Research Paper

Bioethanol production from mixed sugars by *Scheffersomyces stipitis* free and immobilized cells, and co-cultures with *Saccharomyces cerevisiae*

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Bioethanol can be produced from several biomasses including lignocellulosic materials. Besides 6-carbon sugars that represent the prevalent carbohydrates, some of these feedstocks contain significant amounts of 5-carbon sugars. One common limit of the major part of the xylose-fermenting yeasts is the diauxic shift between the uptake of glucose and xylose during the fermentation of mixed syrups. Thus, optimized fermentation strategies are required.

In this paper the ability of *Scheffersomyces stipitis* strain NRRLY-11544 to ferment mixed syrups with a total sugar concentration in the range 40–80 g/L was investigated by using mono cultures, co-cultures with *Saccharomyces cerevisiae* strain Bakers Yeast Type II and single cultures immobilized in silica-hydrogel films. The experimental design for the fermentations with immobilized cells included the process analysis in function of two parameters: the fraction of the gel in the broth and the concentration of the cells loaded in the gel. Furthermore, for each total sugars level, the fermentative course of *S. stipitis* was analyzed at several glucose-to xylose ratios.

The results indicated that the use of *S. stipitis* and *S. cerevisiae* in free co-cultures ensured faster processes than single cultures of *S. stipitis* either free or immobilized. However, the rapid production of ethanol by *S. cerevisiae* inhibited *S. stipitis* and caused a stuck of the process.

Immobilization of *S. stipitis* in silica-hydrogel increased the relative consumption rate of xylose-to-glucose by 2–6 times depending on the composition of the fermentation medium. Furthermore the films performances appeared stable over three weeks of continuous operations. However, on the whole, the final process yields obtained with the immobilized cells were not meaningfully different from that of the free cells. This was probably due to concurrent fermentations operated by the cells released in the broth. Optimization of the carrier characteristics could improve the performances of the process with immobilized cells.

Introduction

During the recent years, the production of lignocellulosic bioethanol has progressively approached the industrial development. In Italy, the most abundant residual biomass includes straws from the agricultural sector that contain significant amounts of C5 sugars, mostly xylose. One possible process scheme for converting this biomass could be the simultaneous hydrolysis of cellulose and hemicellulose in the pretreated raw material to achieve mixed sugar syrups. Diauxic lag is a major problem associated with mixed sugars utilization by the major part of pentose-fermenting yeasts,

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either wild and engineered strains [1]. In particular, the glucose present in the medium represses enzymes and transporters necessary for the xylose metabolism. To avoid this drawback the optimization of the process strategy is necessary. Remarkable breakthroughs in the pentoses fermentation were achieved through the production of engineered microorganisms [2–4]. On the other side, the investigation of wild microorganisms is still considered attractive [5,6] also because the knowledge of their response as function of diverse process conditions could help the development of future strategies for strains improvement. Among the wild type yeasts capable to ferment xylose, *Pichia stipitis*, today renamed *Scheffersomyces stipitis* [7], is considered the most interesting [8,9]. The use of this microorganism in co-cultures with other yeasts species with complementary metabolism has been considered as a way to provide the simultaneous conversion of mixed sugars syrups and increase the substrate utilization rate [10,11]. An important prerequisite of co-cultures is the absence of any inhibitory effect over each other [12,13]. Furthermore, similar culture characteristics are necessary for the maximum activity of each strain [14]. The most commonly used yeast species combination is *S. stipitis* and *Saccharomyces cerevisiae* because the most part of the tested strains are able to withstand and to ferment sugars at similar pH and temperature [10].

