min⁻¹) till 40 min, when a certain and progressive decrease in temporal productivity is appreciated in Fig. 2. HMF increases from 20 min of reaction with an increasing productivity or production rate. From 4.19 g·L⁻¹ of glucose and 0 g·L⁻¹ of HMF at time zero, a processing time of 60 min shows the maximum difference between the concentration of glucose (66.45 g·L⁻¹), the target product, and HMF (0.12 g·L⁻¹), producing 0.13 g of glucose per g of potato residue and 0.036 g of HMF per g of this waste. Higher processing times only render a high to very high amount of HMF while glucose increment is limited. As mentioned before, HMF is generated due to the dehydration reaction of glucose, via a chain reaction. Therefore, at a certain operation time the glucose

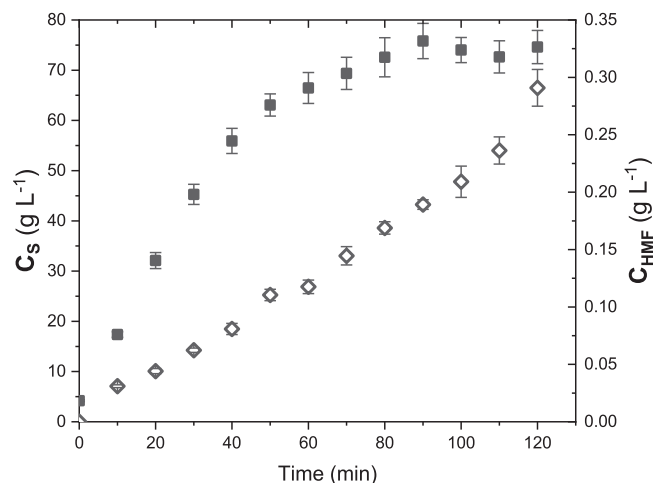


Fig. 2. Evolution over time of the concentration of substrate (S), glucose in this case, and of HMF during the acid hydrolysis of potato residues. Data points: ■ substrate -glucose (S); ♦ HMF.

concentration value not only stops increasing, but eventually begins to slowly decrease due to its dehydration to HMF. To avoid an excessive formation of this by-product that can have a deleterious action of HMF on the microorganism (Tan et al., 2021), a 60 min processing time was chosen as the hydrolysis time to obtain the glucose solution for SA bioproduction, maximizing the difference between the target substrate, glucose, and the undesired by-product.

3.2. Production of succinic acid in bottles from glucose and potato hydrolysate

As a first test, succinic acid was produced both from pure glucose (40 g·L⁻¹) and from potato acid hydrolysate with a similar concentration of glucose using anaerobic conditions and closed bottles as indicated in Section 2.2. Results are collected in Table 1, where it is observed that the outcome regarding acid final titers and yields are really similar, while productivity decreases by 30% and selectivity to SA is also reduced by 16% when replacing a pure glucose medium by potato hydrolysate. This decrease in productivity is probably due to the deleterious effect of the phenolic compounds present in this type of waste (Samaniego et al., 2020). In previous works of this group, it was determined that the increase in biomass concentration favors the selectivity to SA (Escanciano et al., 2023). Taking into account that, probably, due to the inhibitory effects of the phenolic compounds, the amount of biomass generated in the fermentation from the hydrolysate was lower than in the experiment carried out with pure glucose (3.4 g·L⁻¹ vs 4 g·L⁻¹), the decrease in the relationship between SA and by-products in the experiment carried out with potato residues could be justified. While the conversion of the experiments carried out in a reactor with pure glucose reached 100% conversion, in the case of carrying them out in a bottle the conversion

Table 1

Succinic acid concentration, yield, productivity and selectivity in its production process from pure glucose and potato hydrolysate in bottles.

Substrate	Pure glucose	Potato hydrolysate
$C_{P,max}$ (g·L ⁻¹)	11.3	10.6
$Y_{S,0}$ (g·g ⁻¹)	0.28	0.27
$Y_{S,cons}$ (g·g ⁻¹)	0.53	0.50
S_{conv} (g·g ⁻¹)	0.53	0.54
$Prod.$ (g·L ⁻¹ ·h ⁻¹)	0.46	0.32
$Sel.$ (g·g ⁻¹)	0.55	0.46

^a $C_{P,max}$: maximum product concentration, $Y_{S,0}$: yield relative to initial substrate concentration, $Y_{S,cons}$: yield relative to initial substrate consumed, S_{conv} : substrate conversion, $Prod.$: productivity, $Sel.$: selectivity.

decreased considerably, a phenomenon also observed in the bibliography by other authors (Shen et al., 2016b; Xi et al., 2013). In spite of this, it should be noted that both the experiments carried out with commercial glucose and glucose from the hydrolysate led to similar conversions: 53% and 54%, respectively.

