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FEASIBILITY OF BIOETHANOL PRODUCTION FROM CIDER WASTE

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BIOETHANOL PRODUCTION FROM CIDER WASTE

ABSTRACT

Wastewater from cider factories (losses during transfers, products discarded due to quality policies, and products returned from the market) exhibit a Chemical Oxygen Demand greater than 170,000 mg O_2/l, mainly due to the ethanol content and carbohydrates that are added to obtain the finished product. These effluents can represent up to 10% of the volume of cider produced, and they must be treated to meet environmental regulations. In this work, a process was developed, based on alcoholic fermentation of the available carbohydrates present in ciders. The impact of inhibitors at different pH, size and reuse of inoculums and different nutrient supplementation on the ethanol yield were evaluated. The use of a 0.5 g/l yeast inoculum and corn steep water as the nutrient source allowed for depletion of the sugars in less than 48 h, which increased the content of ethanol to more than 70 g/l.

Keywords: Cider waste; Bioethanol production; Preservatives; Corn steep water

INTRODUCTION

The cider production process comprises the alcoholic fermentation of apple juice, which is mediated by yeasts that naturally occur in the fruit or are incorporated as inoculums at the beginning of the process [1]. Once the fermentation is finished, the liquid is pre-conditioned and sweetened by adding sugar, usually corn syrup or sucrose, to obtain the final product, called “hard cider”. The cider market in Argentina is approximately 80 million liters per year, and there is a significant commitment from local industries to expand this market in upcoming years. Wastes and wastewater generated during the cider-making process were identified as potential sources to obtain added-value products, such as ethanol, via alcoholic fermentation mediated by yeast. These wastewaters comprise the purges from the fermentation process, cider losses during transfers, products discarded due to quality policies, and products that have returned from the market past the expiration date. These wastewaters exhibit a high

Chemical Oxygen Demand (COD), with values greater than 170,000 mg O_2/l, due to its elevated sugar and ethanol content, and usually represent approximately 10% of the volume of cider produced. Therefore, they must be treated prior to discharge into the environment. Treatment of the wastewaters is usually performed in high-rate anaerobic reactors, such as a UASB (Upflow Anaerobic Sludge Blanket) or EGSB (Expanded Granular Sludge Blanket), which require high processing times and hence, a high volume and cost. As an alternative to conventional treatment processes, these sugar-containing wastewaters can be regarded as a raw material for ethanol production via alcoholic fermentation mediated by yeast [2-5]. However, they contain products that can inhibit the fermentation process: a) the ethanol itself and b) preservatives that are added to the cider to avoid spoilage by bacteria and yeast. The Argentinian Alimentary Code (AAC) specifically includes sorbic acid or its salts and sulfur dioxide as permissible preservatives. Several reports on the effects of these preservatives on yeast performance have been previously published; however, they focus on an immediate or environmental contamination, in which the microorganism concentration is approximately $1x10^3$-$1x10^4$ CFU/ml [6-10]. The effects of these preservatives in productive cultures (10^8 CFU/ml) were not reported. In addition, cider wastes contain ethanol at a concentration of approximately 4-6% v/v, which could impact yeast performance [11-13]. Therefore, studies to minimize or eliminate these effects are necessary for successful fermentation. The feasibility of ethanol production using sugar-sweetened beverages and certain brewery wastes has been demonstrated [3, 4], but there was no sulfur dioxide or initial ethanol in these wastes. As the activity of these preservatives depends on the pH of the medium, the effect of several concentrations of potassium sorbate and sulfur dioxide in the range permissible by the AAC for ciders were assayed at the pH of the wastes (3.5) and at pH 5.0, which is close to the optimum pH for yeast growth [14]. In addition, the effects of the initial ethanol concentration were also evaluated for a wide range of concentrations at pH 3.5 and 5.0.

A previous report on the kinetics of nitrogen consumption by yeast during the cider-making process highlights that a minimal concentration of nitrogen sources is available for yeast at the end of the process [15]. Since there is no difference in the composition of the wastes under study and cider, it is expected that the wastes contain a low available nitrogen content for the yeast. Therefore, nutrient supplementation could be necessary for a successful alcoholic fermentation. Corn steep water (CSW), a co-product of the corn wet milling industry, is widely used as a nitrogen and minerals source in several cheap culture media [16-19]. This waste and a defined supplement developed by Comelli et al. [20] which contains a mix of mineral salts, were assayed as nutrient sources for the alcoholic fermentation of cider waste.

