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ORIGINAL PAPER

Study of sugarcane pieces as yeast supports for ethanol production from sugarcane juice and molasses

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Abstract Due to the environmental concerns and the increasing price of oil, bioethanol was already produced in large amount in Brazil and China from sugarcane juice and molasses. In order to make this process competitive, we have investigated the suitability of immobilized *Saccharomyces cerevisiae* strain AS2.1190 on sugarcane pieces for production of ethanol. Electron microscopy clearly showed that cell immobilization resulted in firm adsorption of the yeast cells within subsurface cavities, capillary flow through the vessels of the vascular bundle structure, and attachment of the yeast to the surface of the sugarcane pieces. Repeated batch fermentations using sugarcane supported-biocatalyst were successfully carried out for at least ten times without any significant loss in ethanol production from sugarcane juice and molasses. The number of cells attached to the support increased during the fermentation process, and fewer yeast cells leaked into fermentation broth. Ethanol concentrations (about 89.73–77.13 g/l in average value), and ethanol productivities (about 59.53–62.79 g/l d in average value) were high and stable, and residual sugar concentrations were low in all fermentations (0.34–3.60 g/l) with conversions ranging from 97.67–99.80%, showing efficiency (90.11–94.28%) and operational stability of the biocatalyst for ethanol fermentation. The results of this study concerning the use of sugarcane as yeast supports could be promising for industrial fermentations.

Keywords *Saccharomyces cerevisiae* · Sugarcane · Ethanol fermentation · Immobilization

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Introduction

Because of environmental concerns over the use and depletion of nonrenewable fuel sources, together with the increasing price of oil and instabilities in the oil markets, there is a need to search for energy substitutes [1]. Bioethanol is now considered a profitable commodity by its increasing use as renewable energy source and car fuel [2]. Sugar cane juice and sugar cane molasses are the substrates of choice for fuel ethanol in Brazil because of its high sugar content and availability [3]. Sugarcane is also a prime economic crop in southern China. It is total planting areas were about 18 million mu in 2006 statistically. It has been already recommended as one of the best raw materials for fuel ethanol production by china government. Therefore, in order to make this process competitive, it is essential to produce ethanol at low cost and in shorten fermentation time.

One such process is yeast cell immobilization because of its technical and economical advantages compared to free cell system [4], which facilitates faster fermentation rates by providing higher cell densities per unit fermentation volume, the in situ removal of cells reduces the cost of recovery. It also helps in protecting cells from toxic effects of low pH, temperature, osmotic, inhibitors, etc. and thereby increasing ethanol yield and reducing the costs required for inoculation development [5]. Further,

the reduction of costs, in bioprocesses involving immobilized cells systems are related to aspects such as the cost of raw materials, the use of cheap, abundant and stable immobilization supports, the high cell concentrations in the bioreactors, the simplicity and low cost of the immobilization techniques, the stability of the immobilized biocatalyst in operating conditions [6]. Based on these purposes, varies immobilization supports for variety of products have been reported such as alginates [5, 7], Apple pieces [8–11], orange peel [12], gluten pellets [13] and delignified cellulosic residues [14, 15]. Generally, the liginicellulose materials were delignified prior to application for the immobilization supports. Natural cellulose materials contained a large number of hydrophilic groups in the form of positive charge, with the absorption of negative charge cells. Through the delignification treatment, the lignin was removed partially and more hydrophilic groups were exposed, which increased the absorption [16]. The delignification treatment with sodium hydroxide solution increased the possibility cells going through and so accommodated their immobilization [15]. These natural biocatalysts were considered more attractive in the ethanol production as well as in wine making and beer production than inorganic supports, such as alginates, in relation with low price of the support and its abundance in nature [11].

Sugarcane is also a natural, abundant, cheap material, and is a suitable substrate for cell growth, therefore, we decided to use sugarcane as support for yeast immobilization and then utilize it for alcohol production from sugarcane juice and molasses. The aim of this research work was to immobilize *Saccharomyces cerevisiae* on sugarcane pieces and evaluate the efficiency and suitability of immobilized *S. cerevisiae* for production of ethanol.