3.3. Production of succinic acid in a bioreactor from different initial concentrations of glucose and development of a simple kinetic model

In Fig. 3, it can be observed that there are similar biomass (X), temporal curves for all glucose or substrate (S) concentrations, Fig. 3D, with identical maximal biomass concentration in the stationary phase of 5–5.5 g·L⁻¹ and equivalent slope values at the inflection points in the exponential phase of the growth curves (maximal biomass production rate). Obtaining similar biomass values in all the experiments carried out is probably due to the range of concentrations studied (20–50 g·L⁻¹). Other authors have studied the production of succinic acid in a wider range of concentrations between 0 and 160 g·L⁻¹ (Lin et al., 2008). It was observed that, at concentrations lower than 10 g·L⁻¹, the value of the specific growth rate of biomass had an increasing trend. Between 10 and 50 g·L⁻¹ this parameter reached its maximum value and the values remained practically constant; at higher sugar concentrations this parameter decreased due to the inhibitory effects of glucose. As in this work the experiments have been carried out between 20 g·L⁻¹ and 50 g·L⁻¹ and, therefore, they are in that interval of 10 g·L⁻¹ and 50 g·L⁻¹ in which the specific growth rate is constant, the final concentration of the biomass is the same in all cases. Likewise, substrate consumption curves are almost lines, Fig. 3C, except for the lag phases, that have practically the same slopes except for the 50 g·L⁻¹ glucose experiment, where the consumption rate is slightly slower, which seems to indicate inhibition by substrate. Very similar trends in the production rate of succinic acid or product (P) are appreciated, Fig. 3A, again except for the 50 g·L⁻¹ experiment, where SA production starts 1–2 h later at a slower pace, suggesting an incipient substrate inhibition. In fact, the experiments carried out at lower concentrations reached a 100% conversion of the substrate, while at 50 g·L⁻¹ the conversion was 97%. As in all cases, the compound that is generated in the greatest amount is SA, the metabolic route that seems to prevail is the generation of oxaloacetate from phosphoenolpyruvate and CO₂ (Fig. 1). The second most prevalent metabolic route of phosphoenolpyruvate generated from glucose after glycolysis is the generation of pyruvate and its consequent transformation into acetic and formic acids. The activity of phosphoenolpyruvate carboxykinase, the main CO₂-fixing enzyme in the metabolism of *A. succinogenes*, seems to be impaired at low glucose concentrations, since Fig. 3B shows that the lower the glucose concentration, the higher the rate of by-product (BP) generation. Glucose is used in less quantity for the generation of biomass, a process that also only takes place during the first 10 h of fermentation.

Similar final by-product values are achieved regardless of the initial biomass concentration, with an increase in selectivity with the initial glucose concentration, 0.52 g·g⁻¹ at 20 g·L⁻¹ of substrate, 0.58 g·g⁻¹ at 30 g·L⁻¹, 0.62 g·g⁻¹ at 40 g·L⁻¹, 0.66 g·g⁻¹ at 50 g·L⁻¹.

Low concentrations of the carbon source were favorable both for the yield and for the productivity of succinic acid, with values of 0.72 g·g⁻¹ and 0.98 g·L⁻¹·h⁻¹ for the runs at 20 g·L⁻¹ and 0.68 g·g⁻¹ and 0.71 g·L⁻¹·h⁻¹ when working with 50 g·L⁻¹ of glucose.

Comparing the results of this study with those obtained by other authors, it is evident that, at the moment, there is no consensus regarding the production trends of succinic acid from glucose in batch-type operations with *A. succinogenes*. (Luthfi et al., 2018) observed an inhibition of cell growth as the initial glucose concentration increased from 20.3 g·L⁻¹ to 123.1 g·L⁻¹. They achieved optimum yield and productivity (0.62 g·g⁻¹, 2 g·L⁻¹·h⁻¹) when fermenting from 60.3 g·L⁻¹ of substrate. Above that concentration runs seemed to suffer from some kind of inhibition by substrate. In addition, they observed a decrease in selectivity as the initial amount of glucose in the culture medium

