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Performance of several *Saccharomyces* strains for the alcoholic fermentation of sugar-sweetened high-strength wastewaters: Comparative analysis and kinetic modelling

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ABSTRACT

This work focuses on the performance of ten commercial *Saccharomyces* yeast strains in the batch alcoholic fermentation of sugars contained in selected industrial wastewaters from the soft drink industry. Fermentation has been applied successfully to treat these effluents prior to their disposal. Although many strains were investigated, similar behaviour was observed between all of the *Saccharomyces* strains tested. When media were inoculated with 2 g L^{-1} of yeast, all strains were able to completely consume the available sugars in less than 14 h. Thus, any of the strains studied in this work could be used in non-conventional wastewater treatment processes based on alcoholic fermentation. However, ethanol production varied between strains, and these differences could be significant from a production point of view. *Saccharomyces bayanus* produced the most ethanol, with a mean yield of 0.44 $g_{ethanol}$ $g_{sugarconsumed}^{-1}$ and an ethanol specific production rate of 5.96 $g_{ethanol}$ $(L\,h)^{-1}$. As the assayed soft drinks wastewaters contain about 105 g_{sugar}/L of fermentable sugars, the concentration of ethanol achieved after the fermentations process was 46.2 $g_{ethanol}$/L. A rigorous kinetic modelling methodology was used to model the *Saccharomyces bayanus* fermentation process. The kinetic model included coupled mass balances and a minimal number of parameters. A simple unstructured model based on the Andrews equation (substrate inhibition) was developed. This model satisfactorily described biomass growth, sugar consumption and bioethanol production. In addition to providing insights into the fermentative performance of potentially relevant strains, this work can facilitate the design of large-scale ethanol production processes that use wastewaters from the sugar-sweetened beverage industry as feedstock.

1. Introduction

Unlike fossil fuels, bioethanol is a renewable energy resource and does not increase atmospheric carbon dioxide levels. Ethanol is blended with gasoline to reduce greenhouse gas emissions [1]. Ethanol production technology has been a focus of scientists and industry for technological, economic and environmental reasons. Ethanol production processes encompass many different techniques and many different feedstocks, mainly sugarcane and corn [2–5]. The choice of ethanol production process depends on crop availability and geographical location.

Currently, lignocellulosic residues [6–8] represent attractive renewable sources for bioethanol production. However, the associated technology is not sufficiently developed. Moreover, the large quantity of wastewater produced by fermentation of lignocellulosic biomass poses a problem for large-scale production [7]. In this scenario, sugar-sweetened wastewaters emerge as an attractive alternative for ethanol production [9–11]. Because sugar sweetened beverages are produced in high quantities (*e.g.*, 6000 million L $year^{-1}$ in Argentina), sugar-sweetened beverage wastewater is generated in large quantities as well. The soft drink industry is the most important actor in the sugar-sweetened beverage sector, producing approximately 75% of all sugar-sweetened beverages. A portion of the beverages produced (2.5–5.0%) is discarded due to quality control practices or is returned from retail stores due to lack of gas or expired product [10]. From an economic point of view, this sugar-sweetened beverage

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wastewater is an interesting raw material for bioethanol production due to its high sugar content (60–180 g L^{-1}) and the fact that the sugars consist of sucrose and/or a mixture of glucose and fructose (provided in the form of high-fructose corn syrup). These simple fermentable sugars can be used directly in fermentations without any need for media preconditioning [9].

Because *Saccharomyces cerevisiae* var. Windsor has already been shown to produce bioethanol *via* fermentation of sugars in wastewaters from the non-alcoholic sugar-sweetened beverage industry [9–11], we focused on the performance of other commercial *Saccharomyces* yeast strains in this study. The main purpose of this work was to compare the fermentative performances of different strains and to identify the *Saccharomyces* strain with the highest ethanol yield and specific production rate. Fermentation assays were performed using ten *Saccharomyces* yeast strains, including *S. cerevisiae*, *S. bayanus* and *S. pastorianus*. A mixture of soft drinks served as the substrate. The concentrations of biomass, sugars and ethanol were monitored over time, and several parameters were calculated to compare strain performance.

A kinetic model for biomass growth, ethanol formation, and sugar consumption rates, which could be of interest for the industrial fermentation of sugar-sweetened wastewater, was also developed. Both structured and unstructured mathematical models were tested for the kinetic modelling of this fermentation process. Although structured models can explain complex microbial systems at the molecular level, relatively simpler unstructured kinetic models such as the Monod model are more widely used for practical applications [12–15]. In this work, an Andrews-based unstructured model was proposed to predictthe behaviour of *Saccharomyces bayanus*, a potentially excellent ethanol producer strain. In addition to kinetic parameters such as maximum specific growth rate, specific rate of ethanol production and specific rate of sugar consumption, the latency time for bioethanol production was also evaluated and modelled in this work.

