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ORIGINAL ARTICLE

Integral use of brewery wastes as carbon and nitrogen sources for the bioproduction of succinic acid

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Abstract
Circular bioeconomy is one of the major socio-economic objectives for the twenty-first century, which includes the use of biomass waste and its transformation through environmentally friendly processes into biorefinery building blocks. Among these compounds, succinic acid (SA) obtained by fermentation stands out. This work demonstrates the feasibility of using beer bagasse and spent brewer's yeast as carbon and nitrogen sources for the bioproduction of SA with *Actinobacillus succinogenes*. The use of a progressive enzymatic treatment liberated simple monosaccharides and peptides that were used by the microorganism, in a subsequent fermentation. Compared to the use of commercial xylose and yeast extract, the used of beer wastes obtained better yields (0.77 g g^{-1}) and selectivity (76%), though with a slightly lower productivity (0.15 g L^{-1} h^{-1}). Finally, an unstructured non-segregated kinetic model was successfully fitted, facilitating the future performance of bioreactor design, techno-economic analysis, scaling of the process, or design of a control system.

Keywords Succinic acid · brewery wastes · *Actinobacillus succinogenes* · kinetic modeling · circular economy

1 Introduction

Currently, the largest beer consumers are the USA, China, Brazil, Russia, and Germany. Although the largest percentage of beer produced still comes from large companies, numerous craft beer businesses have been promoted since 1970, becoming a sector that has gaining an important market power, especially since the COVID-19 pandemic began, at which time drinking habits underwent severe changes. Despite the fact that in the developed regions the consumption of beer remained constant or even in decline, in the twenty-first century, there has been a large increase in beer consumption globally due to the impact on its demand from developing regions and, therefore, intensifying the amount of waste generated by this growing industry [1–3]. Beer bagasse, also known as brewer's spent grain, amounts to 85% of the total solid waste in brewery, with a total yearly production of 34–35 million tons in Europe. Of this residual bagasse, approximately 70% is used for livestock feed, 10% is used for biogas, and 20% is deposited in landfills (releasing 513 kg CO_2 equivalent of greenhouse gases per ton of bagasse) [4–6]. Brewer's spent yeast is obtained at a rate of 0.125 million tons/year [5]; however, despite its high protein content and versatility of applications, this yeast generally ends up being mixed with wastewater and discharged for treatment (releasing 83 kg CO_2 equivalent per ton of waste treated) [6, 7]. Although these residues can be used for human consumption, as well as in the pharmaceutical industry, more solutions are needed to cope with these high volumes, which will continue to increase over the coming decades [8]. For this reason, in recent years, numerous studies have been carried out focused on the use of these residues in fermentation processes with fungi and actinobacteria to produce high value products such as β-glucans, proteins, amino acids or succinic, glutamic, lactic, and γ-aminobutyric acids [9, 10].

The generation of food waste is a problem that is gaining more and more prominence. According to the Food and Agriculture Organization (FAO), each year, 1300 million tons of food is wasted [11, 12] and the lack of efficiency in the use of food resources jeopardizes the fulfillment of several of the Sustainable Development Goals (SDGs) [13].

Biorefineries represent a great solution to part of this problem and, therefore, meet several of these objectives [14]. According to the United States Department of

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Energy (US DOE), succinic acid is one of the 12 main platform chemicals [15, 16]. Traditionally, succinic acid has been used for the production of resins, coatings, and pigments. It also has many applications in the food industry as an acidulant, flavoring, and sweetener, as well as in the pharmaceutical industry. It is worth noting its great potential as a replacement for maleic anhydride, acting as a chemical platform for the generation of a multitude of compounds. Furthermore, one of its most promising applications in the bioeconomy era is the production of biodegradable polymers, such as polyamides and polyesters [17–19].

Since succinic acid is an intermediate compound of the tricarboxylic acid cycle (TCA), it can be synthesized by almost all cells, both plant and animal. The use of fungi for the production of this compound has been extensively studied; however, the use of these microorganisms presents numerous difficulties both during fermentation and in the separation and purification processes. To date, bacteria isolated from the rumen of cattle are considered the best candidates to produce this acid, being its final product during anaerobic fermentation. The most promising strain is *Actinobacillus succinogenes*, a bacterium with the distinctive ability to produce a relatively large amount of succinic acid under anaerobic conditions from a wide variety of carbon sources such as arabinose, cellobiose, fructose, glucose, lactose, maltose, mannitol, mannose, sorbitol, sucrose, or xylose [20–24].