MATERIALS AND METHODS

Characterization of ciders and corn steep water

The cider wastewater does not differ in composition from the commercial cider [21]; therefore, ciders purchased in the local market were used in the assays. Ciders from several factories and the corn steep water were characterized through determination of the COD, FAN (free amino nitrogen) compounds, reducing sugars, ethanol, glycerol, magnesium, inorganic phosphorus, potassium sorbate and sulfur dioxide. The COD was measured using the standard technique [22]. The FAN compounds were measured using the EBC-ninhydrin method [23]. The reducing sugar content was measured using the Miller colorimetric method [24]. The sugar concentration was calculated indirectly using a standard curve constructed from different concentrations of D-glucose (Merck, NJ, USA). The ethanol content in the ciders, and experiments were determined by gas chromatography [25]. The glycerol was determined using an enzymatic kit (SB Lab., Santa Fe, Argentina) and the magnesium and phosphorus concentrations were determined using colorimetric kits (Wiener Lab., Rosario, Argentina), each calibrated to their respective standards. The sorbic acid content in the cider and

fermented cider was determined using capillary electrophoresis and the free SO_2 content was measured using the Ripper method, with starch as the indicator. A calibration curve was previously generated with sodium metabisulfite as the in situ SO_2 generator to obtain the desired concentration of free SO_2. The biomass concentration was determined by turbidity measurement at 600 nm using a VIS spectrophotometer (DR/2010, HACH, Loveland, USA), correlated to a dry weight calibration curve built using the standard technique with several suspensions of yeast in distilled water [22].

Alcoholic fermentations

Fermentation assays were conducted in glass flasks (500 ml or 300 ml of work volume), in batch mode under anaerobic conditions at 30±0.1°C and were performed in triplicate. Although the fermentations were initiated under microaerophilic conditions, the dissolved oxygen in the medium and the oxygen in the flask head space were negligible compared with the carbohydrate content in the media (approximately 90 g/l), ensuring complete anaerobic metabolism of the sugars. During fermentation, samples (1 ml) were taken in duplicate and immediately centrifuged at 4000 x g for 5 min. The pellet (yeast) was washed and resuspended in distilled water to the initial starting volume. The initial supernatant was transferred to a sterile 1.5-ml tube and stored at -20°C until analytical determination. The biomass, reducing sugar and ethanol were determined as previously described. Glycerol was determined at the beginning and end. From the experimental results, the ethanol and glycerol yields were calculated accordingly:

$$Y_{Et}\left[\frac{g_{Et}}{g_{Sugar}}\right] = \frac{(Final\ Ethanol - Initial\ Ethanol)\ [g/l]}{(Final\ Reducing\ Sugars - Initial\ Reducing\ Sugars)\ [g/l]} \quad (1)$$

$$Y_{Gly}\left[\frac{g_{Gly}}{g_{Sugar}}\right] = \frac{(Final\ Glycerol - Initial\ Glycerol)\ [g/l]}{(Final\ Reducing\ Sugars - Initial\ Reducing\ Sugars)\ [g/l]} \quad (2)$$

Impact of pH on the inhibitory effects of ethanol and preservatives present in cider

The effect of pH over the initial concentrations of ethanol (2, 4 and 6% v/v), sulfur dioxide (50, 100 and 200 mg/l of free SO_2) and potassium sorbate (100, 200 and 300 mg/l) on yeast performance were evaluated. The fermentations were performed using a synthetic medium composed of an aqueous solution of glucose (45 g/l) and fructose (45 g/l) at two pH values (3.5 and 5.0) and supplemented with 15 g/l of yeast extract. The yeast was inoculated at 2 g/l, and controls without inhibitors were also included. An approximate molecular SO_2 concentration was determined from the chemical equilibrium between free and molecular SO_2 as a function of the dissociation constant and the medium pH using equation (3). Using the pK_{a1} value (1.81), the molecular SO_2 concentrations for 50, 100 and 200 mg/l of free SO_2 were approximately 0.03, 0.06 and 0.12 mg/l; and 1.00, 2.00 and 4.00 mg/l for pH 5.0 and 3.5 respectively.

$$SO_{2\,(molecular)} = \frac{SO_{2\,(free)}}{1+10^{(pH-pKa)}} \qquad (3)$$

Finally, four experiments were performed to evaluate the impact of pH over the effect of other compounds present in ciders in alcoholic fermentation. A comparison using synthetic medium supplemented with inhibitors at the concentrations present in ciders and the cider waste was performed at pH values of 3.5 and 5.0. Experiments “a” and “b” contained synthetic medium that was supplemented with ethanol (40 ml/l), potassium sorbate (200 mg/l) and free SO_2 (50 mg/l) at pH 3.5 and 5.0, respectively; experiments “c” and “d” contained cider at its original pH value (3.5) and pH 5.0, respectively. All media were supplemented with yeast extract (15 g/l) and inoculated with yeast at 2 g/l.