2. Materials and methods

2.1. Strains, media and fermentations

Ten commercial *Saccharomyces* strains were used in this work (Table 1). Axenic (bacteria-free) cultures were obtained *via* streaking and stock cultures were stored at −80 °C. A mixture of soft drinks based on Argentinian market volumes (65% cola, 28% lemon-lime and 7% orange) was used in the fermentation assays. A mineral-based supplement [11] was added for to ensure successful alcoholic fermentation, consisting of: $(NH_4)_2HPO_4$ (10.6 g L^{-1}), $MgSO_4{\cdot}7H_2O$ (6.4 g L^{-1}) and $ZnSO_4{\cdot}7H_2O$ (7.5 mg L^{-1}). The initial concentration of yeast in each assay was 2.00 ± 0.10 g L^{-1}. Fermentation assays were performed according to previous works [9,11]. The pH value of the media was initially adjusted to 4.50 ± 0.10 to avoid salts precipitation in the form of struvite (magnesium ammonium phosphate).

2.2. Analytical procedures

Biomass concentrations were indirectly determined *via* turbidity measurements at 600 nm and a calibration curve as described previously [11]. Total sugar contents were determined *via* the phenol-sulfuric acid colorimetric method [16] and ethanol concentrations were monitored with a SnO_2 sensor, as described previously [9].

3. Results and discussion

3.1. Performance of commercial Saccharomyces strains

Fermentation assays were performed using a mixture of soft drinks supplemented with a mineral-based media [11] and ten commercial *Saccharomyces* strains (Table 1). While most of the strains are reported as *S. cerevisiae* (strains 7–10), the use of the *lager* denomination for some *S. cerevisiae* strains is not taxonomically adequate. In addition, robust evidence does not exist regarding the genetic background of each strain or the existence of hybridization events with the ale yeast *S. cerevisiae* (except for the GV3 strain). Hybridization is a common practice used in the alcoholic beverage industry to select the best strains to be used as inoculums. The notion of "*Saccharomyces* strains" is therefore a generic notion.

Yeast cell growth, ethanol production and fermentable sugar consumption were monitored over time in each experiment. The performance of each yeast strain was evaluated using several previously defined parameters, including latency times, specific rates and product yields [9,10].

The experimental results for each *Saccharomyces* strain are shown in Fig. 1. For comparative purposes, the performance parameters calculated for each strain are also reported in Table 2. Despite the fact that strains were from different sources (countries) and were different species, the similarity in behaviour between the *Saccharomyces* strains evaluated in this work can be clearly seen. Typical growth curves were obtained for all yeast strains. The lag time (λ) for growth was approximately 2.5 h, with slight variations between each yeast strain (Fig. 1A). Despite slight

Table 1
Commercial yeast strains used in this study.

ID (this paper)	Product name	Strain info[a]	Manufacturer
S.cer-W	Windsor	*Saccharomyces cerevisiae*. British Ale yeast, production of ale beers.	LallemandBrewing Co., Felixstowe, UK
S.cer-ER	Ethanol Red	*S.cerevisiae*. Industrialethanol industry.	Fermentis Ltd., MarcqenBaroeul, France
S.cer-04	Safale s-04	*S.cerevisiae*. English Ale yeast, production of ale beers.	
S.cer-23	Saflager s-23	*S.cerevisiae*. German lager yeast, production of beers.	
S.bay	Cider yeast	*S. bayanus*. Lager yeast. Production of ciders	Young's Group, Bradley, Bilston West Midlands, UK
S.pas	Brau-Pau	*S. pastorianus*. Lager yeast, production of pilsner style beers.	Brau-Partner, Heilbronn, Germany
GV3	GV3-Yellow Label	*S. cerevisiae (bayanus)* from the Pasteur Institute, production of stronger sparkling wines	Muntons PLC, Stowmarket, Suffolk, UK
GV5	GV5-White Label	*S. cerevisiae*. French yeast, production of white wines	
GV6	GV6-Orange Label	*S. cerevisiae*. Yeast lager strain, production of light dessert wines.	
S.cer-PG	Premium Gold	*S. cerevisiae*.English Ale yeast, production of ale beers.	

[a] Available in the datasheet provided byeach manufacturer.