In recent years, great efforts have been devoted to the investigation of succinic acid production from residues through fermentation processes (Table 1) [25–30]. It is worth noting the work of [31], in which they used winery wastes (grape pomace, stalks, and wine lees) to produce bacterial cellulose, value-added fractions, and succinic acid; they worked with *A. succinogenes* as a biocatalyst for the production of this last compound [18] also used this microorganism and olive pits and sugarcane bagasse as carbon sources, two residues rich in xylose [32] compared the performance of *A. succinogenes* and *Basfia succiniproducens* in the production of succinic acid from vine shoots and surplus grape and, although both species had similar yields from vine shoots, fermentation from grape with *A. succinogenes* obtained much better results than with *B. succinoproducens*. Despite its high costs, as a general rule, in the production of succinic acid, yeast extract (YE) [17, 21, 32, 33] is the usual source of nitrogen [34], although its substitution by corn steep liquor (CSL) [35, 36] or a mixture of both nutrient sources [37] have also been studied. Jiang et al. [38] used brewer's spent yeast for the production of succinic acid from glucose. They compared the effect of brewer's yeast pretreatment by autolysis or by enzymatic hydrolysis, observing better fermentation performance from enzymatic hydrolysate. However, to achieve complete glucose consumption, they

Table 1 Comparison of the bibliographic results of succinic acid production through the action of *A. succinogenes* with those corresponding to this study in a reactor using BBH or commercial xylose as carbon source and SBYH or YE as nitrogen source

Type of operation	Cell state	Carbon source	Nitrogen source	C_{SA}(g L^{-1})	Y_{SA} (g g^{-1})	P_{SA} (g L^{-1} h^{-1})	Reference
Batch	Growing, free	Xylose	YE	3.94	0.42	0.15	[47]
Batch	Growing, free	Xylose	YE	36.7	0.27	0.51	[18]
Batch	Growing, free	Lignocellulosic sugars	YE	27.0	0.55	0.22	[48]
Batch	Resting, free	Xylose	None	8.51	0.43	0.18	[45]
Continuous	Growing, immobilized	Xylose	YE, corn steep liquor	29.4	0.68	3.4	[56]
Fed-batch	Growing, free	Olive pits	YE	33.7	0.27	0.50	[18]
Batch	Growing, free	Napier grass	YE	17.54	0.58	0.79	[33]
Repeated batch	Growing, immobilized	Tequilana agave bagasse	YE	33.6	0.39	1.32	[50]
Batch	Growing, free	Glucose	SBYH	47.6	0.68	0.63	[38]
Fed-batch	Growing, free	Grape stalks and pomace	YE	40.2	0.67	0.79	[49]
Fed-batch	Growing, free	Grape stalks and pomace	Wine lees	37.2	0.64	0.79	[31]
Batch	Growing, free	Xylose	YE	10.6	0.53	0.38	This study
Batch	Growing, free	BBH	YE	13.3	0.61	0.41	This study
Batch	Growing, free	Xylose	SBYH	12.8	0.61	0.14	This study
Batch	Growing, free	BBH	SBYH	15.6	0.77	0.15	This study

needed to supplement the medium with vitamins. In summary, although the state of the art shows the feasibility of producing succinic acid by biotechnological processes, it has a high cost mainly associated to the cost of the carbon source and, especially, of the nitrogen source.

Therefore, this paper is focused on batch producing succinic acid by biotechnological means at a lower cost by promoting circular economy concepts. The novelty is based on using brewery wastes as source of secondary raw materials to replace both carbon and nitrogen sources in the fermentation process. Beer bagasse, rich in xylose, is used as carbon source and brewer's spent yeast as nitrogen source, without vitamin supplementation. The results are compared with fermentations carried out using commercial xylose and yeast extract. Finally, a kinetic model will be fitted to experimental data on the evolution of the species involved in the fermentation carried out from hydrolysates facilitating the future performance of bioreactor design, techno-economic analyses, scaling of the process, or design of a control system.

2 Methods

2.1 Beer bagasse hydrolysis

Beer bagasse, obtained from a local brewery (La Cibeles S.L—Madrid, Spain), was dried at 40°C for 48 h, ground, and sieved until reaching a size of 0.75 mm. Afterwards, it was mixed with water in a 1:6 w:v ratio and adjusted to pH 5.5 with H_3PO_4. Subsequently, enzymatic hydrolysis was carried out in three steps. The first was carried out at 90°C and 180 rpm for 1 h, adding 1 mL of Termamyl® SC DS for each liter of the mixture of water and bagasse. In the next stage, the temperature was lowered to 55°C and hydrolysis was carried out for 1 h at 180 rpm with the addition of 0.3 mL L^{-1} Saczyme® Yield, 0.3 mL L^{-1} FAN Boost™, and 0.3 mL L^{-1} Ultraflo® XL. In the last stage, the pH was adjusted to 5 (H_3PO_4) and the sample was hydrolysate for 10 h at 180 rpm and 45°C with 15 mL L^{-1} of Celluclast® 1.5 L [39, 40]. The obtained hydrolysate was centrifuged for 20 min at 4°C and 8000 rpm. Due to the high amount of volatiles in the hydrolysate [41], the supernatant was subjected to air blowing for 6 h at 50 °C to eliminate volatile compounds. Finally, it was purified with activated charcoal (AC) for 1 h in a proportion of 4 g per 100 mL of hydrolysate to remove phenolic compounds that can inhibit cell growth [42–44]. This sample is considered the beer bagasse hydrolysate (BBH) used as carbon source.

2.2 Spent brewer's yeast hydrolysis

The spent brewer's yeast has also been kindly donated by La Cibeles S.L. The yeast was mixed with water at a ratio of 10% w/v and adjusted to pH 6.8 by addition of NaOH. Enzymatic hydrolysis was performed with Alcalase® in a proportion of 2 g kg^{-1} dry brewer's yeast (brewer's yeast had a moisture of 43%) for 12 h at 60 °C and 200 rpm [38]. The sample was centrifuged for 15 min at 4 °C and 8000 rpm and the supernatant collected, the spent brewer's yeast hydrolysate (SBYH), was used as nitrogen source.