Evaluation of the feasibility of replacing yeast extract with mineral defined salts supplement. Impact of different initial biomasses

In previous work, a supplement containing $(NH_4)_2HPO_4$, $MgSO_4$ and $ZnSO_4$ allowed for the successful fermentation of a synthetic medium with 100 g/l of simple sugars, when the initial concentrations of these salts were 10.6 g/l, 6.4 g/l and 7.5 mg/l, respectively [20]. As cider contains phosphorus and magnesium salts, a different behavior is expected. Four assays using cider at pH 5.0 were conducted to evaluate the feasibility of the use of salts to replace yeast extract. The first was a positive control, and the medium was supplemented with yeast extract at 5 g/l. In the second experiment, the medium was supplemented with mineral salts at previously reported optimal concentrations: $(NH_4)_2HPO_4$, 10.6 g/l; $MgSO_4$, 6.4 g/l; and $ZnSO_4$, 7.5 mg/l. For the third assay, the medium was supplemented with 5.0 g/l $(NH_4)_2HPO_4$, 2.5 g/l $MgSO_4$ and 5.0 mg/l $ZnSO_4$. The fourth experiment was conducted without supplementation. The media were inoculated with 0.5±0.1 g/l of yeast.

In addition, the effect of inoculum concentration on fermentation time was explored. Four experiments using yeast concentrations of 0.25, 0.50, 0.75 and 1.00 g/l were performed on cider supplemented with 5.0 g/l $(NH_4)_2HPO_4$, 2.5 g/l $MgSO_4$ and 5.0 mg/l $ZnSO_4$ at pH 5.0. To evaluate the best concentration of initial biomass, ethanol productivity, such as the quotient between ethanol production at a certain time, and the biomass present in the medium at that time were determined.

Effect of CSW as nutrient source on alcoholic fermentation of sugars present in ciders.

A widely available agroindustrial waste was also explored as a nutrient source to replace the yeast extract. Corn Steep Water (CSW) (generously provided by Glutal S.A., http://www.glutal.com.ar/), at concentrations of 1.25, 2.50, 3.75, and 5.00% v/v, was evaluated. The fermentations were performed in triplicate on cider at pH 5.0, and yeast *S. cerevisiae* var. Windsor was inoculated at 0.5±0.1 g/l. The specific consumption of sugars

was determined to select the minimal supplementation ratio that allow carrying out a successful fermentation.

Finally, an economic analysis of the supplements: yeast extract, mineral defined salts and corn steep water, was performed.

Impact of biomass reuse in alcoholic fermentation of sugars present in ciders.

Finally, the ability to reuse the biomass in alcoholic fermentation of ciders was evaluated using CSW as a supplement at 2.50%v/v. The pH of the cider was adjusted to 5.0, and yeast was inoculated at 0.5±0.1 g/l. At the end of cider fermentation, the biomass was recovered by centrifugation, and a portion was reused as the inoculum of the next fermentation to obtain an initial concentration of 0.50 g/l. This procedure was repeated up to five times.

Statistical analysis

Analysis of variance (ANOVA) and LSD multiple comparison tests were performed using the statistical software STATGRAPHICS Centurion XV.II, with the obtained data. A 95% significance level was used for ANOVA analysis.

RESULTS AND DISCUSSION

Cider and corn steep water characterization

The most relevant parameters of the four ciders from different Argentinian companies and of corn steep water (CSW) are provided in Table I of the supplementary information. The cider characterization confirms a low nitrogen content for both ammonium and FAN compounds; however, the ciders showed a similar Mg^{2+} content with the CSW, which was assayed as the supplement. This waste showed higher concentrations of organic nitrogen compounds. Their FAN content was approximately 1200 mg/L, approximately 100 times higher than the content

in cider. In addition, the CSW showed a higher content of $P\text{-}PO_4^{3-}$, which is an essential nutrient for successful alcoholic fermentation using the yeast S. cerevisiae, with respect to that observed in the cider [16, 21, 26].