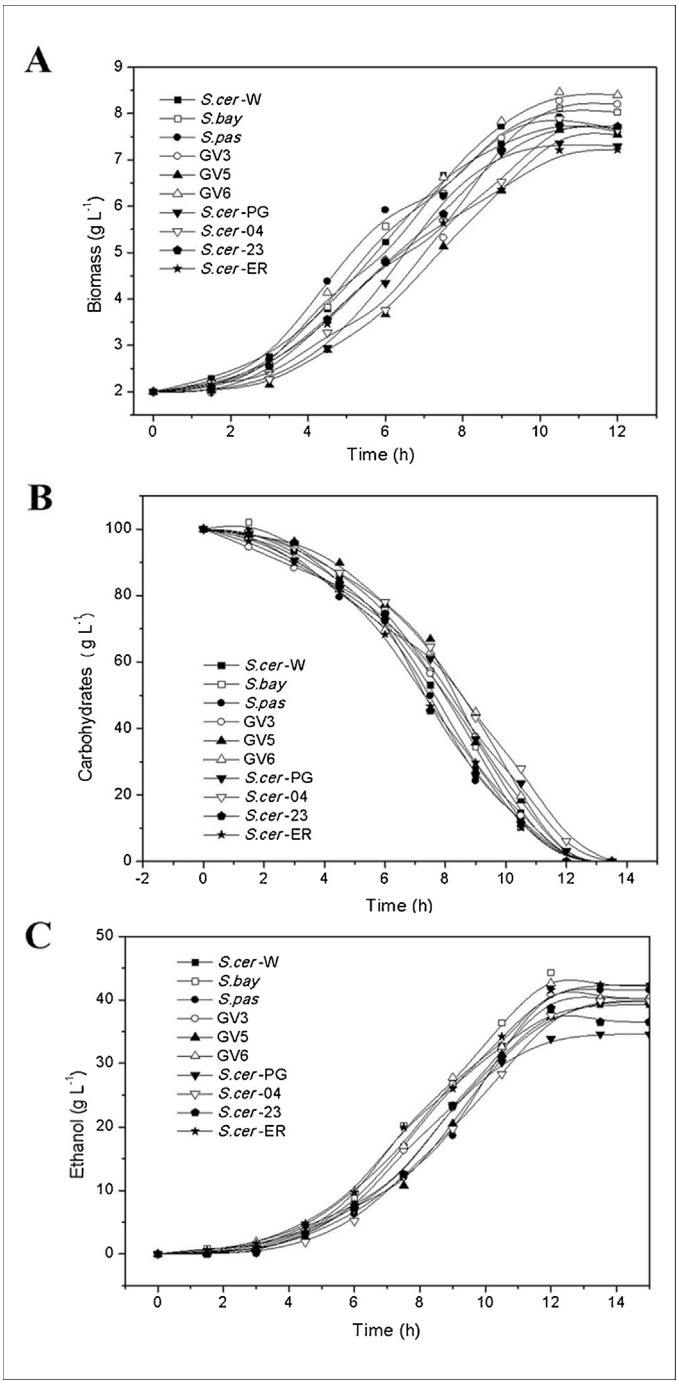

Fig. 1. Comparison of the performance of ten commercial *Saccharomyces*strains (see Table 1 for strain details).Evolutionof biomass [A], sugar [B] and ethanol [C] concentrations during batch fermentation assays performed on a mixture of soft drinks (65% cola, 28% lemon-lime and 7% orange). All media were supplemented with $(NH_4)_2HPO_4$ 10.6 g L^{-1}; $MgSO_4 \cdot 7H_2O$ 6.4 g L^{-1} and $ZnSO_4 \cdot 7H_2O$ 7.5 mg L^{-1}. The reported values are the means of three independent experiments performed with each strain.

differences in lag phase duration, the time elapsed between the beginning and end of the exponential growth phase (ϕ–λ) was nearly the same for all strains (7.6 ± 0.4 h). Due to the acclimatization of the yeast to the medium, biomass yields (Y_b) and specific growth rates (r_b) were similar for the yeast strains studied. All strains likely possess similar susceptibilities to the inhibitors (*e.g.*, sodium benzoate) present in the wastewaters of interest. The influence of these compounds on the performance of *S. cerevisiae* var. Windsor (referred to as *S.cer-W* in this paper) has been reported previously [9].

When media were inoculated with 2 g L^{-1} yeast, all strains were able to completely consume the available sugars in fewer than 14 h (Fig. 1B), with specific sugar consumption rates between 9.9 and 13.0 g_{sugar} $(Lh)^{-1}$. *S.bay* had the highest specific sugar consumption rate, while GV6 had the lowest. Interestingly, no significant differences were observed in the length of time (approximately 8.6 ± 0.8 h) between the start of sugar consumption and sugar depletion (α - ω) or in the length of time (approximately 9.7 ± 0.6 h) between the end of the lag phase and sugar depletion (α - λ). These similarities may be due to the similar latencies for the initiation of sugar consumption (ω) between thestrains.

Maximum ethanol production (e^{max}) varied between the evaluated strains: *S.bay* is at the upper end of ethanol production (44.28 $g_{ethanol}$ L^{-1}), while *S.cer*-PG is at the lower end (34.60 $g_{ethanol}$

Table 2
Comparison of yeasts performance in fermentations performed on a mixture of soft drinks[a].