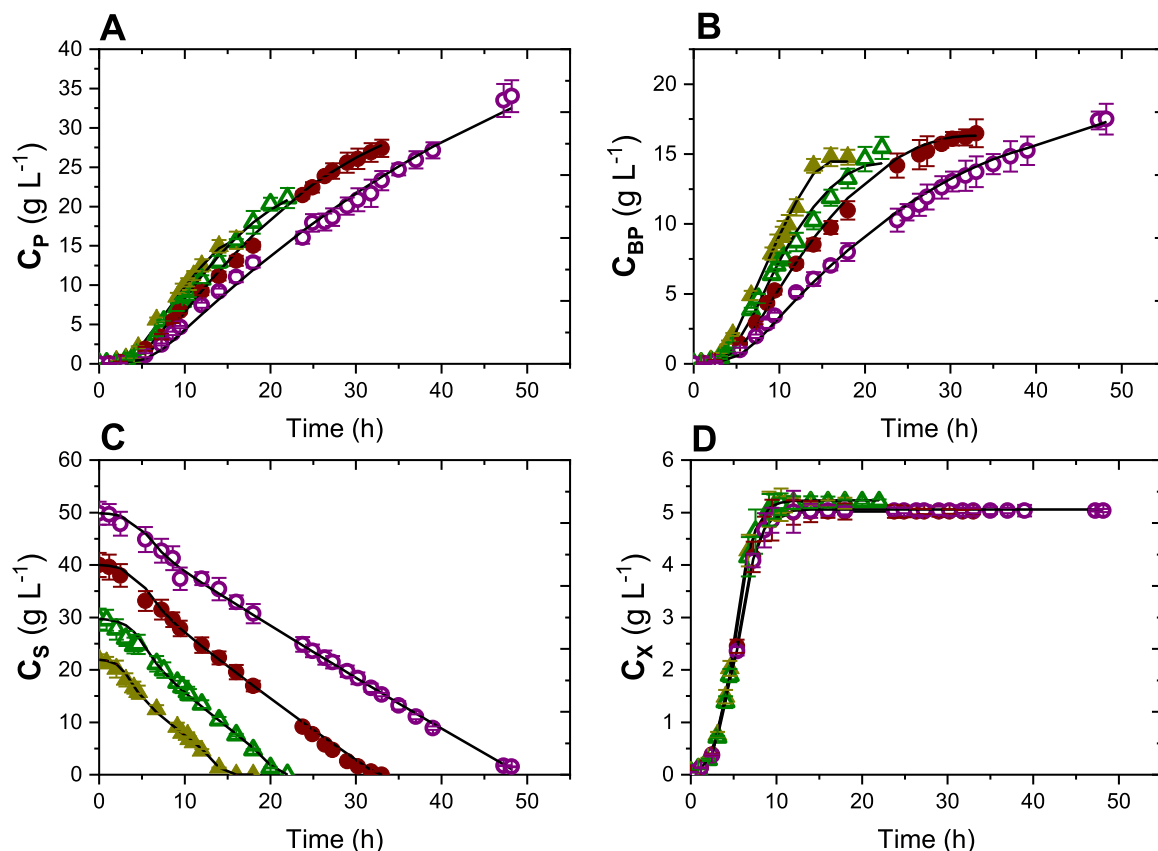


Fig. 3. Kinetic modeling in runs at different initial glucose concentrations. Evolution of SA or product (P) concentration (Figure A), evolution of by-products (BP) concentration (Figure B), evolution of glucose or substrate (S) concentration (Figure C), evolution of biomass (X) concentration (Figure D). Data points: \blacktriangle run 1 – 20 g·L⁻¹ glucose; \triangle run 2 – 30 g·L⁻¹ glucose; \bullet run 3 – 40 g·L⁻¹ glucose; \circ run 4 – 50 g·L⁻¹ glucose. Model predictions shown as lines.

increased. On the other hand, Ferone et al. studied the influence of the initial concentration of this sugar in a range between 5.4 and 80.7 g·L⁻¹ (Ferone et al., 2017). Although the yield results showed oscillations, a downward trend could be deduced as the initial amount of the carbon source increased, coinciding with this study. Despite this, they established a concentration of 42.7 g·L⁻¹ of glucose as the optimum value, a value at which they observed the optimal values of productivity and succinic acid/acetic acid ratio (0.36 g·L⁻¹·h⁻¹, 0.74 g·g⁻¹). It is also worth noting the work of Salvachua et al., who studied the effect of the initial glucose concentration between 40 g·L⁻¹ and 100 g·L⁻¹ (Salvachúa et al., 2016). Although the maximum concentration of glucose was reached at 100 g·L⁻¹, productivity was clearly impaired from 80 g·L⁻¹ of substrate, a concentration at which there was also no complete consumption of this sugar.

Considering data collected in Fig. 3, a modified version of a kinetic model previously published was applied to these runs (Escanciano et al., 2023). The robustness of this model was demonstrated by its application in fermentations carried out under different conditions and operating variables (yeast extract concentration, biomass concentration, CO₂ flow, agitation, and batch and fed-batch type operations).

The development of a kinetic model is a key stage in the design phase of the equipment involved in the fermentation process, especially the bioreactor, to ensure high operational stability and process reproducibility. The use of kinetic models for the design of bioreactors is the safest and most economically efficient way to carry out a good scaling of the process. Since it also provides a large amount of valuable information about the course of the reaction, a more precise process control system can also be developed (Bisotti et al., 2022; Miller and Block, 2020; Wang et al., 2020; Xia et al., 2021).

This model is based in a simple reaction scheme (shown in Fig. 4), on

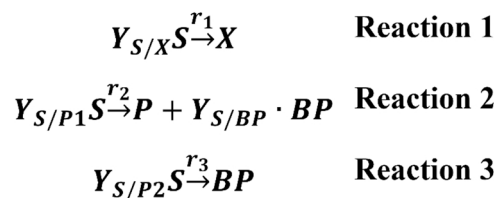


Fig. 4. Reaction network of the kinetic model.

the logistic equation –Eq. (9)– for the biomass growth and on potential kinetic equations to explain substrate consumption and product and byproducts onsets. This kinetic model is of the unstructured non-segregated type, considering the bacteria as a single component (no differences between diverse bacterial cells) and only major substrates and products in the broth containing the cells. The reaction network is presented through a scheme depicting a first reaction considering glucose (S) consumption (reaction 1) to obtain biomass (X), another reaction –reaction 2– to explain SA (P) and by-products (BP) generation, and a final reaction –reaction 3– that describes independent byproduct (BP) creation from glucose. Thanks to previous work carried out by this research group (Escanciano et al., 2022a), it was possible to demonstrate the possibility of producing SA efficiently with cells in a resting state, that is, in the absence of nutrients and, therefore, without cell growth. Due to these conclusions, a reaction scheme has been proposed in which the production of product and by-products is not associated to the growth of biomass. Each of the reactions in the reaction network proceeds at a certain rate described by the reaction rates r_i . The rate equations of the reactions 1, 2 and 3 are respectively r_1 (Eq. (9)), r_2 (Eq. (10)), and r_3 (Eq. (11)). The logistic equation (Eq. (9)) describes the