Alcoholic fermentation

Impact of pH on the inhibitory effect of ethanol and preservatives present in cider

The ciders contain ethanol and preservatives, such as sulfur dioxide and potassium sorbate, which could present an inhibitory effect on alcoholic fermentation mediated by yeast [27-29]. The isolated effects of several concentrations of ethanol, sulfur dioxide and potassium sorbate at pH 3.5 and 5.0 using synthetic medium are provided as Figure I, Figure II and Figure III of the supplementary information, respectively, and the fermentation parameters are provided in Table II of the supplementary information. Although ethanol and potassium sorbate exerted an inhibitory effect at pH 3.5, higher for higher assayed concentrations, a total consumption of sugars was observed in less than 12 h. Only sulfur dioxide completely inhibited alcoholic fermentation at pH 3.5 for all concentrations tested. Conversely, at pH 5.0, the inhibition was much lower, allowing a complete consumption of sugars in less than 12 h for the three inhibitors, and all concentrations evaluated.

The impact of pH on the joint effect of ethanol and preservatives was evaluated using synthetic medium and cider. The performance of yeasts on synthetic medium supplemented with ethanol, potassium sorbate and sulfur dioxide at the concentrations present in cider and the cider are shown in Figure 1.

As expected, the yeast was not able to grow at pH 3.5 in both assayed media. Free SO_2 was added at 50 mg/l, producing a molecular SO_2 concentration of approximately 1 mg/l, which could be the principal cause of the inhibition of yeast growth [8]. On other hand, depletion of

the sugars was observed in less than 12 h in both experiments conducted at pH 5.0. The fermentation parameters are listed in Table 1. A slightly better performance was observed for the fermentation of cider than for the synthetic medium, exhibited by the average values of the fermentation parameters, but these differences were not significant at the 95% confidence level. These results could be due to the higher availability of magnesium and phosphate ions in the cider. These ions, which are present at low concentrations in the yeast extract [30], are two of the principal macronutrients required for yeast biomass growth and fermentation of sugars [26].

No significant differences were observed in the ethanol yield for the control experiments (synthetic medium without ethanol or preservatives) at pH values of 3.5 and 5.0. However, at a pH of 3.5, the fermentations carried out on cider medium and synthetic medium supplemented with ethanol and preservatives were strongly inhibited. Although there was a small initial consumption of sugars and ethanol production, the fermentation parameters were not calculated to avoid confusion since no subsequent consumption of sugars was observed during the experience, rendering the process unfeasible under these conditions. Conversely, the ethanol yields were greater than 85% for fermentations carried out at a pH of 5.0, and significant differences with the control experience at this pH were not shown. The latency time and the biomass growth rate were not significantly different between experiences and in the control (see Table 1) and were similar at values previously reported for yeast [3].

The results demonstrate that pH adjustment is the key to avoid preservative effects and confer viability for the use of sugars in cider residues as a raw material for alcoholic fermentation mediated by yeast.

Evaluation of the feasibility of replacing yeast extract with a mineral defined salts supplement. Impact of different initial biomass

Yeast extract is a widely used nutrient source in laboratory assays but is an expensive additive for industrial applications. Thus, exploring alternative and cheaper nutrient sources would reduce the cost of the process [14, 17, 21]. Therefore, several mineral and organic nutrient sources were assayed to replace yeast extract in the alcoholic fermentation of cider sugars. Initially, the effect of replacing the yeast extract with mineral salts was explored, and the results are shown in Figure 2.

In the positive control (yeast extract supplemented at 5.0 g/l) and in the experiences with both concentrations of mineral salt evaluated, sugar depletion in less than 48 h was observed. The biomass growth, sugar consumption and ethanol production as well as ethanol yield were similar for all cases, highlighting the feasibility of replacing the yeast extract with mineral salts as the nutrient source.

The lower salt concentration evaluated (5.0 g/l $(NH_4)_2HPO_4$, 2.5 g/l $MgSO_4$ and 5.0 mg/l $ZnSO_4$) was sufficient to carry out a successful fermentation of sugars in cider in less than 48 h. This concentration was lower than that previously optimized for a similar fermentation of waste containing sugars at 100 g/l [20] and could be due to the content of phosphates and magnesium present in the ciders. Thus, using this salt concentration, the effect of inoculating a different initial biomass (0.25; 0.50; 0.75 and 1.00 g/l) was evaluated. The results are shown in Figure 3.

In all the experiments, the yeast depleted the sugars and exhibited a similar ethanol yield, which did not show significant differences. The experience when biomass was added at 0.50 g/l showed a higher specific sugar consumption and ethanol productivity. Thus, a concentration of 0.50 g/l yeast (10^6 cell/ml approximately) was adopted for the next assays, a value that is in agreement with the recommendations of the principal suppliers of dried and liquid yeasts.