Parameter[b]	Yeast strain[c]										Average (SD)
	S.cer-W	*S.cer*-ER	*S.cer*-04	*S.cer*-23	*S.bay*	*S.pas*	GV3	GV5	GV6	*S.cer*-PG	
λ (h)	2.1	2.0	2.8	2.4	2.2	1.7	2.6	3.2	2.2	2.8	2.4 (0.4)
ϕ (h)	9.3	10.1	10.9	9.8	9.5	9.5	10.5	10.8	10.1	9.8	10.0 (0.6)
r_b($g_b L^{-1} h^{-1}$)	0.81	0.65	0.73	0.77	0.85	0.79	0.79	0.75	0.82	0.76	0.80 (0.05)
b^{max} ($g_b L^{-1}$)	7.8	7.2	7.9	7.7	8.1	7.9	8.3	7.6	8.5	7.4	7.9 (0.4)
Y_b($g_b g_s^{-1}$)	0.056	0.051	0.056	0.055	0.058	0.056	0.060	0.054	0.062	0.051	0.056 (0.003)
ω (h)	3.5	3.2	3.6	3.5	3.9	3.2	3.5	4.1	3.1	3.1	3.5 (0.3)
α (h)	11.6	11.4	13.4	11.3	11.6	11.4	12.0	12.2	13.1	12.9	12.1 (0.7)
r_s($g_s L^{-1} h^{-1}$)	12.4	12.2	10.2	12.8	13.0	12.1	11.7	12.3	9.9	10.2	11.7 (1.1)
β (h)	4.5	4.0	5.1	4.9	4.4	5.2	4.7	5.0	4.1	4.8	4.7 (0.4)
ε (h)	11.5	12.0	12.8	11.9	11.8	12.6	12.1	12.4	12.2	11.3	12.1 (0.5)
r_e ($g_e L^{-1} h^{-1}$)	5.6	5.3	5.1	5.5	6.0	5.7	5.5	5. 4	5.3	5.3	5.5 (0.2)
e^{max} ($g_e L^{-1}$)	39.2	42.4	39.9	38.7	44.3	42.1	40.8	39.7	42.6	34.6	40.4 (2.6)
Y_e ($g_e g_s^{-1}$)	0.392	0.424	0.399	0.387	0.443	0.421	0.408	0.397	0.426	0.346	0.404 (0.03)

[a] Based on the marketing volumes in Argentina: 65% cola type, 28% lemon-lime and 7% orange. All media were supplemented with $(NH_4)_2HPO_4$ 10.6 g L^{-1}; $MgSO_4 \cdot 7H_2O$ 6.4 g L^{-1} and $ZnSO_4 \cdot 7H_2O$ 7.5 mg L^{-1}.

[b] λ, lag phase duration; ϕ, end of exponential growth phase; r_b, biomass (b) specific growth rate; Y_b, biomass yield; ω, latency to initiate sugar (s) consumption; α, time of complete sugar consumption; r_s, sugar consumption specific rate; β, latency to initiate ethanol (e) production; ε, completion time for ethanol production; r_e, ethanol production specific rate; e^{max}, maximal ethanol production; Y_e, ethanol yield.

[c] Please see Table 1 for strain details. The values denote the mean of triplicate independent biological experiments. Standard Deviations were intentionally excluded to simplify the reading.

L^{-1}). Despite this difference in maximum ethanol production, the time elapsed between the end of the lag phase and the end of ethanol production (ε - λ) was similar between all of the *Saccharomyces* strains investigated. It is worth noting that the length of time required for maximum ethanol production coincided with the length of time required for complete sugar depletion ($\varepsilon/\alpha = 1.0 \pm 0.1$ for all strains). The time for the end of ethanol production (ε - β) was approximately an hour longer (7.4 ± 0.4 h) than the aforementioned time for complete sugar depletion(α - ω). Interestingly, the time elapsed between the beginning of ethanol production and the beginning of the exponential growth phase (β - λ) was approximately $2.3 \pm 0.$ 5 h for all of the *Saccharomyces* strains tested. This period was an hour longer(1.1 ± 0.4 h) than the period between the initiation of sugar consumption and the initiation of the biomass growth stage (ω - λ). However, the period between the initiation of the stationary phase and the end of sugar consumption (ϕ - α) or the end of ethanol production (ϕ - ε) was negative (-2.1 ± 0.6 h and -2.1 ± 0.5 h, respectively), indicating that sugar consumption and ethanol production continued for approximately 2 h after the end of the exponential growth phase. Ethanol and biomass yields (Y_e and Y_b) were similar to values reported previously for *S. cerevisiae* var. Windsor [10,11]. However, if only "alcoholic parameters" are considered, *S.bay* clearly exhibits better performance both the Y_e and r_e for *S.bay* were approximately 10% higher than the "average" Y_e and r_e (Table 2, last column). This difference in performance is more evident in a *face-to-face* comparison between the strains.

Despite the apparent differences between "top-fermenting" (ale) and "bottom-fermenting" (lager) yeast and the differences between different *Saccharomyces* species, similar behaviour was observed for all strains. This result is consistent with the genetic background shared by commercial *Saccharomyces* strains. Yeast taxonomy supports this hypothesis: a comparison of genomes from *S. cerevisiae*, *S. eubayanus*, *S. uvarum*, *S. pastorianus* and *S. bayanus* revealed that *S. cerevisiae* (ale yeast) and *S. eubayanus* (a cold-tolerant lager yeast) are pure species, while the remaining strains (all lager yeasts) are a either double or triple hybrid strains. *S. pastorianus* is a hybrid of *S. cerevisiae* and *S. eubayanus*, while *S. bayanus* is the result of hybridization events between *S. uvarum*, *S. eubayanus* and *S. cerevisiae*. Most commercial strains from the brewery industry are hybrid strains with *S. cerevisiae* as the host [17,18].