microorganism growth based on two relevant kinetic parameters: the maximum value of the biomass concentration $-C_{Xm}$ and the specific growth rate $-\mu$. The equations describing the evolution of substrate, SA and by-products are independent of the logistic equation and they are of potential type (Eqs. (10) and (11)). Eq. (10) describes the SA or product (P) formation and the by-products (BP) generation. Eq. (10) is characterized by the kinetic constant k_{P1} . Eq. (11) describes the formation of the sum of the by-products independently of the formation of SA. Eq. (11) is characterized by the kinetic constant k_{P2} . In the *A. succinogenes* metabolic pathway (Fig. 1) there are by-products such as formic acid and acetic acid that can be formed independently of the presence of CO_2 in the culture medium. However, for the activation of succinic acid generation, CO_2 insufflation is required. That is, by-products are always present in the metabolic pathway of *A. succinogenes*, while for the formation of succinic acid activation of this metabolic pathway is required by adding CO_2 , reason for which an independent equation has been assigned to the formation of SA. Finally, several macroscopic yields link biomass, substrate, SA, and by-products: $Y_{S/X}$ (relationship between substrate and biomass in reaction 1) $Y_{S/P1}$ (relationship between substrate and product and by-products in reaction 2) $Y_{S/BP}$ (relationship between substrate and by-products in reaction 2), and $Y_{S/P2}$ (relationship between substrate and by-products in reaction 3) in Eqs. (12) to (15) of production and consumption rates. Eq. (12) describes substrate consumption in reactions 1, 2 and 3 taking into account the macroscopic yields $Y_{S/X}$, $Y_{S/P1}$ and $Y_{S/P2}$. Eq. (13) describes SA production and, since in the reaction scheme the generation of product (P) only occurs during reaction 2, the rate of that reaction (r_2 , Eq. (10)) is the same as the rate of SA production (Eq. (13)). Eq. (14) shows the biomass growth rate that coincides with the rate of reaction 1 (r_1 , Eq. (9)). Since, as mentioned above, the BP generation takes place during the course of reactions 2 and 3 of the scheme shown in Fig. 4, its production rate corresponds to the sum of the rates of reactions (10) and (11) taking into account the macroscopic performance $Y_{S/BP}$.

$$r_1 = \mu \cdot C_X \cdot \left(1 - \frac{C_X}{C_{Xm}}\right) \quad (9)$$

$$r_2 = k_{P1} \cdot C_S \cdot C_X \quad (10)$$

$$r_3 = k_{P2} \cdot C_S \cdot C_X \quad (11)$$

$$\frac{dC_S}{dt} = -Y_{S/X} \cdot r_1 - Y_{S/P1} \cdot r_2 - Y_{S/P2} \cdot r_3 \quad (12)$$

$$\frac{dC_P}{dt} = r_2 \quad (13)$$

$$\frac{dC_X}{dt} = r_1 \quad (14)$$

$$\frac{dC_{BP}}{dt} = Y_{S/BP} \cdot r_2 + r_3 \quad (15)$$

Until now, most of the kinetic models developed are of the unstructured-non-segregated type, being limited, moreover, to the prediction of the evolution of biomass or succinic acid. However, some authors have proposed models that allow studying the kinetics of biomass, succinic acid, and by-products simultaneously in the fermentation carried out by different microorganisms (*A. succinogenes*, *Basfia succiniciproducens*, *Mannheimia succiniciproducens*, and *Yarrowia lipolytica*) (Li et al., 2022; Lin et al., 2008; Pateraki et al., 2016; Song et al., 2008; Vlysidis et al., 2011). The biomass formation rate in these studies was based on a Monod type model, considering, in addition, that it could be susceptible to substrate inhibitions of the Haldane-Andrews type and/or Luong type and product inhibitions of the Luong type. In addition, they agreed on the decision to include the Pirt's maintenance coefficient in the rate of the carbon source consumption equations and on the use of the Luedeking-Piret expression to estimate the evolution of succinic acid

and by-products concentrations (Escanciano et al., 2022b).

These models are highly accurate and take into account a large number of inhibitions due to substrate or product, however, they imply the need to incorporate a large number of parameters, needing to estimate up to 19 kinetic parameters. This is quite inconvenient from the point of view of chemical engineering, due to its possible application in carrying out process scaling or techno-economic analysis. Therefore, it is necessary to limit the use of these parameters as much as possible (Escanciano et al., 2022b; Li et al., 2022; Lin et al., 2008; Pateraki et al., 2016; Song et al., 2008; Vlysidis et al., 2011).

In all cases, the estimation of the Pirt's coefficient obtained extremely low values (Li et al., 2022; Song et al., 2008; Vlysidis et al., 2011) and, since, as previously discussed, there is a wide range of initial substrate concentration values that do not generate inhibition (Ferone et al., 2017; Luthfi et al., 2018; Salvachúa et al., 2016), on many occasions sugar consumption can be estimated without the need for this type of parameter. Regarding the inhibition by products and by-products, Lin et al. observed that formic acid was the compound that generated the greatest inhibition in the production of SA (Lin et al., 2008). They determined that the critical concentrations for formic acid, ethanol, acetic acid, pyruvic acid and succinic acid would be, respectively: 16 g·L⁻¹, 42 g·L⁻¹, 46 g·L⁻¹, 74 g·L⁻¹, and 104 g·L⁻¹.