2.3 Microorganism reactivation, adaptation, and inoculum

The microorganism used was *A. succinogenes* DSM 22257 (German Collection of Microorganisms and Cell Cultures GmbH). For its reactivation, cells maintained at − −80°C in a Tryptic Soy Broth (TSB)/glycerol 50% v v^{-1} mixture were thawed and incubated at 37°C and 200 rpm for 24 h in bottles with TSB medium. TSB composition was as follows (in grams per liter): 17 tryptone, 3 soytone, 2.5 glucose, 5 NaCl, 2.5 K_2HPO_4. The air in the bottles had been previously displaced by N_2 bubbling.

To achieve high yields and a good reproducibility, the cells were reactivated and adapted to the carbon source [45]. For this, successive cell growths were carried out under anaerobic conditions at 37°C, 200 rpm in bottles with increasing concentrations of xylose (until reaching 20 g L^{-1}) and 60 mL of production medium, whose composition was as follows (in grams per liter): 3 K_2HPO_4, 0.43 $MgCl_2{\cdot}6H_2O$, 0.2 $CaCl_2$, 1 NaCl, 10 yeast extract (YE). In addition, both $NaHCO_3$ and the amount of xylose in the medium were added and the pH was adjusted to 6.8. In the event that a fermentation with BBH was subsequently carried out, an adaptation was performed by means of consecutive growths in bottles, under the conditions indicated for the adaptation stage to xylose, with increasing percentages of the hydrolysate until it completely replaced the carbon source. This stage was not necessary to carry out fermentations with SBYH as a nitrogen source. After the adaptations, the last growth of the microorganism was carried out in the inoculum stage. This growth was done under the same operating conditions and with the same composition as in the last step of adaptation to the carbon source, starting from an initial suspended biomass concentration of 0.05 g L^{-1}.

2.4 Bottle production under different dilution factors (DF) of SBYH

In order to determine the best SBYH concentration, a series of experiments was carried out in duplicate in bottles at 37°C, 200 rpm with 20 g L^{-1} of commercial xylose and the previously described production medium but substituting the commercial YE for SBYH. Starting with 0.05 g L^{-1} of biomass, fermentations were carried out with culture media whose composition was 50% SBYH, DF 2, up to media whose proportion of SBYH was 8.3% (DF 12).

As a control, a production of bottles with 10 g L^{-1} YE as a nitrogen source was also carried out.

2.5 Succinic acid bioproduction in a batch reactor

The production of succinic acid in a batch reactor was performed in a 2-L stirred tank BIOSTAT B-Plus (Sartorius AG, Germany). The runs were carried out in duplicate. The operating conditions were as follows: 37°C, 300 rpm, pH 6.8 (5 M NaOH), CO_2 flow rate of 0.1 vvm and a working volume of 1 L. The previously described production medium was used with 20 g L^{-1} of xylose but without addition of $NaHCO_3$. In the pertinent experiments, commercial xylose was substituted by BBH in sufficient quantity to reach 20 g L^{-1} of xylose (containing, furthermore, 0.29 g L^{-1} of maltose, 0.10 g L^{-1} of glucose, 0.03 g L^{-1} of arabinose, 0.72 g L^{-1} of mannose, and 0.29 g L^{-1} of fructose). Experiments were also carried out in which YE was substituted for SBYH with the DF previously determined to be optimal. The fermentations began after the inoculation of 0.05 g L^{-1} of biomass.

2.6 Analytical methods

Biomass concentration was determined by UV–vis spectrophotometry (Shimadzu UV–Vis spectrophotometer UV-1603, Japan) at 600 nm.

Substrate and product concentration were quantified through an Agilent Technologies 100 series equipment, USA, by high-performance liquid chromatography (HPLC). For the sugars analysis, a BP-800 Pb column (8%, 300×× 7.8 mm, Benson) was used. For the determination of the acids concentration, a BP-800 H column (8%, 300×× 7.8 mm, Benson) was chosen. Both columns worked at 80°C with a H_2SO_4 5 mM solution at a flow rate of 0.5 mL min^{-1}. The refraction index detector temperature was 55°C.

2.7 Theory/calculation

In order to compare the results obtained, various fermentation parameters such as titer (C_{SA}, g L^{-1}), yield (Y_{SA}, g g^{-1}), productivity (P_{SA}, g L^{-1} h^{-1}), and selectivity (S_{SA}, g g^{-1}, considering byproduct formation) are used throughout this work, according to the following equations:

$$Y_{SA} = \frac{C_{SA}}{C_{S_0}} \tag{1}$$

$$Y_{SA,Scons} = \frac{C_{SA}}{C_{S_{cons}}} \tag{2}$$

$$P_{SA} = \frac{C_{SA}}{t} \tag{3}$$

$$S_{SA} = \frac{C_{SA}}{C_{SA} + C_{FA} + C_{AA}} \tag{4}$$

where C_j is the concentration of compound "j" in gL^{-1} (j = SA is succinic acid, S0 is substrate at time zero, S_{cons} is the consumed substrate, FA is formic acid, and AA is acetic acid) and t is time, in h.