The slight differences observed between strains could be significant depending on "the perspective of the process". Because all strains are able to completely consume the sugars present in less than 14 h, all strains are suitable from an "environmental" point of view, *i.e.*, fermentation by yeast as a wastewater treatment process. The main principle of biological treatment processes is the conversion of dissolved organic matter into easily removable compounds: gases and biomass, which separate spontaneously or *via* decantation or filtration, respectively. The proposed treatment processes mediated by yeast also produce ethanol, which can be separated by distillation. Ethanol is produced from the sugars (primarily sucrose and/or high fructose corn syrup (HFCS)) contained in sugar-sweetened beverages. All strains investigated are able to completely remove the sugars from the medium. Thus, all strains are good agents for the treatment of high-strength wastewater [10]. However, from a "productive" point of view, *S.bay* is the best strain, as it exhibits higher specific rates of sugar consumption and ethanol production as well as the highest ethanol yield of the strains evaluated in this work. In a biorefinery that uses sugar-sweetened wastewater as a raw material, *S.bay* would be a suitable platform for ethanol production. Despite all the assays were performed using a nutritional supplement deficient in vitamins (defined mineral media), the ethanol yield of 0.443 $g_{ethanol}/g_{sugar}$ is very close to the yields on a corn mash reported in the literature: 0.451 $g_{ethanol}/g_{sugar}$ for Ethanol Red strain and 0.456 $g_{ethanol}/g_{sugar}$ for Microbiogen strain [19]. Because *S.bay* could be of interest for industrial applications, we selected this strain and performed a more detailed analysis in an attempt to develop a kinetic model of the fermentation process.

3.2. Kinetic modelling and parameter determination

Kinetic modelling is an important step in fermentation process development, because models facilitate process design and control, reducing process costs and increasing product quality. The logistic equation is increasingly used to describe microbial growth in many different biological systems. This model only describes the behaviour of biomass and does not include

substrate consumption as modelled based on the Monod equation [14,15]. In addition, other terms are used to describe the sugar consumption or ethanol production. For example, a widely used model for product formation is the modified Gompertz model, which includes parameters for lag time, maximum bioethanol production rate and potential maximum product concentration [19,20]. Articles describing the behaviour of the three key variables involved in the fermentation process (biomass, substrate and product) in a coupled manner are unusual. In this work, a rigorous methodology for kinetic modelling was applied, using coupled mass balances with a minimal number of parameters. The number of parameters was minimized to avoid the "lazy" approach so well-described by John von Neumann: *"with four parameters I can fit an elephant and with five I can make him wiggle his trunk"* [21]. In other words, a model can be made to fit any data set if sufficient parameters are included. Fermentations of a mixture of soft drinks were performed in duplicate using a $2\,g\,L^{-1}$ inoculum of *S. bayanus* and a defined inorganic nutritional supplement [11].Samples were taken every 30 min to better observe the behaviour of various variables over time and to avoid failures related to the relationship between the number of experimental points and model parameters (a mistake frequently observed in other works).The methodology for kinetic modelling included the following steps:

i) Initially, a coupled mass balance for the main process variables (biomass, sugar and ethanol) was proposed and described by Eqs. (1)–(3).

$$\frac{dx}{dt} = \mu x \tag{1}$$

$$\frac{ds}{dt} = -\frac{\mu}{Y_{x/s}} x \tag{2}$$

$$\frac{de}{dt} = Y_{e/x}\mu x + \gamma x \tag{3}$$

where μ is the biomass specific growth rate and X, S and e are the concentrations of biomass, sugars and ethanol, respectively, all expressed in $g\,L^{-1}$. $Y_{x/s}$ is the biomass yield coefficient ($g_{biomass}\ g_{sugar}^{-1}$), $Y_{e/X}$ is the ethanol/biomass yield coefficient ($g_{ethanol}\ g_{biomass}^{-1}$) and γ the constant for ethanol production by maintenance ($g_{ethanol}\ g_{biomass}^{-1}\ h^{-1}$).

ii) The values of μ over time were obtained by applying Eq. (4) to biomass measurements from each experiment, and the relationship between μ and sugar content was analysed. Several models were evaluated for their ability to describe the experimental values of μ. The classical Monod Eq. (5) was not able to describe the experimental results, while the Andrews expression (6) [22] suitably represented the experimental values.

$$\mu = \frac{d\,ln(x)}{dt} \tag{4}$$

$$\mu = \mu_{max}\frac{s}{s + K_s} \tag{5}$$

$$\mu = \mu_{max}\frac{s}{s + K_s + K_i s^2} \tag{6}$$

iii) Once an expression for biomass specific growth rate was identified, biomass yield ($Y_{x/s}$) was determined by plotting $(x_{(t)} - x_{(t=0)})$ *vs.* $(s_{(t)} - s_{(t=0)})$. As shown in Fig. 2 A, a constant relationship was observed between biomass formation and substrate consumption over the course of each experiment. These results confirm the linear relationship between biomass growth and sugar consumption assumed in the model and expressed as $(Y_{x/s})$.