In Table 2, the kinetic parameters estimated with the proposed model for the experiments carried out under different initial concentrations of commercial glucose are shown. As previously mentioned, the biomass values in the range of glucose concentrations studied (20–50 g·L⁻¹) did not show significant differences (conclusion also observed by (Lin et al., 2008), which is why μ and $Y_{S/X}$ remain constant in all estimations. The estimated macroscopic yields ($Y_{S/P1}$, $Y_{S/P2}$, $Y_{S/BP}$) for fermentations carried out at different initial amounts of glucose present variations within their confidence intervals, which agrees with the observations made in Fig. 3, where it was observed that, regardless of the initial amount of glucose, the generation of by-products in the fermentations was similar in all cases. This implies that the reaction of pyruvate from phosphoenolpyruvate (and its consequent transformation into acetic and formic acids) proceeds in a similar way in all cases. For this reason, k_{P2} , kinetic parameter of the rate of reaction 3 for the generation of by-products, also does not suffer variations outside the confidence interval and can be considered constant. Therefore, in this work, no statistically significant variations have been observed depending on glucose initial concentrations for most of the estimated kinetic parameters, except for k_{P1} , parameter that would be modified due to the variations in production rate that took place under different initial amounts of sugar, as well as the variations in the yield of succinic acid.

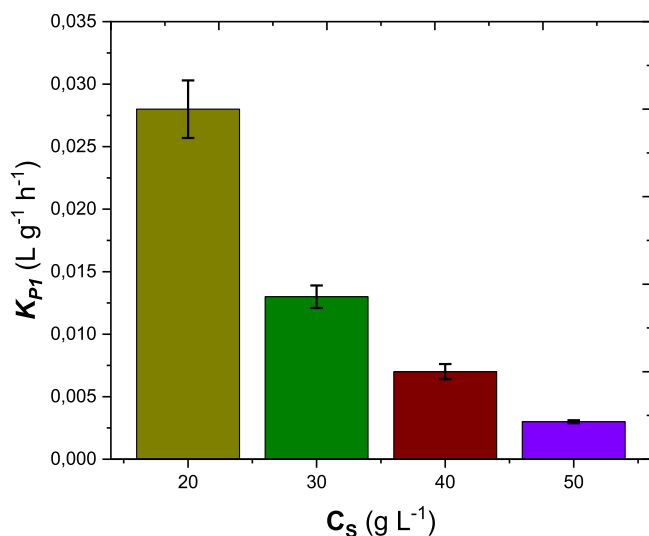
This kinetic constant k_{P1} , as shown in Fig. 5, clearly decreases as the substrate concentration increases, confirming the substrate inhibition firstly appreciated in Fig. 3A and C. As previously mentioned, this inhibition affects the decrease in phosphoenolpyruvate carboxykinase activity and, therefore, the fixation of CO_2 for the formation of oxaloacetate, a compound that is subsequently reduced to generate succinate (Fig. 1). Therefore, for the approach of a model that would take this parameter into account, which affects both the rate of formation of products and by-products, and which does not drastically increase the number of kinetic parameters, Eq. (16) was proposed. Thus, this parameter is calculated as a function of an exponential equation that incorporates the constants α and β , allowing the simultaneous adjustment of all the experiments to different initial glucose concentrations. Thanks to this equation that allows to integrate the main inhibitory effect, the one corresponding to the carbon source, it is possible to carry out a joint estimation of the kinetic parameters of fermentation from the data of all the experiments carried out at different initial glucose concentrations. Table 2 shows the results both in the individual estimations of each one of the fermentations (runs carried at different initial glucose concentrations), as well as in the joint estimation of all the fermentations carried out at different concentrations of this sugar. Eq. (16) is included in this joint estimation, so, in this last case, instead of presenting the

Table 2

Kinetic parameter values calculated by fitting the kinetic model to experimental data of succinic acid production from different initial glucose concentrations individually and together.

C_s (g L ⁻¹)	20			30			40			50			All runs		
C_{Xm} (g L ⁻¹)	5.209	±	0.019	5.232	±	0.032	5.019	±	0.021	5.060	±	0.045	5.095	±	0.027
k_{p1} (L g ⁻¹ h ⁻¹)	0.028	±	0.002	0.013	±	0.001	0.007	±	0.001	0.003	±	0.0001			
k_{p2} (L g ⁻¹ h ⁻¹)	0.017	±	0.002	0.017	±	0.004	0.018	±	0.002	0.018	±	0.001	0.017	±	0.001
μ (h ⁻¹)	0.859	±	0.015	0.854	±	0.023	0.845	±	0.043	0.839	±	0.025	0.923	±	0.016
$Y_{S/P1}$ (g g ⁻¹)	0.818	±	0.023	0.891	±	0.022	0.823	±	0.062	0.888	±	0.020	0.830	±	0.066
$Y_{S/P2}$ (g g ⁻¹)	1.696	±	0.310	1.625	±	0.109	1.773	±	0.132	1.646	±	0.157	1.667	±	0.089
$Y_{S/BP}$ (g g ⁻¹)	1.138	±	0.149	1.112	±	0.111	1.099	±	0.091	1.194	±	0.091	1.102	±	0.043
$Y_{S/X}$ (g g ⁻¹)	1.174	±	0.129	1.632	±	0.141	1.115	±	0.082	1.532	±	0.136	1.120	±	0.087
α (L g ⁻¹ h ⁻¹)													0.152	±	0.008
β (L g ⁻¹)													0.079	±	0.001