Another option for the fermentation of mixed syrups is the use of immobilized cells technology [15,16]. The hypothesis is that glucose is preferentially consumed by the cells on the film surface, while the cells entrapped within the carrier are forced to use the residual xylose. As a consequence, a higher consumption of xylose would be expected. The general advantages of the immobilized cells processes are: high cells load that enhances the fermentation productivity and feasibility of continuous processing without any interruption [17]. Some of the authors have also reported an effect of the entrapping matrix on improving the yeast resistance to microbial inhibitors [18]. In general, the use of an entrapping matrix might change the physiological status of the cells and consequently affect the microorganism metabolism. Ca-alginate beads are one of the most used hydrogel for the immobilization of microorganisms and enzymes [16,19]. However this carrier has shown poor resistance thus limiting its use in industrial applications. The sol–gel method for encapsulation of biomolecules has gained increasing interest in biotechnology during the past decades [20,21]. Silica-hydrogel offers some advantages including improved mechanical strength and thermal and chemical stability [22]; absence of swelling phenomena in aqueous or organic solvent that could cause leaching of entrapped biomolecules [23,24]. Furthermore, silica is not metabolized by microorganisms and is not toxic. Some of the authors have demonstrated a high activity and longevity of bacteria cells entrapped in silica [25–27]. This is due to the fact that the gel matrix protects the cells and prevents their lysis even in the absence of nutrients [28]. Finally the gel porosity facilitates nutrients permeation within the carrier and the product release into the external medium [29,30].

The aim of the present paper was to compare the fermentation of mixed syrups by *S. stipitis* by using two different process strategies single cultures, as free and immobilized cells, and co-culture with *S. cerevisiae*. In particular, silica-hydrogel was used as carrier for the yeast cells entrapping. To the best of our knowledge, the use of *S. stipitis* immobilized in silica hydrogel has never been

investigated before. Both the process strategies have been tested with synthetic media containing different concentrations of total sugars and different glucose-to-xylose ratios. The final purpose was to analyze the potentialities and limits of these approaches under comparable process conditions.

Materials and methods

Microorganisms and inoculums
S. cerevisiae Bakers Yeast Type II (Sigma Aldrich) and *S. stipitis* NRRL Y-11544, obtained from DSMZ in Germany, were used in this study.

S. cerevisiae culture was maintained at $4^\circ C$ on YPD agar plates containing (g/L): D-glucose 20, yeast extract 10, peptone 20 and agar 20. *S. stipitis* culture was maintained at $4^\circ C$ on agar plates containing (g/L): D-xylose 20, yeast extract 3, malt extract 3, peptone 5 and agar 20.

The culture liquid medium contained (g/L): D-glucose 20, yeast extract 10, peptone 20 at pH 5.5 ± 0.2 for *S. cerevisiae* and D-xylose 50, D-glucose 5, yeast extract 3, malt extract 3 and peptone 5 at pH 5.5 ± 2 for *S. stipitis*. Before inoculation, it was sterilized for 15 min at $121^\circ C$. To prepare the inoculum, 1000 mL Erlenmeyer flasks containing 200 mL of the medium were inoculated with cells from 24 hours agar plates and incubated at $26 \pm 2^\circ C$ in a rotary shaker at 150 rpm. After 48 hours, yeast cells were harvested by centrifugation at 6000 rpm for 10 min. The cells were washed twice and resuspended in sterile saline solution (0.9% NaCl). The obtained suspensions were used for the preparation of the silica-hydrogel films and the inoculum in the tests performed with free cells. To evaluate the cell mass concentration, 3 mL of the suspension was filtered on 0.45 μm filters and dried at $60^\circ C$ overnight until no weight change was observed.

Preparation of the immobilizing supports
The preparation of the immobilizing supports is based on the sol–gel technique. The first step was the precursors hydrolysis followed by jellification at neutral pH. 10 g sodium silicate ($Na_2O \cdot 2SiO_2$) was gradually dissolved in 100 g water. The initial pH was reduced until 10 by adding (H)-ion-exchange-resin (Dowex Marathon Sigma). The solution was filtered first on a 6 μm membrane to remove the resin and then on a 0.45 μm filter to reduce any microbial contamination. Finally, the pH was adjusted at 7 by adding 6N H_2SO_4. The yeast suspension was then mixed with the silicic acid solution formed at neutral pH. Jellification typically occurred in few minutes at room temperature. Following this step, the yeast cells remained entrapped in a cage tailored to their size. The yeast concentration in the experiments with immobilized cells has been estimated on the basis of the cell mass mixed with the gel before jellification and on the amount of gel loaded.