Effect of CSW as nutrient sources on alcoholic fermentation of sugars present in ciders.

For the mineral salts and yeast extract supplement, nitrogen is a principal nutrient, and this compound had a large impact on the alcoholic fermentation of cider sugars. The CSW have high nitrogen compound contents and are a cheap supplement source. Several ratios of CSW supplementation were evaluated: 1.25; 2.50; 3.75; and 5.00%v/v. The evolution of biomass, sugars and ethanol are shown in Figure 4.

The use of CSW as a supplement allowed for total sugar consumption in less than 48 h for all supplement ratios assayed, with ethanol yields greater than 85% of the theoretical value (see Table 2). The FAN compounds were consumed in the first 24 h in all experiments (data not shown), which is in agreement with the report by Gutiérrez et al. [31]. The results demonstrated the feasibility of the use of CSW as a supplement for the fermentation of cider sugars in replacing mineral salts and yeast extracts with similar yields. However, the sugar consumption for the ciders supplemented at 1.25% v/v finished very close to the time limit established for the fermentation (48 h). In addition, the experience supplemented with a ratio of 2.50% v/v of CSW showed higher specific sugar consumption; therefore, this relationship was used to perform an economic analysis of the different supplements assayed in this work. The Table III supplementary information shows the bulk price of each supplement or those components of the supplement (yeast extract, mineral salts and corn steep water). The average cost to supplement 1 m^3 of cider waste with corn steep water (1.65 U$D/$m^3$ of cider waste) is approximately 13 times cheaper than yeast extract (22.5 U$D/$m^3$ of cider waste) and represent less than half the cost of the mineral salt supplement (4.185 U$D/$m^3$ of cider waste). These data are in agreement with previous reports of the economic analysis of this nutrient source [32]. Thus, CSW was used to assay the biomass reuse on the alcoholic fermentation of cider waste.

Impact of biomass reuse

Biomass reuse was evaluated using CSW as a supplement at 2.50%v/v. The evolution of biomass, sugars and ethanol for the first and fifth experiments is shown in Figure 5. Slight extensions of the time needed for sugar depletion as well as a diminished biomass growth were observed as the number of fermentations increased. However, the ethanol yield was similar in the experiments, with values greater than 85% of the theoretical value (see Table 2). The inoculum used in the first fermentation was grown in YPG medium, a rich and expensive medium containing glucose, yeast extract and peptone. In this regard, the feasibility of recycling yeast five times using CSW as the sole nutrient source could reduce the process cost. This approach was previously used to reduce the overall ethanol production cost, [33] and an additional economic analysis should be performed to determine the influence of this strategy in the valorization process.

In this work, an alcoholic fermentation process was proposed as an alternative process to enhance the ethanol content of cider discards. However, the ciders contain some preservatives that are added to prevent spoilage. The pH adjustment was found to be the key factor to avoid these inhibitory effects. Insufficient attention to the control of pH is one of the most common factors of failures of fermentation by brewers, whisky and gin distillers and vintners. In agreement with the results of this work, Lin et al. [34] reported that an improper fermentation pH, although it does not interrupt the process, can greatly influence the processing time and yield. Another important factor for successful fermentation is the correct supplementation of the medium. A defined supplement, yeast extract, and corn steep water were shown to be adequate for the fermentation of the sugars present in ciders. The corn steep water was the cheaper supplement that was able to carry out successful fermentation, reducing the global cost of the proposed process.

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Fig. 1 Effects of pH on the alcoholic fermentation of simple sugars in synthetic medium supplemented with ethanol, preservatives (SO_2 and potassium sorbate), and cider waste. **A-C** represent the evolution of biomass, sugars and ethanol, respectively. Squares and circles represent the experiences on synthetic medium supplemented with 4% v/v ethanol, 50 mg/l free SO_2, and 200 mg/l potassium sorbate at pH 3.5 and 5.0, respectively; and the triangles and inverted triangles represent experiences carried out using cider waste at pH 3.5 and 5.0, respectively.

Fig. 2 Effect of replacing yeast extract with mineral salts as supplement on the alcoholic fermentation of sugars present in cider waste. **A-C** represent the evolution of biomass, sugars and ethanol, respectively, when the cider was supplemented with: 10.6 g/l $(NH_4)_2HPO_4$, 6.4 g/l $MgSO_4$ and 7.5 mg/l $ZnSO_4$ (square symbols); 5.0 g/l $(NH_4)_2HPO_4$, 2.5 g/l $MgSO_4$ and 5.0 mg/l $ZnSO_4$ (circle symbols); yeast extract at 5 g/l (triangle symbols) and non-supplemented medium (inverted triangle symbols).