With the aim of advancing the potential scaling-up of the succinic acid, bioproduction from brewery waste was fitted to data of fermentation with hydrolyzed used simultaneously as carbon and nitrogen sources. The aforementioned model is a simple kinetic model of the unstructured-non-segregated type, previously developed by the research group [46]. Its robustness was proven through its application in succinic acid production processes carried out under different operating conditions and variations in its culture medium composition [46]. It is made up of the equations included in Eqs. (5) to (14), with Eqs. (5) to (7) corresponding to the simplified reaction scheme, in which, for its application in this work, all the consumed sugars have been lumped in a single compound, called S, and lumping has also been applied to the two acids (formic and acetic) that are obtained as by-products, calling the compound BP. The model is made up of three reactions, whose kinetic equations are collected in expressions (8) to (10). Finally, Eqs. (11) to (14) collect the set of differential equations used in the statistical adjustment of the model to the experimental data.

Reaction network:

$$Y_{S/X}S \xrightarrow{r_1} X \tag{5}$$

$$Y_{S/P1}S \xrightarrow{r_2} SA + Y_{S/BP} \bullet BP \tag{6}$$

$$Y_{S/P2}S \xrightarrow{r_3} BP \tag{7}$$

Reaction rates

$$r_1 = \mu \bullet C_X \bullet \left(1 - \frac{C_X}{C_{Xm}}\right) \tag{8}$$

$$r_2 = k_{P1} \bullet C_S \bullet C_X \tag{9}$$

$$r_3 = k_{P2} \bullet C_S \bullet C_X \tag{10}$$

Production and consumption rates

$$R_S = \frac{dC_S}{dt} = -Y_{S/X} \bullet r_1 - Y_{S/P1} \bullet r_2 - Y_{S/P2} \bullet r_3 \tag{11}$$

$$R_{SA} = \frac{dC_P}{dt} = r_2 \tag{12}$$

$$R_X = \frac{dC_X}{dt} = r_1 \tag{13}$$

$$R_{BP} = \frac{dC_{BP}}{dt} = Y_{S/BP} \bullet r_2 + r_3 \tag{14}$$

In these equations, r_i (g L^{-1} h^{-1}) is the reaction rate of reaction "i," R_j (g L^{-1} h^{-1}) is the consumption or formation rate of compound "j," μ (h^{-1}) is the specific biomass growth rate, C_{Xm} (g L^{-1}) is the maximum biomass concentration, k_{P1} and k_{P2} (L g^{-1} h^{-1}) are the kinetic constants, and $Y_{S/X}$, $Y_{S/P1}$, $Y_{S/BP}$, and $Y_{S/P2}$ (g g^{-1}) are the macroscopic yields.

3 Results and discussion

The experimentation carried out in the present work includes several aspects: the hydrolysis of BBH and its characterization (considering different hydrolysis and/or detoxification techniques) for using it as a carbon source in the production of succinic acid with *A. succinogenes* DSM 22257; the hydrolysis of SBYH and the determination of the best concentration of this hydrolysate as a nitrogen source in the process; the study of the feasibility of using each of the beer wastes to replace the commercial carbon (xylose) and nitrogen (YE) sources separately; and, finally, the use of both wastes as carbon (BBH) and nitrogen (SBYH) sources in the bioprocess. Furthermore, with the aim of providing information for further studies of the integral production process of succinic acid from beer wastes, a kinetic model developed by the research group was applied to check its capacity to describe the process based on beer solid wastes.

3.1 Characterization of the beer bagasse hydrolysate (BBH)

In the hydrolysis of BBH, the action of α-amylase allowed the hydrolysis of the α-1,4 glycosidic bonds in amylose and amylopectin and the starch was rapidly broken down into soluble dextrins and oligosaccharides. Then it was possible to apply a cocktail of endo-β-glucanases that hydrolysed (1,3) or (1,4) linkages in β-D-glucans and xylanases that hydrolysed (1,4)-β-D-xylosidic linkages in xylans. Endoproteases hydrolysed internal peptide bonds, and glucoamylases broke dextrins down to simple sugars. Finally, thanks to a mixture of endo- and exo-glucanases, the cleavage of the cellulose polymer into smaller sugars and oligomeric polysaccharides was achieved, obtaining a hydrolysate whose composition is shown in Table 2.

Table 2 Sugar concentration of beer bagasse after hydrolysis, after removal of volatiles by aeration and after the addition of a purification step with activated carbon (AC)

Compound	Concentration (g L^{-1})
Sucrose (SAC)	0.00
Maltose (MALT)	1.57
Glucose (GLUC)	0.57
Xylose (XYL)	105
Galactose (GALACT)	11.8
Arabinose (ARAB)	0.19
Mannose (MANN)	3.87
Fructose (FRUCT)	1.56

In the detoxified and volatile-free hydrolysate, a final sugar concentration of 124.6 g L^{-1} was obtained, of which the majority was xylose. The preponderance of this pentose is in agreement with the results obtained by other authors [40]. The rest of the sugars were released in a much lower proportion; in fact, the sum of all of them barely accounts for 16% of the total sugar composition.