iv) With the value of $Y_{x/s}$ fixed, the biomass experimental data were fit to Eqs. (1), (2) and (6). Model parameters (μ_{max}, K_S and K_i) were determined by a nonlinear regression of the experimental points. The relative average quadratic deviation between experimental and predicted biomass values was chosen as the objective function to be minimized. Calculations were performed using MATLAB 7.9.0 R2009b [23].

v) Using the set of parameters obtained in step (iv), the goodness of fit of the model for sugar consumption was evaluated. To validate parameters, experimental sugar values and model-predicted values were plotted together (Fig. 3B).

vi) After identifying a suitable expression for μ and determining the parameters of the expression, ethanol yield ($Y_{x/e}$) was determined by plotting $(e_{(t)} - e_{(t=0)})$ *vs.* $(x_{(t)} - x_{(t=0)})$. As shown in Fig. 2B, a nonlinear relationship exists between ethanol and biomass, mainly at the beginnings of experiments. Because a latency time was observed for ethanol production, the addition of a term to model this delay was proposed. Latency time has been used in several models and depends on a large number of variables, with inoculum size being the most significant [24–26]. For this reason, the inclusion of a time-dependent term in the model was considered meaningless, as all subsequent expressions would depend on inoculum size. The inclusion of a time-dependent term would limit the validity of the model to the experiments and assay conditions described in this work. To account for latency in ethanol production, a term dependent only on sugar concentrations was included in the ethanol mass balance (7). Although the rate of sugar consumption depends on inoculum size, the latency time observed for ethanol production is directly related to the initial sugar content of the medium. In a previous work, latency times (in hours) of approximately 1.1 ± 0.2; 1.5 ± 0.1; 1.9 ± 0.2; 2.3 ± 0.3 and 3.0 ± 0.3 were observed in fermentations with media initially containing 60, 75, 105, 110 and $120\,g\,L^{-1}$ of sugars, respectively [10].

$$\frac{de}{dt} = Y_{e/x}\mu x e^{-(pS)^q} + \gamma x \tag{7}$$

vii) Finally, the values of μ_{max},K_S,K_i and $Y_{x/s}$ determined in steps *i* to *vi* were used to calculate the parameters $Y_{x/e}$, p. q and γ in Eq. (7). Nonlinear regression of the experimental results was performed; the relative average quadratic deviation between the experimental and predicted ethanol concentrations in the coupled balance was used as the objective function to be minimized. Calculations were performed using MATLAB 7.9.0 R2009b [23].

The experimental data (biomass, sugar and ethanol concentrations) and model-predicted values are shown in Fig. 3. The values of the corresponding kinetic parameters are reported in Table 3. The model consisting of Eqs. (1), (2), (6) and (7) successfully describes yeast biomass growth, sugar consumption and ethanol production. The values obtained for μ_{max} and $Y_{x/s}$ were consistent with those reported in the literature for *S. cerevisiae* grown on simple sugars such as glucose and fructose [26–29]. Notably, the Michaelis-Menten saturation constant (K_s) values determined in this work were higher than those reported in the literature [27,28]. Despite the availability of simple and readily fermentable sugars, soft drinks contain several compounds that can affect yeast metabolism, such as preservatives [9]. It is quite possible that the overall effect of such compounds on yeast growth is reflected in a higher K_s value compared to media free of such compounds.