Fig. 3 Impact of the inoculum on the alcoholic fermentation of sugars present in ciders. **A-C** represent the evolution of biomass, sugars and ethanol, respectively, when the initial biomass was 1.00 g/l (square symbols); 0.75 g/l (circle symbols); 0.50 g/l (triangle symbols); and 0.25 g/l (inverted triangle symbols), using mineral salts at 5.0 g/l $(NH_4)_2HPO_4$, 2.5 g/l $MgSO_4$ and 5.0 mg/l $ZnSO_4$ as the supplement.

Fig. 4 Effect of corn steep water as a nutrient source on the alcoholic fermentation of sugars present in ciders. **A-C** represent the evolution of biomass, sugars and ethanol, respectively, when the cider was inoculated at 0.50 g/l of yeast and supplemented with CSW at 1.25% v/v

(square symbols); 2.5% v/v (circle symbols); 3.75% v/v (triangle symbols) and 5% v/v (inverted triangle symbols).

Fig. 5 Feasibility of biomass reuse in the alcoholic fermentation of sugars present in cider. **A-C** represents the evolution of the biomass, sugars and ethanol, respectively for the first (square symbols) and the fifth (circle symbols) cycle of biomass reuse.

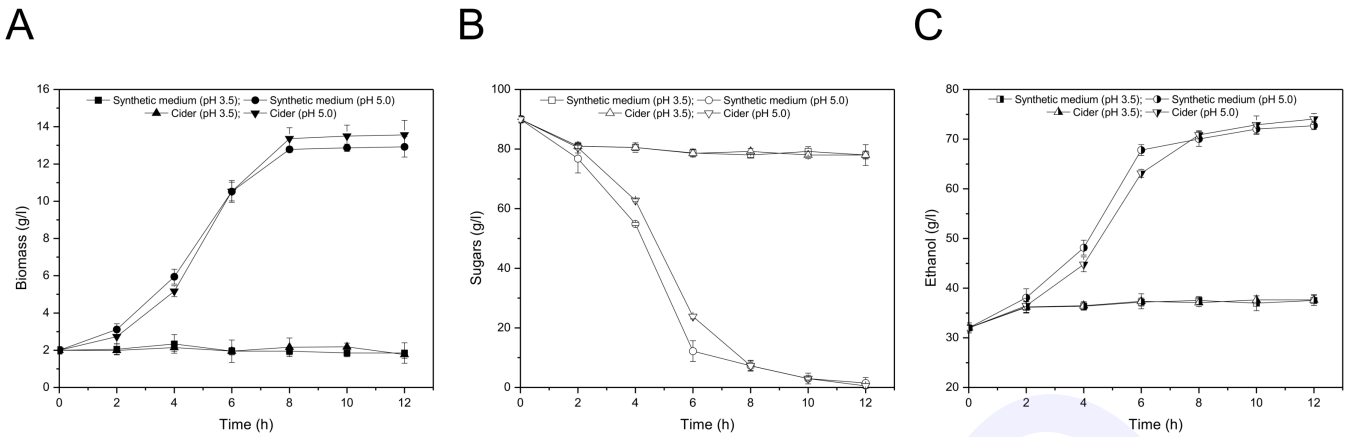
Table 1: Effects of ethanol and preservatives on fermentation parameters in experiences carried out on synthetic medium and cider waste.

Fermentation parameters	Synthetic medium (Control)	Cider	Synthetic medium with ethanol and preservatives
		pH 3.5	
λ(h)	1.30±0.40[a]	ND	ND
$r_{b,max}$ (g_b/L h)	2.13±0.16[a]	ND	ND
Y_{et} (g_{Et}/g_{Sugar})	0.46±0.02[a]	ND	ND
Y_{gly} (g_{Gly}/g_{Sugar})	0.011±0.002[b]	ND	ND
		pH 5	
λ(h)	1.26±0.30[a]	1.53±0.12[a]	1.84±0.14[a]
$r_{b,max}$ (g_b/L h)	2.29±0.27[a]	1.87±0.27[a]	1.97±0.19[a]
Y_{et} (g_{Et}/g_{Sugar})	0.48±0.01[a]	0.47±0.02[a]	0.46±0.02[a]
Y_{gly} (g_{Gly}/g_{Sugar})	0.018±0.002[a]	0.014±0.002[ab]	0.010±0.002[b]

(ND) not determined. Values followed by different letters, within the same parameter row, indicate significant differences ($P < 0.05$) according to LSD test.