3.2 Determination of the dilution factor of the spent brewer's yeast hydrolysate (SBYH)

Jiang et al. [38] carried out a study to determine the most appropriate concentration of SBYH to produce succinic acid. They compared the production of succinic acid from SBYH and yeast extract in quantities such that total nitrogen concentrations coincided [38]. However, they were not able to achieve the same process performance. This is probably due to the difference in nitrogen bioavailability of YE and SBYH, the differences in the content of minerals, vitamins, and the presence of potential inhibitors in their composition. Therefore, considering the bioavailability of the nitrogen source more important than the amount of nitrogen itself, five dilutions of the aforementioned hydrolysate were performed and subsequently used in fermentation experiments, determining the amount of SBYH necessary to carry out a fermentation with the same performance as with commercial YE. Results in Table 3 show the comparison with a control experiment carried out using 10 g L^{-1} YE as a nitrogen source in terms of titer, yield, selectivity, and productivity after 24-h fermentation. As it can be seen, the results obtained for DF6 are the closest to those ones obtained when using the commercial YE.

The decrease in the dilution factor of SBYH leads to a reduction in selectivities. Despite this, the highest values of yield and productivity were not reached with the lowest dilution factor (DF 12), but with the next one that was studied

Table 3 Succinic acid concentration, yield, selectivity, and productivity after 24 h of fermentation with SBYH at different dilution factors (DF) and commercial YE as nitrogen sources

Fermentation parameters	SBYH					YE
	DF 12	DF 6	DF 4.5	DF 3	DF 2	
C_{SA} (g L^{-1})	3.45	4.41	4.17	3.98	3.69	4.39
S_{SA} (g g^{-1})	0.69	0.67	0.61	0.40	0.36	0.52
Y_{SA} (g g^{-1})	0.16	0.23	0.21	0.21	0.18	0.22
$Y_{SA/S.cons}$ (g g^{-1})	0.58	0.60	0.54	0.38	0.33	0.60
P_{SA} (24 h) (g L^{-1} h^{-1})	0.14	0.18	0.17	0.17	0.15	0.18

(DF 6). When using a DF 12, the yield of succinic acid in relation to the xylose consumed was very similar to that corresponding to the fermentation with DF 6. However, the differences between the yields of succinic acid depending on the amount of initial xylose are much greater (16% for DF 12 and 23% for DF 6), which seems to indicate an insufficient quantity of nutrients with a DF 12. The yield and productivity values with DF 6, DF 4, and DF 3 are very similar, but, as with DF 6, the amount of by-products generated is lower and also a smaller amount of SBYH is used. Therefore, it was considered that the best option was to perform fermentation with this amount of hydrolysate.

3.3 Succinic acid bioproduction with xylose or BBH as a carbon source

Keeping yeast extract as nitrogen source at a concentration of 10 g L^{-1}, two experiments were performed to study the effect of carbon source: xylose (pure reagent) and beer bagasse hydrolysate (whose main sugar is xylose, as shown in Table 2). Figure 1 shows the results obtained in batch experiments. It is observed that the growth when beer bagasse is used is somewhat faster and a higher biomass concentration is reached (Fig. 1C). It should be noted that the hydrolyzed bagasse does not only have xylose as a sugar in its composition, so in Fig. 1B, the consumption of the rest of the sugars can be observed: maltose, glucose, arabinose, mannose, and fructose are completely consumed around 17-h fermentation; however, the microorganism is not able to metabolize galactose. As for xylose consumption (Fig. 1B), the consumption of xylose as a pure reagent is somewhat faster, but it is practically exhausted around 27 h for both xylose sources (pure and BBH). Finally, the succinic acid production achieved (see Fig. 1A) is higher when BBH is used (13.3 g L^{-1}), although it is not much higher than that obtained with 20 g L^{-1} of pure xylose (10.6 g L^{-1}), as shown in Table 1. As for the two by-products obtained in the process (formic acid and acetic acid), no difference is observed between the use of pure xylose or BBH. Regarding productivity (see Table 1), when using BBH, its value suffers a slight increase compared to that obtained with pure xylose (0.41 versus 0.38 g L^{-1} h^{-1}). It is concluded that the use of BBH favors the selectivity of succinic acid production, being