^a $C_{X,max}$: maximum biomass concentration, k_{p1} : kinetic constant of Eq. (10), k_{p2} : kinetic constant of Eq. (11), μ : specific growth rate, $Y_{S/P1}$: macroscopic yield substrate/products in reaction 2, $Y_{S/P2}$: macroscopic yield substrate/products in reaction 3, $Y_{S/BP}$: macroscopic yield substrate/by-products in reaction 2, $Y_{S/X}$: macroscopic yield substrate/biomass in reaction 1, α : proportional parameter in Eq. (16), β : exponential parameter in Eq. (16).

**Fig. 5.** K_{p1} constant as a function of substrate concentration.**Table 3**

Statistical parameter values calculated by fitting the kinetic model to experimental data of succinic acid production from different initial glucose concentrations individually and together.

C_s (g L ⁻¹)	F_{95}	RMSE	SSR	VE (%)
20	30,616	0.84	7.06	99.3
30	29,587	0.87	8.76	98.6
40	41,242	0.67	6.47	99.5
50	33,646	0.58	7.70	99.1
All runs	20,191	1.28	9.63	97.8

^a C_s : substrate concentration

^b F_{95} : Fisher's F value (95% confidence), RMSE: Root Mean Square Error, SSR: sum of variances or squared residues, VE variation explained.

values of k_{p1} , the values of α and β are shown. The statistical parameters of these experiments (estimations of each fermentation carried out at different initial glucose concentrations individually as well as the joint estimation of all the experiments) are presented in Table 3.

$$k_{p1} = \alpha \cdot e^{-\beta \cdot C_{s0}} \quad (16)$$

Fig. 3 shows how the model fits very accurately to all relevant data. In addition, it should be noted that the values of the estimated Fisher's F (20,191–41,242) shown in Table 3 are much higher than its tabulated value at 95% confidence (8.55), which determines a high degree of fidelity of this kinetic model. RMSE, a parameter whose proximity to zero implies a high degree of adjustment of the concentrations estimated by the model to the experimental data, presents values between 0.58 and 1.28 and VE, a parameter whose proximity to 100% implies a high degree of similarity between the calculated and experimental rates, is between 97.8% and 99.5%. Compliance with all these statistical parameters reflects a high goodness-of-fit of the kinetic model and the parameter estimation carried out.

3.4. Production of succinic acid in a bioreactor from potato hydrolysate

Regarding the production of succinic acid, Table 4 shows a notably higher final product concentration when potato hydrolysate (reaching an initial glucose concentration of 35 g L⁻¹) is used as a carbon source instead of commercial glucose. The experiment based on the hydrolysate increases the yield to SA by 37% compared with the experiment with 40 g L⁻¹ pure glucose, also the selectivity increases by 22.5% (reaching a selectivity value of 0.80 g g⁻¹), which is even more relevant. This increase in yield and selectivity are probably due to the composition of this tuber which, as has already been determined by other authors, contains high levels of potassium, vitamin C (12–40 mg g⁻¹ fresh weight) and proteins (0.14–0.16%) (Kita, 2002; Leonel et al., 2017; Samaniego et al., 2020). Specifically, it contains high levels of asparagine, reaching between 0.094% and 1.137% (Granda et al., 2005). However, productivity decreases by 24% and the substrate conversion is high but not complete (97%), probably due to the presence of phenolic compounds, present in potato in a concentration between 0.41 and 3.25 g kg⁻¹ dry weight (Samaniego et al., 2020). Table 4 summarizes the production results obtained in this study together with those obtained by other authors

Table 4

Comparison of the bibliographic results of succinic acid production from biomass through the action of *A. succinogenes* with those corresponding to this study in a reactor using glucose and potato residues as substrate.

Type of operation	Substrate	C _p (g·L ⁻¹)	Y _{S,0} (g·g ⁻¹)	Prod. (g·L ⁻¹ ·h ⁻¹)	Reference
Fed-batch	Grape pomace and stalks	40.2	0.67	0.79	(Filippi et al., 2021)
Repeated batch	Tequilana agave bagasse	33.6	0.39	1.32	(Corona-González et al., 2016)
Fed-batch	Olive pits	33.7	0.27	0.50	(Oreoluwa Jokodola et al., 2022)
Batch	Napier grass	17.5	0.58	0.79	(Lee et al., 2022)
Fed-batch	Citrus peel waste	22.4	0.73	0.45	(Patsalou et al., 2020)
Batch	Palm oil hydrolysate	36.5	0.57	1.95	(Luthfi et al., 2018)
Batch	Grape must	88.9	0.66	0.93	(Hijosa-Valsero et al., 2022)
Batch	Sweet sorghum bagasse	17.8	0.61	0.89	(Lo et al., 2020)
Batch	Sugarcane juice	57.9	0.89	1.21	(Shen et al., 2016a)
Batch	Macroalgae <i>Saccharina latissima</i>	36.8	0.92	3.90	(Marinho et al., 2016)
Batch	Macroalgae <i>Laminaria digitata</i>	24.4	0.86	0.50	(Alvarado-Morales et al., 2015)
Fed-batch	Fresh cassava root	151	1.51	3.22	(Thuy et al., 2017)
Batch	Glucose – 20 g L ⁻¹	15.7	0.72	0.98	This study
Batch	Glucose – 30 g L ⁻¹	21.1	0.71	0.96	This study
Batch	Glucose – 40 g L ⁻¹	27.4	0.68	0.83	This study
Batch	Glucose – 50 g L ⁻¹	34.0	0.67	0.71	This study
Batch	Potato wastes	32.2	0.92	0.64	This study