Fermentation tests
Fermentations were carried out in 250 mL Erlenmeyer flasks at $30^\circ C$, 150 rpm and the pH was adjusted at 5.5. The composition of the fermentation medium was (g/L): yeast extract 1.5; peptone 3; KH_2PO_4 2; $MgSO_4$ 0.5; $(NH_4)_2SO_4$ 1; acetic acid 3. Three concentrations of total sugars were investigated, namely 40, 60 and 80 g/L. Unless otherwise specified, the inoculum size was 6 g/L on dry weight basis. Before fermentation, the media were sterilized by membrane filtration. All the tests were carried out in duplicate and

the results were the mean of four values (two replicates of the process and two replicates of the analysis).

In the co-cultures tests with *S. stipitis* and *S. cerevisiae*, two different internal dosages of *S. stipitis*-to-*S. cerevisiae* were used (*Schef/S* = 2; *Schef/S* = 4 on the mass basis).

The experimental design to optimize the fermentation with immobilized cells included the analysis of the process yields at increasing the inoculum amount in the fermentation broth (1.1–8.3 g/L) and/or the gel-to-broth ratio. Furthermore, for each sugars level, the yeast fermentative course was analyzed at six different proportions of xylose to total sugars (0.2, 0.4, 0.5, 0.6, 0.7, 0.8).

Analysis of the fermentation broths

Sugars and fermentation metabolites were analyzed by using a HPIC DX 300 system equipped with a Nucleogel 300 column. The detector was a Shodex RI101 refractive index.

Results and discussion

Fermentation with free cells of *S. stipitis*

S. stipitis has been widely investigated for its ability to convert xylose to ethanol [31–34]. However, one drawback in the use of this yeast is the preferential consumption of glucose when both glucose and xylose are available, a phenomenon known as diauxic shift [35]. The main purpose of the present paper is to investigate some of the process strategies enabling the simultaneous consumption of glucose and xylose in mixed hydrolyzates. Two process schemes were considered: the use of co-cultures with *S. cerevisiae* strain, and the process with immobilized cells of *S. stipitis* in monoculture.

The *S. stipitis* performance was initially tested by using synthetic broths containing three different concentrations of sugars (40, 60, 80 g/L). The fermentation curves are shown in Fig. 1. The results indicate that the sugars consumption occurred sequentially and xylose uptake significantly increased only when the glucose concentration was below 2 g/L. This effect was much more evident at increasing the overall sugars concentration. The data also show that in the medium containing the lowest sugars concentration the xylose consumption was completed in 50 hours, in that at intermediate level was completed in 100 hours, while in that at the highest level stopped at 72 hours. This metabolism could be due to the glucose repressor effect on *S. stipitis* already reported by other authors and attributed to the inhibition of xylose reductase (XR) and xylitol dehydrogenase (XDH) [36–38]. Furthermore, glucose may also compete with xylose for the transporter binding sites. In particular, Kilian and van Uden [39] have found that *S. stipitis* exhibits both low-affinity and high affinity proton symport systems that operate simultaneously. The low-affinity transport system is shared between glucose and xylose for sugar transport and it is used when sugar concentrations are high.