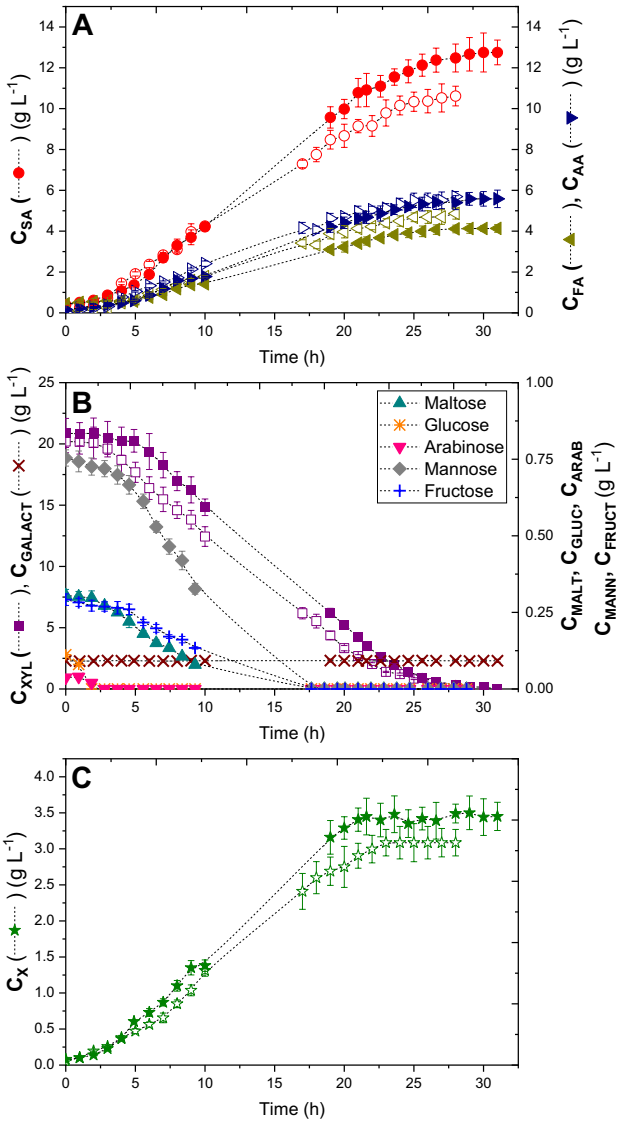

Fig. 1 Time course of **A** succinic, acetic, and formic acids, **B** sugars, and **C** biomass in runs using commercial xylose (open symbols) and BBH (close symbols) as carbon source and YE as nitrogen source

57% with this residue compared to 50% in the case of using commercial xylose.

As can be seen in Table 1, the yield of the operation with BBH (61%) not only exceeds that achieved in the

fermentation with pure xylose (53%), but also the values reached in other works in which they operated in batch using this pentose as a substrate and YE as nitrogen source, such as [47], who managed to obtain yields of 42% also using *A. succinogenes* as a biocatalyst. [18] reached lower yields (27%) but achieved one of the highest batch production rates from pure xylose, with a productivity of 0.51 g L^{-1} h^{-1}. [48] simulated the behavior of a fermentation from lignocellulosic waste carrying out a batch-type operation from a mixture of sugars representative of what could be obtained after hydrolysis of this type of biomass, obtaining yields very similar to those of this work with pure xylose (55%). In recent years, the volume of publications focused on the production of succinic acid from residues has grown rapidly, although the use of those rich in xylose is still a minority. Among the latest studies, it is worth highlighting that of [18], who managed to produce 33.7 g L^{-1} of succinic acid from olive pits reaching exactly the same performance as when they used pure xylose, or the work carried out by [33] who, despite the fact that batch operations do not usually allow high reaction rates, produced 0.79 g L^{-1} h^{-1} of succinic acid from Napier grass. With a fed-batch type operation, [49] managed to reach the same productivity value that the latest authors from grape stalks and pomace, but with a higher yield (67%). Thanks to a repeated batch operation with immobilized cells in agar, [50] were able to considerably increase the production speed (1.32 g L^{-1} h^{-1}), although with a yield that was limited to 39%.

3.4 Succinic acid bioproduction with YE or SBYH as nitrogen source

To compare the influence of YE (10 g L^{-1}) or SBYH (DF 6) on the process, two experiments were performed using pure xylose at a concentration of 20 g L^{-1} as carbon source. The results of the evolution over time of the different compounds present in the processes (xylose, biomass, and succinic, acetic and formic acids) are shown in Fig. 2. In the aforementioned figure, it can be observed that, due to the fact that the consumption of xylose when using YE is faster (Fig. 2A), the process in the experiment carried out with the aforementioned nitrogen source ends after 30 h. However, when diluted SBYH is used, the speed of the whole process (substrate consumption and acid production) slows down. It should be noted that the growth of the microorganism (Fig. 2C) is not affected by the nitrogen source used, being carried out at the same rate in the two experiments carried out.

With respect to acid production (Fig. 2B), it is noteworthy that the use of the residue as a source of nitrogen reduces the production of by-products (reaching a selectivity of 68%, which implies an increase of 36% compared to the equivalent process with YE), while, although its use slows down the rate of succinic acid production, the titer and yield obtained are higher (12.8 g L^{-1}; 0.61 g g^{-1}) than that corresponding to the use of YE (10.6 g L^{-1}; 0.53 g g^{-1}), as can be observed in Table 1. However, the productivity with SBYH (0.14 g L^{-1} h^{-1}) turns out to be half that with YE (0.38 g L^{-1} h^{-1}).