a. C_p: product concentration, Y_{S,0}: yield relative to initial substrate concentration, Prod.: productivity.

from biomass in recent years.

Among the studies devoted to the production of SA from biomass, it is worth highlighting the work of Marinho et al., who achieved a productivity of 3.90 g·L⁻¹·h⁻¹ with a batch operation from macroalgae *Saccharina latissima*, one of the highest productivities achieved from a non-commercial carbon source (Marinho et al., 2016). Thuy et al. carried out the fermentation process of fresh cassava root with a very high production speed (3.22 g·L⁻¹·h⁻¹) through a fed-batch operation (Thuy et al., 2017). In addition, these authors reached a SA yield of 0.92 g·g⁻¹, reaching a reaction yield of 1.51 g·g⁻¹.

In recent years, the number of studies focused on the production of succinic acid from food residues has been increasing (Morone et al., 2019; Sayury Nishida et al., 2021). Filippi et al. used grape pomace and stalks in fed-batch operation, achieving a yield of 67% and a productivity of 0.79 g·L⁻¹·h⁻¹ (Filippi et al., 2021). This same type of operation was used by Oreoluwa Jokodola et al. valorizing olive stone, but the yield was limited to 27% and the productivity to 0.5 g·L⁻¹·h⁻¹ (Oreoluwa Jokodola et al., 2022). Lo et al. carried out a batch type fermentation with the same microorganism to produce succinic acid from sweet sorghum bagasse, achieving yields of 61% and productivities of 0.89 g·L⁻¹·h⁻¹ (Lo et al., 2020). The use of citrus residues has also been studied in fed-batch conditions obtaining relatively low SA productivity values (0.45 g·L⁻¹·h⁻¹), but the yield (0.73 g·g⁻¹) was appreciable (Patsalou et al., 2020). Although one of the highest yields of succinic acid from food residues (0.92 g·g⁻¹) has been achieved in the present study, other authors have managed to achieve considerably higher productivities: Corona-González et al. achieved 1.32 g·L⁻¹·h⁻¹ using a fermentation of Tequilana agave bagasse through a discontinuous operation, though

obtaining considerably lower yield values (0.39 g·g⁻¹) (Corona-González et al., 2016).

3.5. Estimation of kinetic parameters in fermentation with potato residues

Fig. 6 shows the evolution of the substrate, succinic acid, by-products (acetic and formic acids) and biomass in the experiment carried out in a reactor using a hydrolysate of potato waste as a carbon source. This figure also represents the fit to the experimental data of the kinetic model developed for pure glucose in Section 3.3, including Eq. (16) (so that k_{p1} becomes a variable dependent on the parameters α , β and the initial glucose concentration).

Likewise, Fig. 6 shows how the fitting of the model (in lines) is very accurate. Again, RMSE values are low, while F values are much higher than the values needed to overcome the null hypothesis at 95% confidence (threshold F value interval tabulated at 95% confidence for the relevant numerator and denominator degrees of freedom: 20–30). Moreover, VE values are near 100%, so the kinetic model easily explains the evolution of all relevant dependent variables with process time. As an example, in Table 5, the overall values for F , VE and $RMSE$, as well as for the Sum of Squared Residuals (SSR) are collected. In this Table, the values of the kinetic parameters are also compiled together with their intervals of error at 95% confidence according to the Student test, showing that the values, as happened in the case of using pure glucose as carbon source or substrate, are statistically significant.

The comparison of the results, obtained with potato hydrolysate and with pure glucose in terms of the values of the corresponding kinetic parameters, shows that there is a higher final biomass concentration (6.587 g·L⁻¹ vs. 5.095 g·L⁻¹) when using the potato hydrolysate, but, at the same time, lower growth rate is appreciated (μ_m is 0.878 h⁻¹ versus 0.923 h⁻¹), showing a higher need, and ability, for the microorganism to adapt to the hydrolysate, probably due to the presence of toxic substances like HMF or the phenolic compound present in potato and potato processing-derived waters and solutions (Akyol et al., 2016). The increase in succinic acid yield is justified due to the increase in $Y_{S/P1}$ (1.337 g·g⁻¹ vs. 0.830 g·g⁻¹ with pure glucose). Very interestingly, less by-product generation is reflected in $Y_{S/BP}$ and $Y_{S/P2}$, which dramatically decrease from 1.102 g·g⁻¹ and 1.667 g·g⁻¹ with pure glucose to 0.363 g·g⁻¹ and 0.585 g·g⁻¹ with the hydrolysate. In addition, k_{p2} also decreases