Methods

Materials, media and microorganism

Sugarcane was obtained from the Zhanjiang Sugarcane Research Center of Guangzhou Sugarcane Industry Research Institute. Yeast AS2.1190 was a commercial *S. cerevisiae* strain commonly used in the molasses alcohol industry of china. Cell growth was carried out at 30 °C in YPD liquid nutrient medium containing 20 g/l glucose, 10 g/l yeast extract, 20 g/l peptone. Sugarcane juice containing total sugar 176 g/l was obtained by squeezing of sugarcane. Molasses was obtained from the Guangdong Dahua Sugar Manufacturing Co., Ltd. The initial concentration was fixed at 10 °Be (about 154 g total sugar/l of molasses) by addition of water and the initial pH was 3.86. All media were autoclaved at 121 °C for 15 min.

Preparation of supports and yeast immobilization

First, sugarcane was defrosted after storage at −20 °C. Sugarcane pieces were obtained by removal of the external part of the hard skin and cutting into small pieces of 1 cm length. Delignification was performed according to Kopsahelis [14] and Bardi [15], by which the sugarcane pieces were mixed with 1% NaOH solution for 4 h to remove the lignin present inside the material. After that the delignified pieces were washed well with water, and then sterilized at 121 °C for 15 min.

Cell immobilization on sugarcane pieces was carried out by suspending about 4×10^9 yeast cells in 200 ml of YPD medium, and mixed with 60 g of sterilized delignified sugarcane pieces. The mixture was allowed for culture about 16 h. The fermented liquid was then decanted, the immobilized biocatalyst was washed twice with 200 ml of fresh YPD medium and sugarcane—supported biocatalyst was used for the following repeated batch fermentation.

Viable cell counts

The determination of viable cells/ml of fermentation broth and viable cells/g sugarcane pieces was made according to S. Plessas et al. [12]. Sugarcane pieces was done with sterilized 1/4 strength Ringers solution (Merck, Germany). At the end of each batch, 10 g of the immobilized biocatalyst were blended in a stomacher with 90 ml of sterilized Ringer's solution at 100 rpm for 30 min. Likewise, 1 ml of fermentation broth was added to 99 ml of sterilized Ringer's solution for the determination of cell leakage. The initial suspensions were submitted to serial dilutions. The cell concentration in the suspension was determined by microscope counting using a Levy hemacytometer or by spreading 100 μl of the diluted suspensions on malt agar plates. The cell viability was determined by counting the clones after 48 h at 30 °C. The calculations were expressed as colony-forming units per gram of immobilized biocatalyst or per milliliter of fermentation broth, respectively.

Anaerobic fermentation

An amount of 60 g of the sugarcane pieces-yeast biocatalyst (wet weight) and 150 ml of sugarcane juice medium was added in a 500 ml shake flask and anaerobic repeated batch fermentations were successively carried out at 30 °C by adding fresh medium at each cycle. The fermentation kinetics was monitored via the weight loss due to CO_2 release. The fermented liquid was decanted at the end of each fermentation batch, the biocatalyst was washed with 200 ml of the sugarcane juice medium and then fresh medium was added for the next fermentation batch.

Samples of the fermented liquids were collected and analyzed for the concentration ethanol and residual sugar. Fermentations of diluted molasses was carried out in the same way at 30 °C. Fermentation of sugarcane juice were also carried out using free *S. cerevisiae* AS2.1190 cells by inoculating 2×10^7/ml free cells in 150 ml sugarcane juice. Triplicate fermentations were carried out for each of batch fermentation.

Analytical methods

Initial sugar and residual sugar were determined by DNS method according to Miller G. L. [17]. The sugar concentration was calculated using standard curves and expressed as gram sugar per liter. And at the end of the fermentation, samples were withdrawn from the fermentation broth and were filtered through 0.2 μm microfilters for ethanol concentration before injection. Ethanol concentration was determined by high pressure gas chromatograph (HPGC), Nitrogen was used as carrier gas at 32 ml/min. The column temperature was 130 °C. The temperatures of the injector and FID detector were 210 and 220 °C, respectively. Butanol-1 was used as internal standard at a concentration of 0.5% (v/v). 2 ml samples were injected into the column and the concentration of ethanol was determined using standard curves. Ethanol concentration was expressed as g/l. Ethanol productivity was calculated as the grams of ethanol per liter liquid volume produced per day.