Batch fermentation with free co-cultures of *S. stipitis and S. cerevisiae*

Co-cultures of *S. stipitis* and *S. cerevisiae* were tested in synthetic broths at three different sugars concentrations (40, 60 and 80 g/L) and two different internal dosages (*Schef/S* = 2; *Schef/S* = 4). The specific process trend (data not shown) indicated that the consumption of the sugars started simultaneously ensuring higher productivities than pure cultures. Table 1 summarizes the ethanol
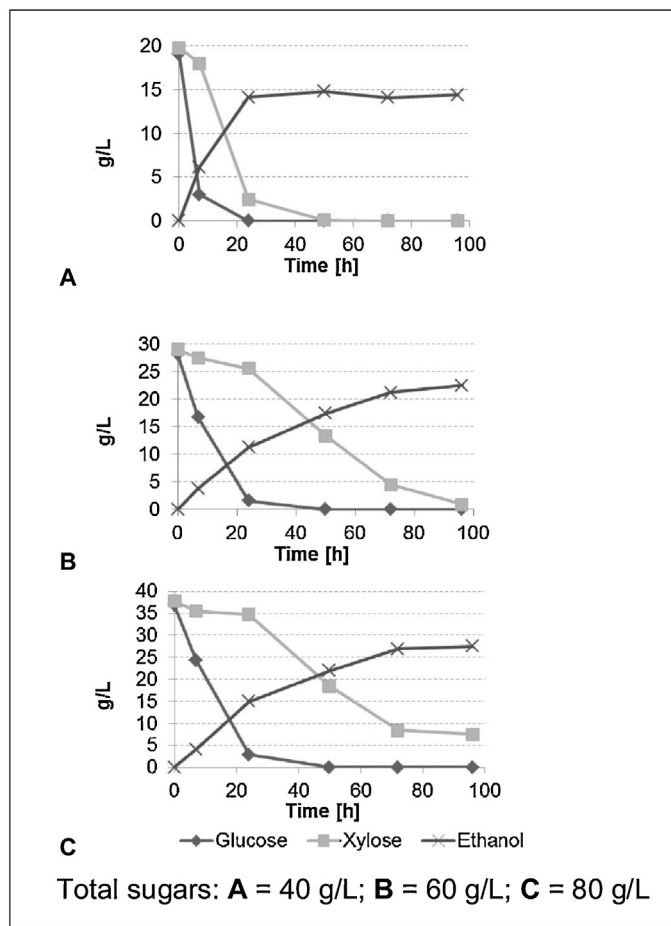

FIGURE 1

Fermentation by *S. stipitis* of synthetic syrups containing three levels of total sugars (40, 60, 80 g/L), and 50% xylose.

yields obtained at increasing the total sugars and the internal *S. stipitis*-to-*S. cerevisiae* ratio. The results indicate that the ethanol yields were not meaningfully influenced by the yeasts ratio at least in the range explored. On the other side, the ethanol yields diminished at increasing the total sugars concentration. This could be due to the osmotic stress exerted by high sugars concentration on yeasts. An indicator of this phenomenon could be the level of glycerol in the fermentation broths [40]. The data in Table 1 show an increase of the glycerol production in the broth containing 80 g/L that was probably due to the cell membrane damaging. Finally, lower productivities were achieved by increasing the concentration of the *S. stipitis* cells.

Fermentation with *S. stipitis* immobilized in silica-gel films

Immobilization of *S. stipitis* was tested to outwit the diauxic behavior. The hypothesis is that the immobilization could affect the nutrient flux through the film and favour a differentiated sugars uptake between the cells on the surface and those within the carrier. In general, immobilization of yeasts in hydrogels (mostly calcium alginate, k-carrageneen) was proved to improve both the process yields and productivities [19,41]. However, one limitation to the application of this technique at industrial scale is the scarce stability of the carrier. In fact, both cells growth and the CO_2 production through the process typically cause some damage

TABLE 1

Fermentation of mixed syrups containing 50% xylose by using co-cultures of *S. stipitis* with *S. cerevisiae*. Effect of the internal ratio *S. stipitis*-to-*S. cerevisiae*

Total sugars, g/L	Yeasts ratio *Schef/Sacch*	EtOH yield[a], $g_{EtOH}/g_{initialSugars}$	Xylose consumption rate[b], g/L/h	Productivity, g/L/h	Glycerol, g/L
40	2	0.40 ± 0.01 (1.42)	0.12 ± 0.01 (0.799)	0.48 ± 0.01	0.78 ± 0.02
40	4	0.455 ± 0.009 (1.62)	0.36 ± 0.01 (2.39)	0.38 ± 0.02	0.91 ± 0.03
60	2	0.314 ± 0.01 (1.12)	0.121 ± 0.007 (0.805)	0.72 ± 0.03	0.76 ± 0.03
60	4	0.337 ± 0.02 (1.20)	0.12 ± 0.01 (0.799)	0.43 ± 0.02	0.87 ± 0.01
80	2	0.276 ± 0.01 (0.98)	0.12 ± 0.02 (0.799)	0.60 ± 0.01	1.11 ± 0.04
80	4	0.286 ± 0.02 (1.02)	0.130 ± 0.005 (0.865)	0.57 ± 0.02	1.90 ± 0.05

[a] The values in brackets are the ethanol yields expressed as molar ratio.
[b] The values in brackets are the xylose consumption rates expressed as mmol/Lh.

to the beads. In previous works we investigated the performances of alginate gel beads [16]. In the present paper, we discuss the results obtained with silica hydrogel films.