Table 2: Yields of ethanol and glycerol for the fermentations of the sugars present in cider.

Parameters	Assayed conditions									
	Different initials biomass using mineral salts as supplement (g L^{-1}).				CSW assayed as supplement (%v/v).				Reuse of biomass as inoculum	
	1	0.75	0.5	0.25	1.25	2.5	3.75	5	First	Fifth
Y_{et} (g_{Et}/g_{Sugar})	0.43±0.01	0.44±0.01	0.44±0.01	0.44±0.01	0.46±0.01	0.44±0.01	0.45±0.01	0.44±0.01	0.45±0.01	0.46±0.01
Y_{gly} (g_{Gly}/g_{Sugar})	0.012±0.003	0.013±0.003	0.014±0.003	0.012±0.003	0.010±0.003	0.012±0.003	0.015±0.003	0.016±0.003	0.014±0.004	0.011±0.003



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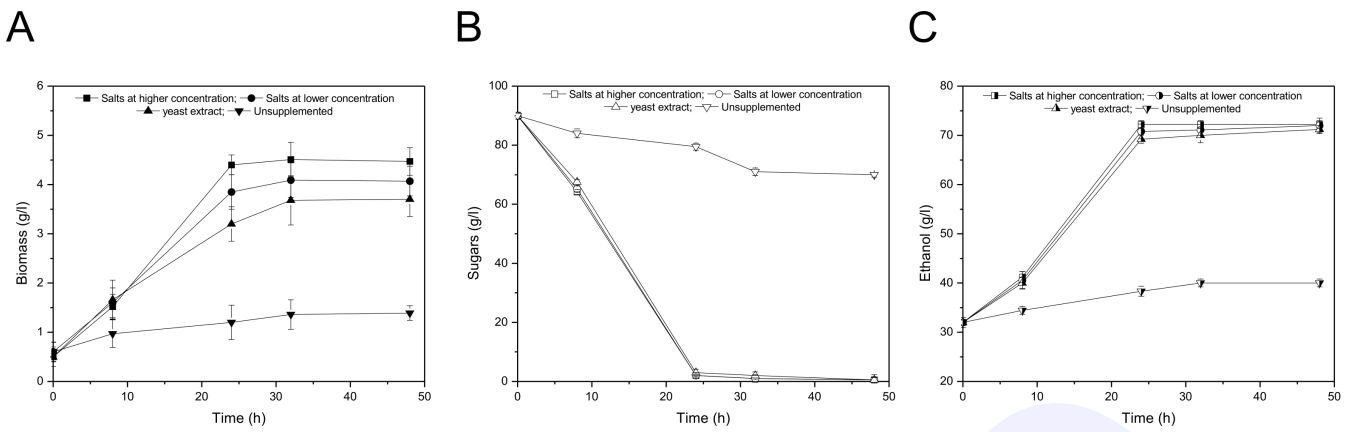

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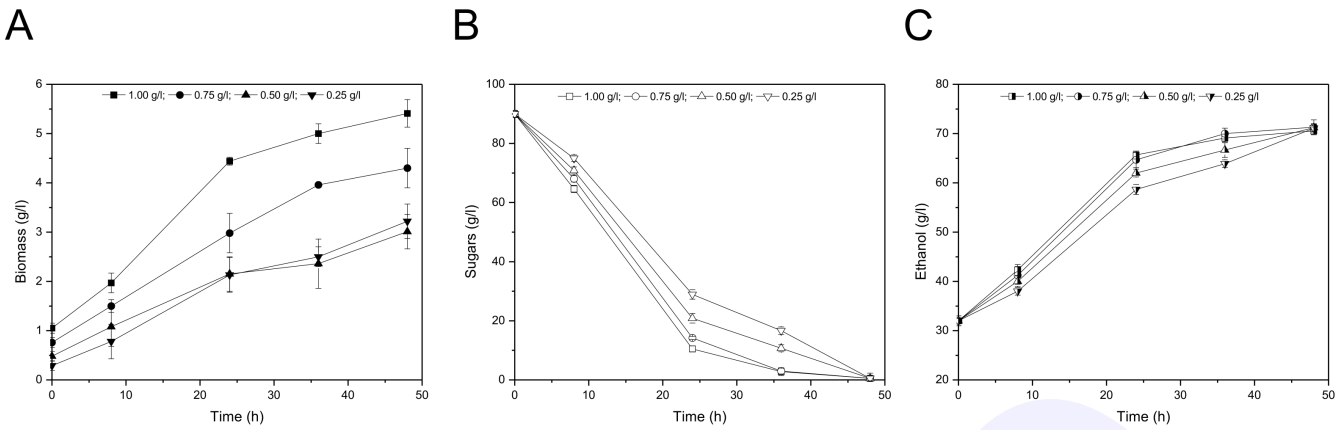