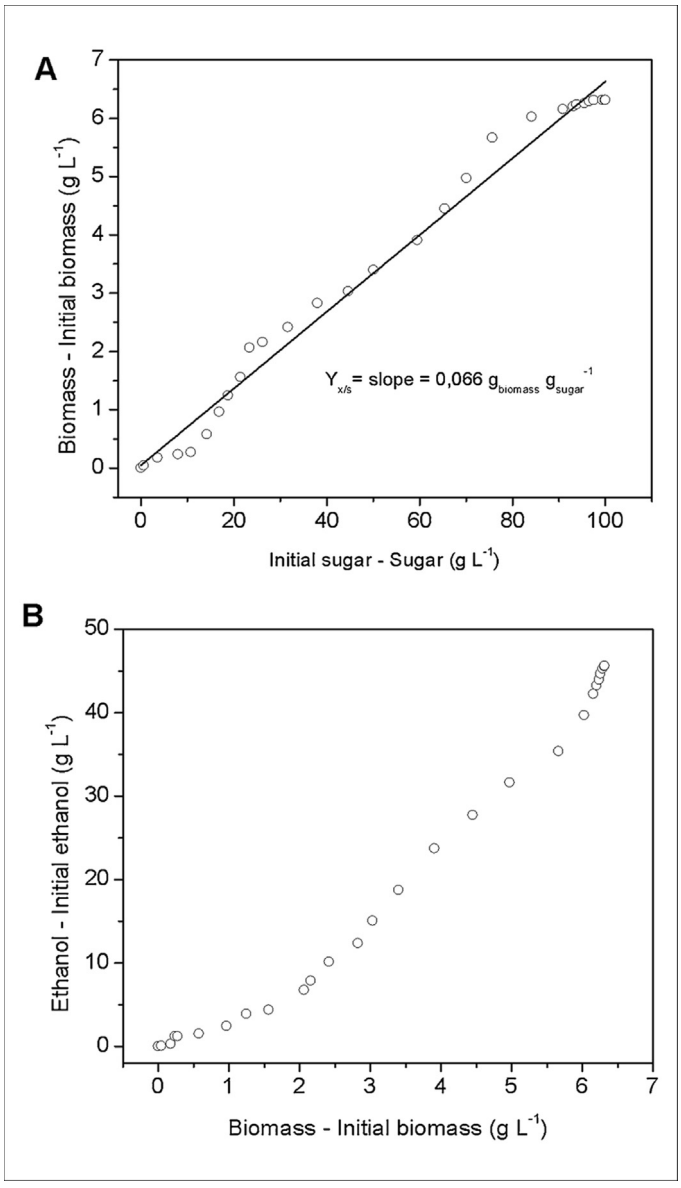

Fig. 2. Evaluation of experimental biomass ($Y_{x/s}$) and ethanol ($Y_{x/e}$) yields *via* a plot of biomass formation *versus* substrate consumption [A] and ethanol production *versus* biomass formation [B], respectively.

The value of the Andrews inhibition constant (K_i) points to significant inhibition at the beginning of the fermentation. This fact is consistent with the high osmotic pressure generated by the initial concentration of sugars in the selected wastewaters. This inhibition rapidly decreases as sugars are consumed [22,30,31]. Ethanol is a product of the primary metabolic pathway used by yeasts to obtain energy under anaerobic conditions. Despite the low value of γ, a portion of the produced ethanol was not associated with growth [32].

The methodology used for kinetic modelling and for parameter estimation allowed the experimental results to be suitably fit with a reduced set of parameters that all make sense physically. This work contributes to the kinetic modelling of fermentations performed by *Saccharomyces bayanus* or strains with the same genetic background. The foundation of this work was the development of a model to describe the behaviour of the three process variables (biomass, substrate and product) in a coupled manner. The methodology described in this work could be used to model the behaviour of other microorganisms on other industrial wastewater feedstock.

The highest final ethanol concentration obtained in the present study was 46.2 g/L. This concentration was reached using 2 g/L of *S. bayanus*. According to previous works [19,33], the specific energy consumption in ethanol distilleries can be estimated by:

$$E\,(MJ/kg_{ethanol}) = 23.025 * [\text{Ethanol Concentration}\,(\%w/v)]^{-0.8255} \quad (8)$$

Using Eq. (8), the energy needed to distil the ethanol produced in the proposed fermentation process would be around 6.7 MJ/kg, while 2.84 MJ/kg is the estimated for ethanol production from steam-pretreated corn stover using simultaneous saccharification and fermentation (SSF) at high gravity conditions [19]. This difference should not be overemphasized, since the steam consumption in distillation represents only a 10–15% of the ethanol costs from corn or lignocellulosic biomass, being the raw material about a 70% and 50% of the costs in these processes,