In the fermentation with immobilized cells, the characteristics of the entrapping carrier along with the dosage of the biocatalyst play an important role as they determine the extent of the mass transfer through the film. A preliminary investigation was carried out by evaluating the effect of different cell concentrations during the film preparation and different percentages of gel loaded during the fermentation. Table 2 summarizes the process yields of two subsequent batches after 150 hours of fermentation. The data show that the process yields increased at raising the yeast concentration and/or the fraction of gel and that the film performances during the second batch were comparable to the first one. These data provided indications for the experimental set-up used during the fermentation tests at different sugars levels. In particular, considering that high gel contents could slow down the process as a consequence of the high mass transfer resistance, a compromise condition was used consisting of loading 11–12% of gel corresponding to an inoculum size of around 6 g/L. An experimental matrix including three levels of total sugars, and six different ratios of glucose-to-xylose was then tested. Figure 2 displays the detailed sugars consumption and products formation for some of the tested conditions. The overall data summarized in Table 3 indicate that, for all the three total sugars concentrations, the ethanol yield decreased at increasing the xylose fraction. On the contrary, the xylose consumption at the maximum ethanol level had different trends depending on the sugars concentration. In particular, at 40 g/L it fluctuated between 83 and 96%, at 60 g/L values of 85 and 92% were achieved only when the xylose fraction in the medium was higher than 0.5. Finally, at the highest sugars level, the xylose consumption increased at raising the xylose concentration in the medium but reached at most 63%. In all the tests the xylose consumption was almost completed in the long-run even in the medium containing the highest sugars concentration. These results are partly in constrast to the data reported by Agbogbo *et al*. [34] that observed an increase in ethanol production upon raising the xylose fraction in the broth. This difference could be due to the use of different strains and/or different conditions adopted for the inocula preparation. In this regard, in their recent investigation, Slininger *et al*. [1] presented numerous evidences on the efficient fermentation of mixed sugars by *S. stipitis* Y-7124 grown on xylose and resuspended in mixed sugar

media, by contrast to the cells grown on glucose. Still the data in Table 3 show that the xylose consumption rate increased at increasing the xylose fraction. This effect was more evident at 60 and 80 g/L. However, increased xylose consumption rates did not correspond to higher ethanol levels in the broths. At 60 g/L, xylitol concentrations in the broths rose with increasing the xylose fraction but the overall amounts were at most few g/L. These data are in agreement with those reported by Agbogbo *et al*. [34]. It must be underlined that, at prolonged process time all the xylose was consumed, but it did not correspond to ethanol or xylitol production. This result could be due to a reabsorption of ethanol in the yeast cells as reported by Skoog *et al*. [42] who found that the reassimilation of ethanol started before all the xylose was completely consumed. In the present paper, the reassimilation process seemed to start already after 7 hours. For instance, in the medium containing 80 g/L total sugars with a fraction of 80% xylose (Fig. 2Cb), the ethanol concentration expected at 7 hours on the basis of the sugars consumption would have been 6.8 g/L. On the contrary, only 1.15 g/L were found. The difference between the ethanol foreseen and that measured in the broth could give an indication of the ethanol adsorbed. In this assumption, the rate of ethanol adsorption was 0.82 g/L/h and similar values were estimated for all the investigated xylose percentages.