Scanning electron microscopy (SEM)

Scanning electron microscopy was performed to observe the support structure and to follow the immobilization process. Pieces of the immobilized biocatalysts were washed twice with distilled water, then the supports were cut into a 0.5 cm side cubes and fixed in 0.1 M sodium cacodylate buffer which contains 2.5% glutaraldehyde at 4 °C, for a minimum of 4 h. After decanting the fixative, the material was dehydrate following a standard serial alcohol dehydration, critical point dried in a high vacuum chamber and mounted onto aluminum stubs. The samples were coated with gold in a Balzers SCD 004 Sputter Coater for 3 min, then observed and photographed in a HITACHI S-3000 N scanning electron microscope (Japan).

Formula

Fermentation efficiency and yield were calculated using the following equations.

$$\text{Efficiency (\%)} = \frac{\text{ethanol equivalent (g)}}{\text{sugar utilized (g)}} \times 100$$

$$\text{Yield (\%)} = \frac{\text{ethanol equivalent (g)}}{\text{initial sugar (g)}} \times 100$$

The alcohol productivity and sugar conversion were calculated using the following equations.

$$\text{Conversion (\%)} = \frac{\text{initial sugar (g)} - \text{residual sugar (g)}}{\text{initial sugar (g)}} \times 100$$

$$\text{Productivity } (\text{g L}^{-1}\,\text{d}^{-1}) = \frac{\text{ethanol formed } (\text{g h}^{-1}) \times 24 \text{ (h)}}{\text{reactor valid volume (L)}}$$

Results and discussion

Immobilization of yeast cells

Micrographs of sugarcane stalks prior to yeast immobilization exhibited the external surface, comprised of parenchyma cells and the opening of vessels (Fig. 1). The vessels are composed of the vascular bundle, anular, spiral vessel and sieve tubes, etc., were apparent throughout the entire stalk. It is necessary to treat the material before immobilization by freezing–thawing process in which the cell structures were destroyed. (Fig. 2). Generally, sugarcane for immobilization supports was storage at −20 °C. While defrosting, an intercellular cavity emerged in sugarcane tissues resulting from cell breakdown, tissue

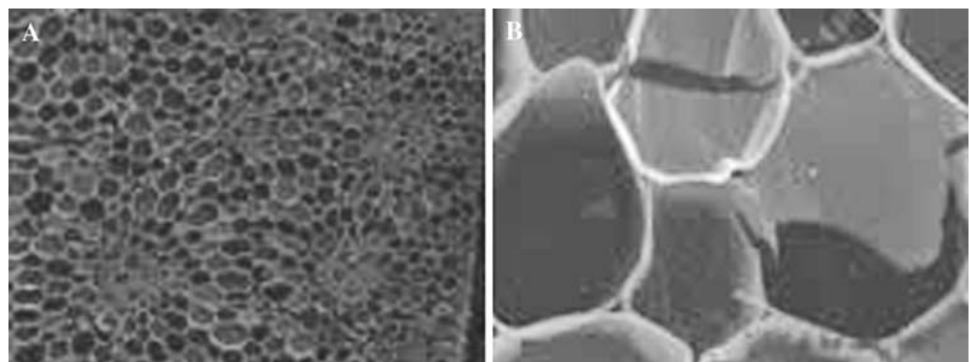

Fig. 1 Scanning electron micrographs of the sugarcane. **a** Cross-sectional view of the sugarcane before cell immobilization. **b** The structure of parenchyma cells of sugarcane after freezing–thawing treatment