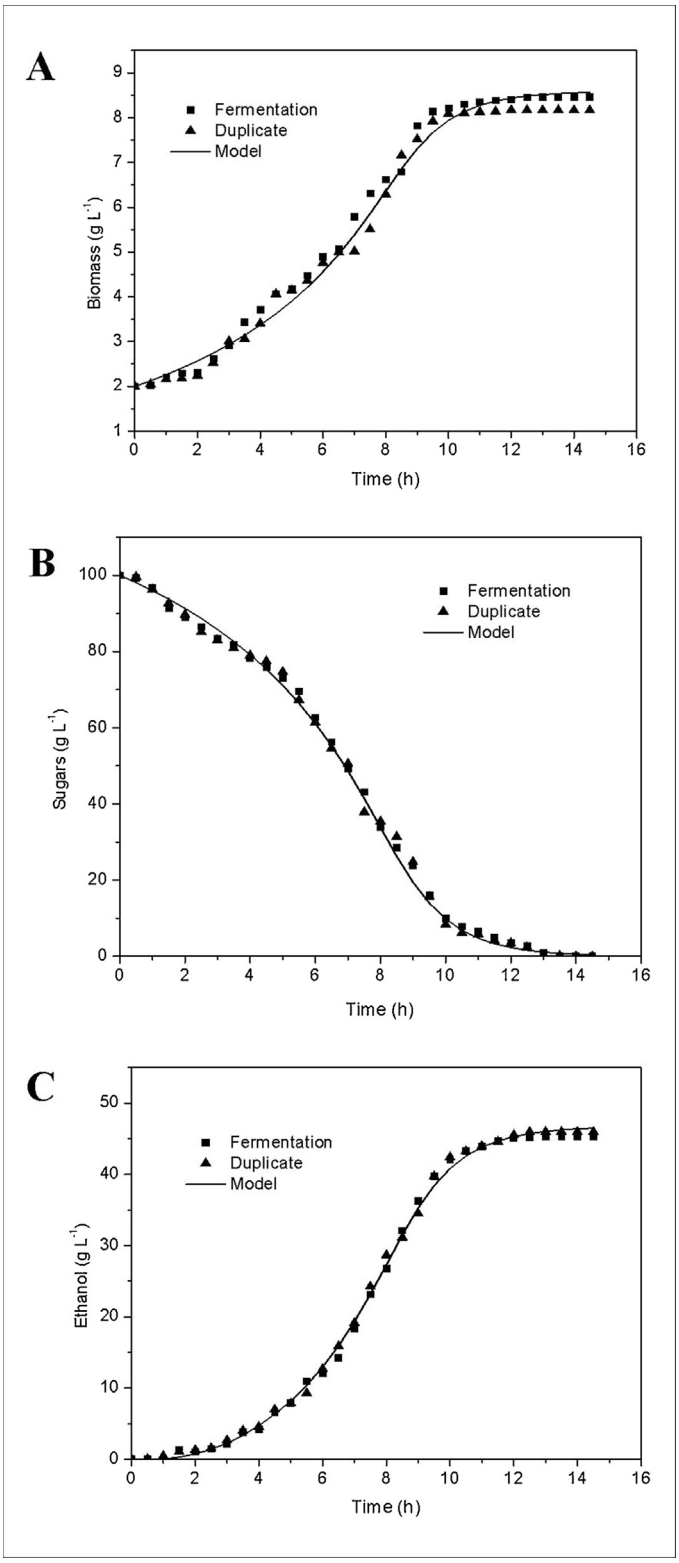

Fig. 3. Fermentative performance of *Saccharomyces bayanus* (*S.bay*). Experimental results (full dots) and model fit (solid line) calculated from Eq. (1), (2), (6) and (7) for biomass [A], sugars [B] and ethanol [C]. Fermentations were performed on a mixture of soft drinks (65% cola, 28% lemon-lime and 7% orange).

respectively [34]. In the proposed process, the raw material has no cost.

The installed capacity of each main bottler in Argentina is about 1000 million L/year, with a potential for wastewaters generation suitable to be fermented of 25–50 million L/year. Thus, the ethanol production from soft drink wastewaters in each facility could be of about 2–3 million L/year of ethanol. This figure is about one tenth of the corresponding to a single small ethanol production facility

Table 3
Parameters of the kinetic model provided by Eqs. (1), (2), (6) and (7). Fermentations were performed on a mixture of soft drinks (65%cola, 28% lemon-lime and 7% orange) using *Saccharomyces bayanus*(*S.bay*) as inoculum.

Parameters[a]	Value
$\mu_{max}(h^{-1})$	0.606
K_s (g_{sugar})	65.535
$Y_{x/s}$ ($g_{biomass}\, g_{sugar}^{-1}$)	0.066
$Y_{e/x}$($g_{ethanol}\, g_{biomass}^{-1}$)	9.227
γ ($g_{ethanol}\, g_{biomass}^{-1}\, h^{-1}$)	0.001
K_i(L g_{sugar}^{-1})	0.029
p(L g_{sugar}^{-1})	1.188e-6
q	3.038
RMS	0.607

[a] μ_{max}, maximum biomass specific growth rate; K_s, saturation constant; K_i, Andrews inhibition constant; γ, kinetic constant of ethanol production by maintenance; $Y_{x/s}$, biomass yield coefficient; $Y_{e/x}$, ethanol/biomass yield coefficient.

[34]. Nevertheless, the economic analysis of the process should not be performed as stand-alone, since it allows to remove the majority of the organic load from soft drinks wastewaters in less than 14 h when 2 g/L of *S. bayanus* is inoculated, producing major savings in the wastewaters treatment plant.

4. Conclusions

The performance of ten commercial *Saccharomyces* strains during batch alcoholic fermentation of wastewater from the soft drink industry was evaluated. Because all strains depleted the available sugars in fewer than 14 h with a 2 gL^{-1} biomass inoculum, any yeast evaluated in this work can be used to treat sugar-sweetened high-strength wastewaters. *Saccharomyces bayanus* was found to be the best ethanol producer, so this strain was selected as potential platform for the large-scale production of ethanol from sugar-sweetened beverage wastewater. A simple unstructured kinetic model was developed to describe alcoholic fermentation by *Saccharomyces bayanus*. This model included substrate inhibition as described by the Andrews model and was able to satisfactorily describe the behaviour of the three measured process variables (biomass, substrate and product) in a coupled manner. The methodology described in this work is robust and could be used to model the behaviour of other yeast strains on various wastewater feedstock by considering the particularities of each fermentative system.

Conflict of interest

The authors declare no conflict of interest and no competing financial interests.

Glossary

Ale (top fermenting) yeast typically ferments at room temperature (15–25 °C). CO_2 adherence to hydrophobic biomass surfaces cause the yeast to emerge at the top of the vessel (*e.g.*, Saccharomyces cerevisiae).

Lager (bottom fermenting) yeast ferment better at cold temperatures (mainly 10 °C).

YPD: Yeast extract, Peptone and Dextrose liquid media.

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