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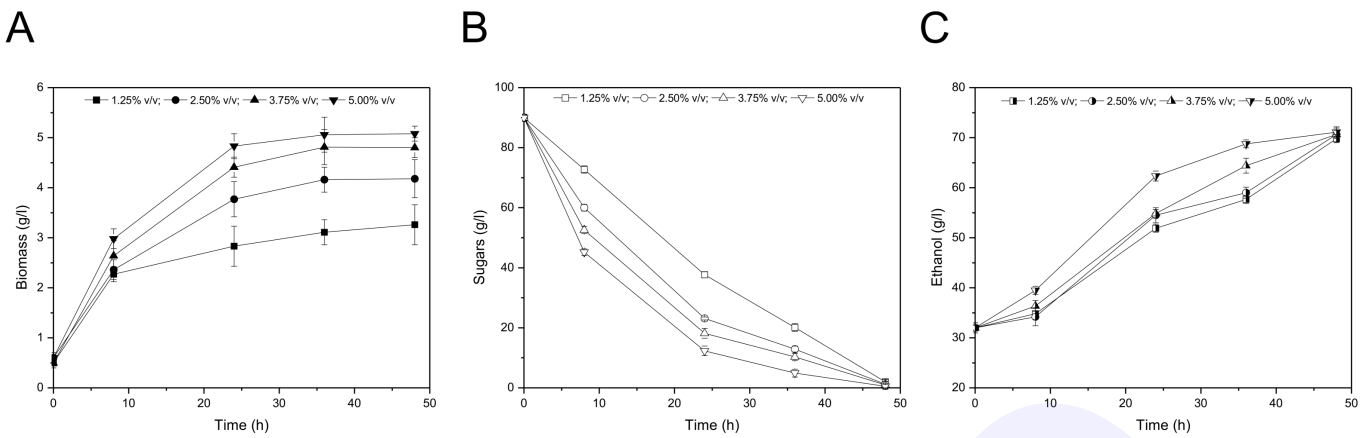

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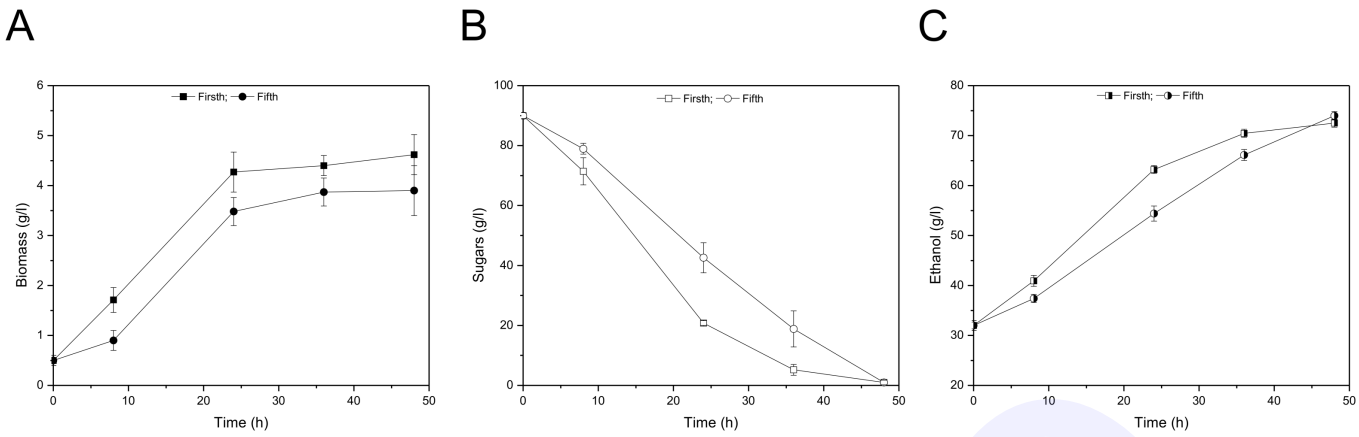

Fig. 5. Feasibility of biomass reuse in the alcoholic fermentation of sugars present in cider. A-C represents the evolution of the biomass, sugars and ethanol, respectively for the first (square symbols) and the fifth (circle symbols) cycle of biomass reuse.