The analysis of the process over several weeks indicated that, with few exceptions, the process maintained similar performance

TABLE 2

Process yields during two subsequent fermentation batches of mixed syrups (60 g/L total sugars, 50% xylose) by *S. stipitis* immobilized in silica-hydrogel films. Effect of the cells concentration in the gel and of the gel-to-liquid ratio

Yeast concentration, g/L	Gel to liquid ratio, %	Ethanol yield[a], $g_{EtOH}/g_{initialSugars}$	
		I batch	II batch
1.1	5.6	0.320 ± 0.010 (1.17)	0.28 ± 0.03 (1.04)
2.2	5.6	0.35 ± 0.02 (1.25)	0.318 ± 0.017 (1.16)
2.2	11	0.330 ± 0.010 (1.20)	0.29 ± 0.02 (1.07)
3.3	17	0.329 ± 0.015 (1.18)	0.34 ± 0.03 (1.23)
4.4	11	0.36 ± 0.015 (1.29)	0.32 ± 0.014 (1.19)
6.7	17	0.34 ± 0.02 (1.26)	0.360 ± 0.010 (1.32)
8.3	10	0.38 ± 0.02 (1.37)	0.365 ± 0.014 (1.30)

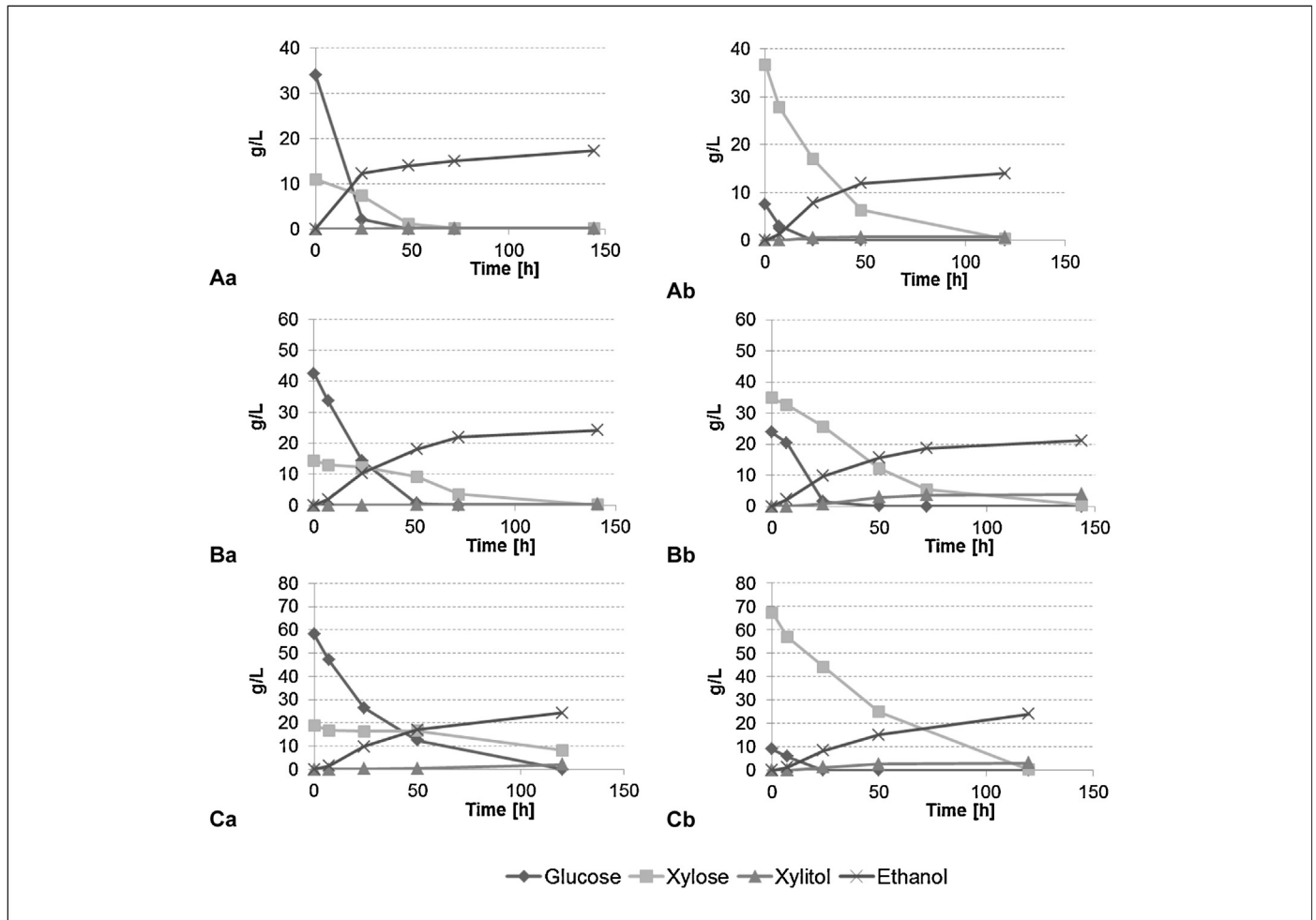[a] The values in brackets are the ethanol yields expressed as molar ratio.