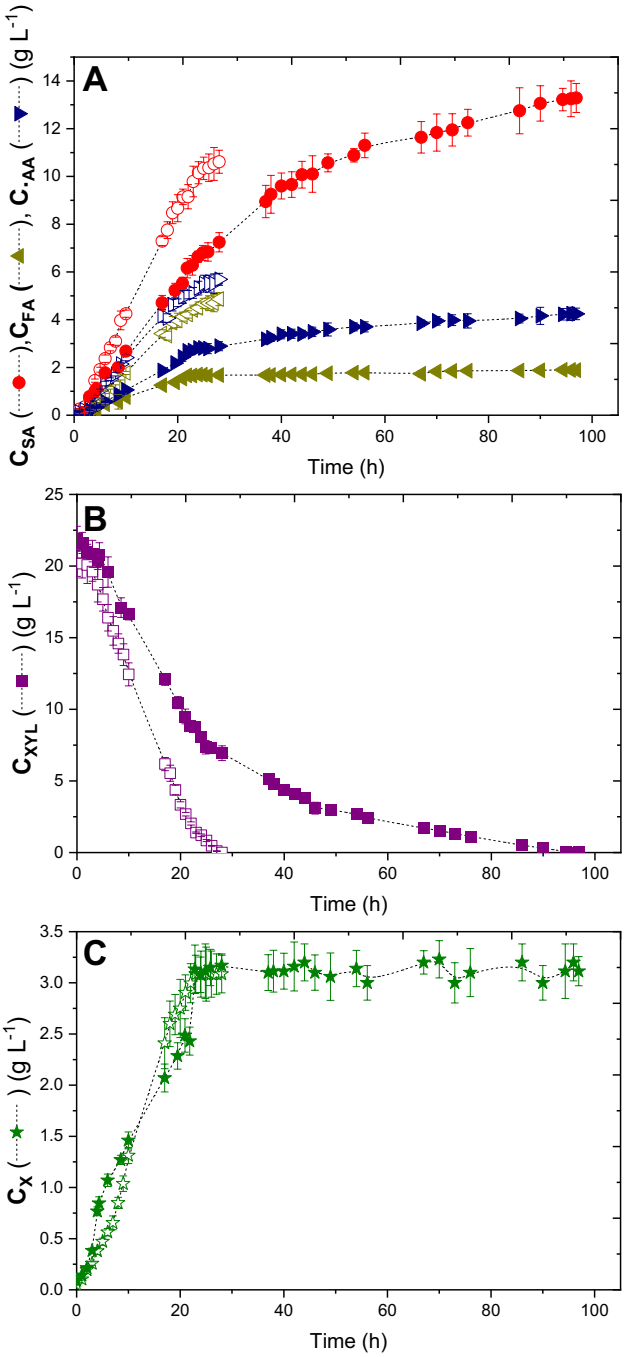

Fig. 2 Time course of **A** succinic, acetic, and formic acids, **B** xylose, and **C** biomass in runs with commercial YE (open symbols) and SBYH (closed symbols) as nitrogen source and commercial xylose as carbon source

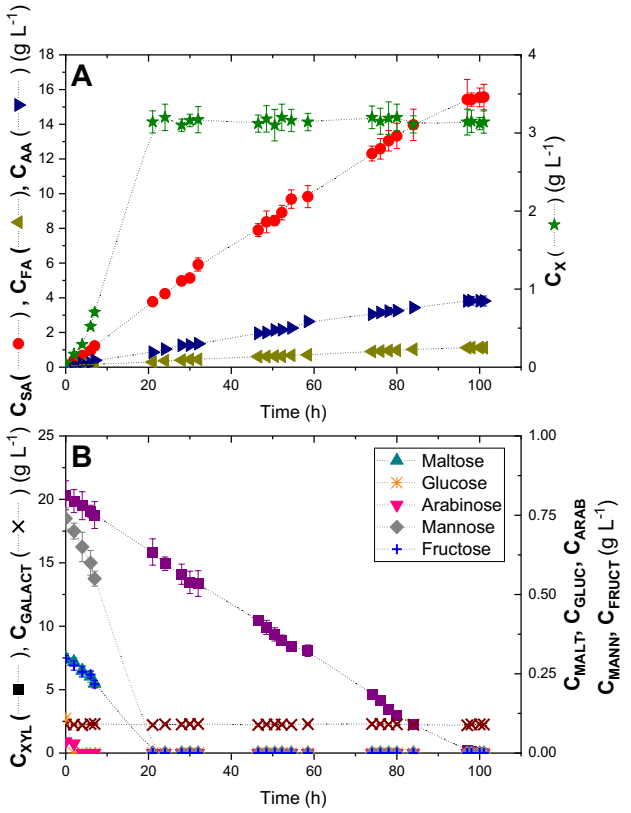

Fig. 3 Time course of succinic acid, by-products, and biomass (**A**) and sugars (**B**) when using BBH as carbon source and SBYH as nitrogen source

It can be mentioned that the replacement of the nitrogen source has hardly been studied in depth, despite being one of the greatest limitations in these types of processes due to its high costs. Table 1 shows the results achieved in two succinic acid production processes with replacement of the nitrogen source by food waste, such as the previously mentioned work by [38], who managed to produce succinic in a batch type operation from glucose and SBYH supplemented with biotin with a yield and productivity of 68 g g^{-1} and 0.63 g L^{-1} h^{-1}, respectively (it should be mentioned that in the productions in which glucose is used as a carbon source, as a rule general, higher values in these type of parameters are obtained [48, 51–56]). In the work of [31], they opted to carry out a fed-batch type operation that would allow optimizing the performance of succinic acid production using wine lees as a nitrogen source, producing 37.2 g L^{-1} of succinic acid in 47 h. In a previous study by this research group, [45] managed to produce succinic acid in the absence of a nitrogen source, using cells in a resting state, reaching a yield of 43% and reducing the by-product formation dramatically compared to the same operation carried out with cells in a growing state.