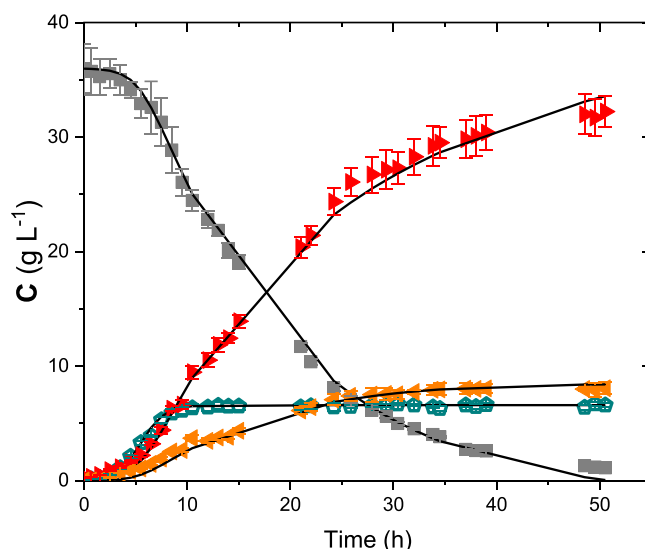


Fig. 6. Kinetic modeling of succinic acid production employing potato wastes as carbon source. Data points: ■ observed substrate; ▲ observed product; ▲ observed by-products; ● observed biomass. Model predictions shown as lines.

Table 5

Kinetic and statistical parameter values calculated by fitting the kinetic model to experimental data of succinic acid production employing potato wastes as carbon source.

C_{Xm} (g·L ⁻¹)	6.587	±	0.043
k_{p2} (L·g ⁻¹ ·h ⁻¹)	0.007	±	0.0003
μ (h ⁻¹)	0.878	±	0.042
$Y_{S/P1}$ (g·g ⁻¹)	1.337	±	0.066
$Y_{S/P2}$ (g·g ⁻¹)	0.585	±	0.045
$Y_{S/BP}$ (g·g ⁻¹)	0.363	±	0.018
$Y_{S/X}$ (g·g ⁻¹)	0.415	±	0.015
α (L·g ⁻¹ ·h ⁻¹)	0.157	±	0.001
β (g·L ⁻¹)	0.073	±	0.001
F_{95}	30,473		
RMSE	0.88		
SSR	6.18		
VE (%)	98.4		

^a $C_{X,max}$: maximum biomass concentration, k_{p1} : kinetic constant of Eq. (10), k_{p2} : kinetic constant of Eq. (11), μ : specific growth rate, $Y_{S/P1}$: macroscopic yield substrate/products in reaction 2, $Y_{S/P2}$: macroscopic yield substrate/products in reaction 3, $Y_{S/BP}$: macroscopic yield substrate/by-products in reaction 2, $Y_{S/X}$: macroscopic yield substrate/biomass in reaction 1, α : proportional parameter in Eq. (16), β : exponential parameter in Eq. (16).

^b F_{95} : Fisher's F value (95% confidence), RMSE: Root Mean Square Error, SSR: sum of variances or squared residues, VE variation explained.

from 0.017 L·g⁻¹·h⁻¹ with commercial glucose to 0.007 L·g⁻¹·h⁻¹ with the hydrolysate. This decrease in k_{p2} may be associated with the presence of HMF, a compound that, although it does not have its own parameter because it has not shown remarkable inhibitory effects on the yield of the process (as previously mentioned, it is found in a very low concentration due to the selected hydrolysis time), may be affecting the reaction rate decreasing the values of the kinetic constants. Another factor that may be affecting the speed of the process is the presence of phenolic compounds reported by other authors (Samaniego et al., 2020). The parameters of k_{p1} only undergo variations within the confidence interval.

4. Conclusions

From fermentation experiments with *A. succinogenes* at different initial glucose concentrations, it has been possible to apply a precise kinetic model to succinic acid production, by-products, biomass, and substrate consumption. Subsequently, after verifying the possibility of producing succinic acid from potato wastes in orbitally shaken bottles, this kinetic model was applied to data from the production of the acid from these residues in a bioreactor batchwise operated. This way it was possible to obtain one of the highest succinic acid bioproduction yields from food residues that can be found in the literature (0.92 g·g⁻¹), even surpassing the one obtained with pure glucose (0.68 g·g⁻¹). However, when employing this monosaccharide, the rate of succinic acid production was higher (0.83 g·L⁻¹·h⁻¹) than when the hydrolysate was used as a carbon source (0.64 g·L⁻¹·h⁻¹). This study shows the potential of potato waste reuse as part of the circular economy concept to develop solutions based on the biorefinery approach.

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Itziar A. Escanciano: Methodology, Software, Investigation, Data curation, Writing – original draft preparation, Visualization. **Victoria E. Santos:** Conceptualization, Software, Validation, Formal analysis, Resources, Data curation, Writing – review & editing, Visualization, Supervision, Funding acquisition. **Ángeles Blanco:** Conceptualization, Validation, Resources, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition. **Miguel Ladero:** Conceptualization, Validation, Formal analysis, Resources, Data curation, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no knowledge of competing financial interests or personal relationships that could have influenced or appeared to influence the work here reported.

Data availability

Data will be made available on request.

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