FIGURE 2

Ethanol production from mixed syrups with an initial concentration of 40 (A), 60 (B), 80 (C) g/L by immobilized cells of *S. stipitis*. Effect of the xylose-to-total sugars ratio a = 20% xylose; b = 80% xylose.

TABLE 3

Description of the process results as function of the medium composition during the fermentation of mixed syrups by immobilized cells of *S. stipitis*

Xylose/total sugars	Total sugars, g/L	Ethanol yields[a], $g_{EtOH}/g_{initialSugars}$	Xylose consumption rate[b], g/L/h	Xylose consumption, %	Xylitol, g/L
0.2	40	0.38 ± 0.02 (1.430)	0.20 ± 0.02 (1.33)	90 ± 9	0.167 ± 0.018
	60	0.43 ± 0.04 (1.586)	0.101 ± 0.007 (0.67)	36 ± 2	0.33 ± 0.02
	80	0.320 ± 0.010 (1.172)	0.11 ± 0.01 (0.73)	13.5 ± 1.3	2.10 ± 0.15
0.4	40	0.35 ± 0.01 (1.278)	0.320 ± 0.010 (2.13)	94 ± 3	0.34 ± 0.03
	60	0.341 ± 0.014 (1.232)	0.331 ± 0.017 (2.20)	69 ± 4	1.00 ± 0.05
	80	0.350 ± 0.013 (1.274)	0.141 ± 0.008 (0.94)	25.2 ± 1.4	0.99 ± 0.08
0.5	40	0.339 ± 0.012 (1.204)	0.278 ± 0.017 (1.85)	96 ± 6	0.487± 0.010
	60	0.359 ± 0.014 (1.272)	0.382 ±0.016 (2.54)	91 ± 4	1.71 ± 0.09
	80	0.339 ± 0.02 (1.204)	0.350 ± 0.010 (2.33)	48.9 ± 1.4	1.74 ± 0.17
0.6	40	0.318 ± 0.011 (1.115)	0.41 ± 0.02 (2.73)	85 ± 4	0.29 ± 0.01
	60	0.360 ± 0.015 (1.258)	0.410 ± 0.017 (2.73)	85 ± 4	3.8 ± 0.4
	80	0.320 ± 0.014 (1.111)	0.56 ± 0.02 (3.73)	58 ± 2	1.86 ± 0.20
0.7	40	0.300 ± 0.012 (1.035)	0.55 ± 0.02 (3.66)	95 ± 3	0.41 ± 0.03
	60	0.326 ± 0.017 (1.120)	0.499 ± 0.016 (3.32)	90 ± 3	2.7 ± 0.2
	80	0.308 ± 0.010 (1.045)	0.67 ± 0.02 (4.46)	56.7 ± 1.7	4.5 ± 0.2
0.8	40	0.32 ± 0.02 (1.059)	0.63 ± 0.04 (4.20)	83 ± 5	0.68 ± 0.01
	60	0.335 ± 0.014 (1.120)	0.65 ± 0.03 (4.33)	92 ± 4	5.2 ± 0.3
	80	0.315 ± 0.016 (1.048)	0.85 ± 0.02 (5.66)	62.9 ± 1.5	3.0 ± 0.2

[a] The values in brackets are the ethanol yields expressed as molar ratio.
[b] The values in brackets are the xylose consumption rates expressed as mmol/Lh.