3.5 Succinic acid bioproduction with BBH and SBYH as carbon and nitrogen sources

Once the possibility of using BBH and SBYH as carbon and nitrogen sources, respectively, in the production of succinic acid was proven, an experiment was carried out using both residues as substitutes for commercial xylose and YE. Figure 3 shows the time course of the results obtained in this experiment for biomass growth, acid production (Fig. 3A), and consumption of sugars present in the BBH (Fig. 3B). It is observed that the succinic acid production using both residues is viable, obtaining a concentration of succinic acid of 15.6 g L^{-1}. This is higher than the yield obtained in the rest of the experiments carried out (see Table 1). Likewise, in the experiment carried out with BBH and YE, the productivity is reduced to approximately half of that observed when pure xylose is used; however, the yield to the target acid obtained (0.77 g g^{-1}) is the highest of all the experiments carried out, including the experiment carried out without residues (0.53 g g^{-1}), with BBH and commercial yeast extract (0.61 g g^{-1}) and with SBYH and commercial pure xylose (0.61 g g^{-1}) as shown in Table 1. It must be taken into consideration that the BBH residue presents a total concentration of sugars higher than the 20 g L^{-1} of xylose used as control. It is worth noting that the simultaneous use of the brewery residues as carbon and nitrogen source, instead of commercial xylose and YE, implies doubling the selectivity of the process, reaching a value of 76%.

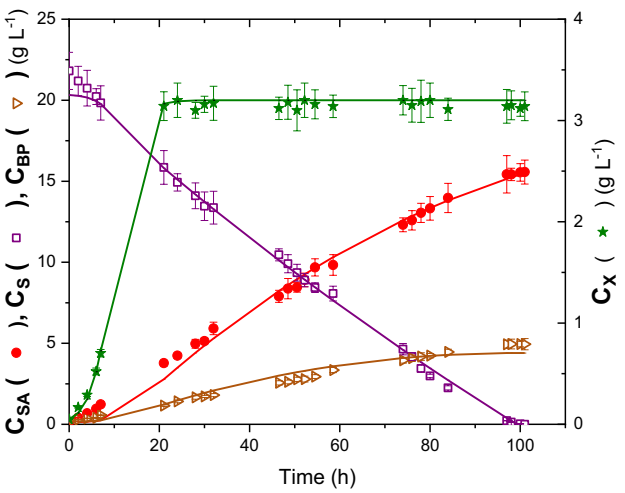

Fig. 4 Simulation of time course of succinic acid, by-products, total sugars, and biomass when using BBH as carbon source and SBYH as nitrogen source by means of a fitting of a proposed kinetic model using parameters from Table 4. Points: experimental data; lines: model predictions

Table 4 Kinetic and statistical parameter values calculated by fitting the kinetic model to experimental data of succinic acid production with BBH as carbon source and SBYH as nitrogen source

C_{Xm} (g L^{-1})	3.20	±	0.07	F_{95}	8,016
kp_1 10^{-3} (L h g^{-1})	3.06	±	0.21	**RMSE**	0.36
kp_2 10^{-2} (L h g^{-1})	8.52	±	0.07	**SSR**	6.52
μ (h^{-1})	0.41	±	0.01	**VE (%)**	99.4
$Y_{S/P1}$ (g g^{-1})	0.39	±	0.02		
$Y_{S/P2}$ (g g^{-1})	1.99	±	0.21		
$Y_{S/BP}$ (g g^{-1})	0.55	±	0.04		
$Y_{S/X}$ (g g^{-1})	0.73	±	0.03		

Considering that approximately 60% of the costs associated with the production of succinic acid are due to the purification stage, selectivity is important [57]. Therefore, the great reduction of byproducts generated during fermentation by simultaneously using SBYH and BBH would allow a great reduction in the costs associated with subsequent separation stages. Furthermore, this fermentation has generated succinic acid with a higher yield than the tests with YE and/or pure xylose. Therefore, it is determined that fermentation with *A. succinogenes* using BBH and SBYH is definitely a process that would allow economizing the production of succinic acid and, at the same time, favoring circularity and food waste valorization.

As mentioned, the experimental data were finally adjusted to a previously developed kinetic model. As has been commented in Section 2.7, the equations have been used to fit the model to the experimental data collected in Fig. 4, in which the evolution of lumped sugars is represented as points, as a single compound, succinic acid, biomass, and by-products. We can appreciate in the aforementioned figure that the fit of the model to the data is very good (represented as lines in Fig. 4). Likewise, Table 4 shows both the values of the statistical fit parameters obtained (which reinforce the goodness of fit), as well as those corresponding to the model parameters, used for the simulation collected in the form of lines in Fig. 4.

4 Conclusions

Bioproduction of succinic acid using wastes from a brewery in an integral manner, as a circular economy concept, is demonstrated. The substitution of the glucose by BBH led to higher yields and productivities. When commercial YE was replaced by SBYH, productivity decreased but both yield and selectivity. Succinic acid production from the secondary raw materials simultaneously obtained the highest yield (77%) and a productivity of 0.15 g L^{-1} h^{-1}. Finally, a simple and accurate kinetic model could be successfully applied in this last run which will facilitate the future performance of techno-economic analyses and scaling of the process.

Author contribution Itziar A. Escanciano: conceptualization, methodology, investigation, data curation, and writing—original draft. Ángeles Blanco, Victoria E. Santos, and Miguel Ladero: conceptualization, data curation, supervision, writing—review and editing, project administration, and funding acquisition.

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Declarations

Competing interests The authors declare no competing interests.

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