TABLE 4

Relative consumption rates of xylose-to-glucose in free and immobilized cells fermentation by *S. stipitis* (all media contained 50% xylose)

Sugars (g/L)	Xylose/glucose relative consumption rate	Process strategy
40	0.31	Immobilization
60	0.72	Immobilization
80	0.15	Immobilization
40	0.11	Free
60	0.13	Free
80	0.09	Free

FIGURE 3

Comparison of the productivity obtained with pure cultures of *S. stipitis* free and immobilized cells, and co-cultures with *S. cerevisiae* (all media contained 50% xylose).

over three weeks of continue operations. In more prolonged time, a reduction of the process yields up to 30% was observed. This could be due to some changes occurring in the immobilizing support. In some cases the films broke and some small parts dispersed in solution. These phenomena could have caused some cells leaks with the consequence of a lower cell density during the subsequent batches. Furthermore, because after each fermentation batch the films were rinsed with water before the addition of fresh broth, this could have washed away part of the surface cells or part of the films. The activity decrease in prolonged operations was more accentuated in the films used for the fermentation of concentrated syrups probably owing to the higher loss of cell viability observed during the fermentation of concentrate syrups. Table 4 lists the relative xylose-to-glucose consumption rates by *S. stipitis* in the free or immobilized cells fermentations at the time of the glucose depletion. The data indicate that the relative consumption rate was higher in the immobilized cells than in the free cells. This result confirms the validity of the fermentation with immobilized cells as mean to increase the simultaneous uptake of both sugars in mixed syrups. However, the maintenance of the carrier characteristics after repeated utilizations is a key factor in the process control. Some measurements of the cells release indicated that, already during the early 7 hours of the second fermentation batch, the cells concentration in the broths was in the range 10^7–10^8 CFU/mL independently of the specific media composition. This could be due to the fact that the cells release was mostly associated to the film thickness while the observed oscillations could be due to several factors, namely the intrinsic reproducibility of the cells distribution in the film and of the cells counting, and the progressive, even if minimal, crumbling of the films after every fermentation batch. This finding indicates that, especially in prolonged use of the immobilized cells, concurrent processes could occur and underlines the importance of further optimization of the carrier features.
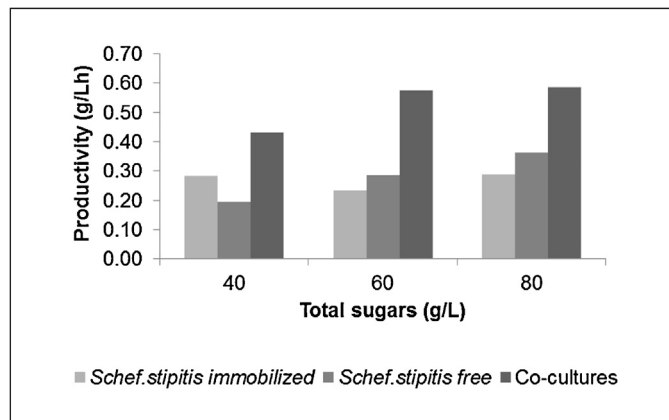
Figure 3 compares the productivities obtained by using single cultures either free or immobilized and, co-cultures of *S. stipitis* with *S. cerevisiae*. The data indicated that the use of *S. stipitis* in co-cultures ensured faster processes with higher productivity than single culture in either free or immobilized cell fermentation. However, the rapid production of ethanol by *S. cerevisiae* inhibited *S. stipitis* and the process stopped prematurely resulting in a lower consumption of xylose than mono-cultures (free or immobilized). This behavior was emphasized when the total sugars increased.

Conclusion

The use of co-cultures or single cultures immobilized in films could represent process strategies to reduce the diauxic limit of wild pentose fermenting yeasts during the fermentation of mixed syrups. In the present paper, fermentation by *S. stipitis* immobilized in silica-hydrolgel was investigated and compared with the fermentation by free cultures and co-cultures with *S. cerevisiae*. The results indicated that immobilization increased the relative consumption rate of xylose-to-glucose from 2 to 6 times depending on the fermentation medium composition. Furthermore, the films showed similar ethanol yields over three weeks of continuous operations. However, the final process yields and productivities appeared similar to the free cells fermentation. This could be due to the occurrence of multiple fermentation processes part of which were performed by the free cells released in the fermentation broths. Further optimization of the films characteristics, including the gel porosity and the carrier shape (i.e. films, beads), are still necessary to improve the process performances.

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