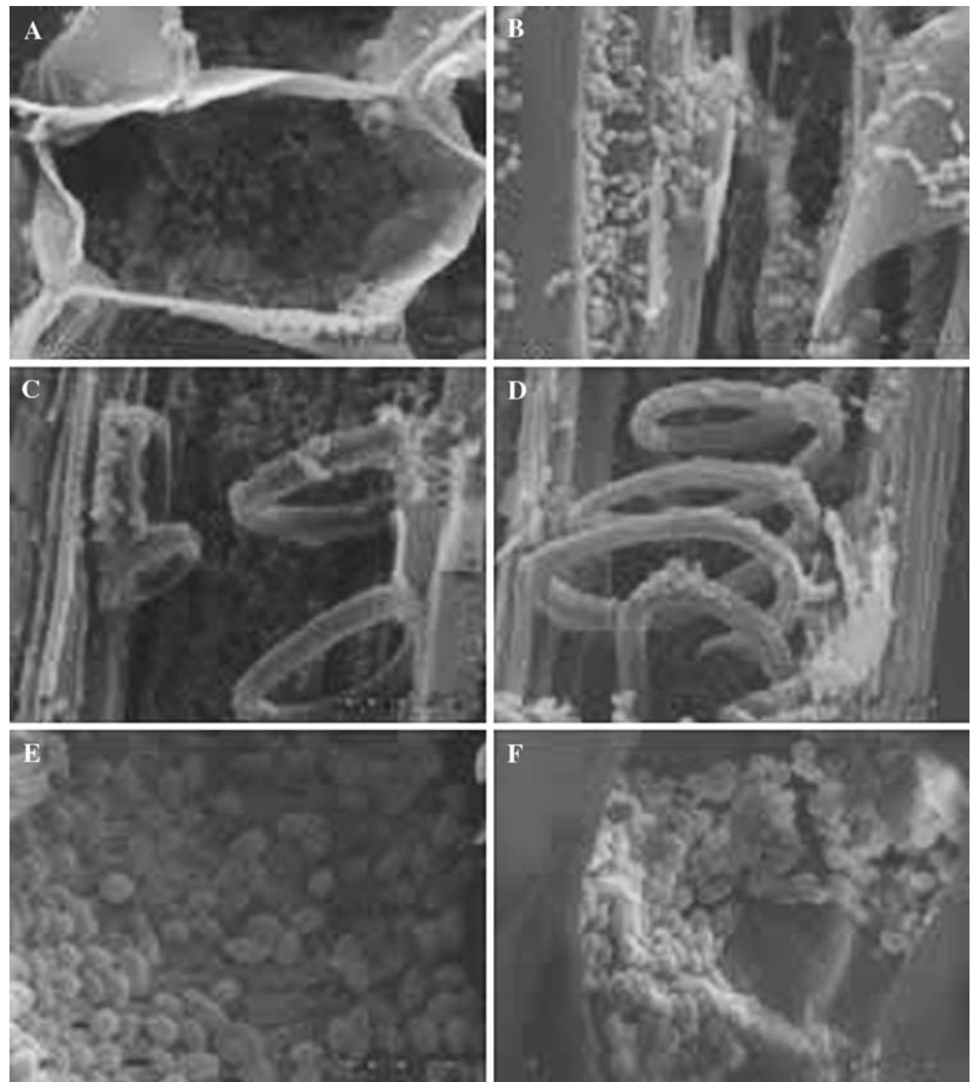

Fig. 2 Scanning electron micrographs of the middle part of the support after yeast immobilization. Cross-sectional view showing adsorbed yeast cells in parenchyma cells (**a**). Longitudinal view showing adsorbed yeast cells into the vessels of the vascular bundle (**b**), anular (**c**), spiral vessel (**d**), aieve tubes (**e**), parenchyma cells (**f**)

splitting, or organized formation. So a large lacuna occupies the center of sugarcane stalk. The pore diameter of the substrate is one more times the major dimension of the cell and have the capacity of accumulating the cells. During the incubation and fermentation, yeast cells can immigrate to the interior of the sugarcane where the cells increased the number by reproduction.

Yeast immobilization was shown by the Scanning electron micrographs. It was observed that high populations of yeast cells homogenously adhered to the surface of sugarcane pieces (Fig. 3). In order to verify that cell immobilization occurred within the whole support and not only on the surfaces exposed to the fermentation media, a sample was observed by a cross-section and by a longitudinal view of its middle part (Fig. 2). Both the cross-sectional area at the vascular bundle structure and the longitudinal area over the surface showed a high population of adsorbed cells. Also our results are in agreement with others in regard that the application of low continuous stirring speeds during the immobilization could lead to better immobilization performance [18].

Immobilization techniques used in general can be grouped into four categories according to Tanaka and Kawamoto [19]. The first one includes those methods which involve the binding of the biocatalyst to a water-insoluble support, by using ionic or covalent chemical links, bio-specific coupling, or junctions due to adsorption phenomena. Natural polymers such as polysaccharides (cellulose, dextran, agarose derivatives), proteins (gelatine, albumin), synthetic polymers (polystyrene derivatives, polyurethane) and inorganic material (sand, clay, ceramics, magnetite) are commonly used for this purpose. A second category includes those methods using multifunctional compounds as glutaraldehyde, toluene or hexamethylene diisocyanate, to form Schiff's bases with functional groups in biocatalysts, thus producing water-insoluble networks. The third category is constituted by those methods involving the trapping of the biocatalyst into a network formed by

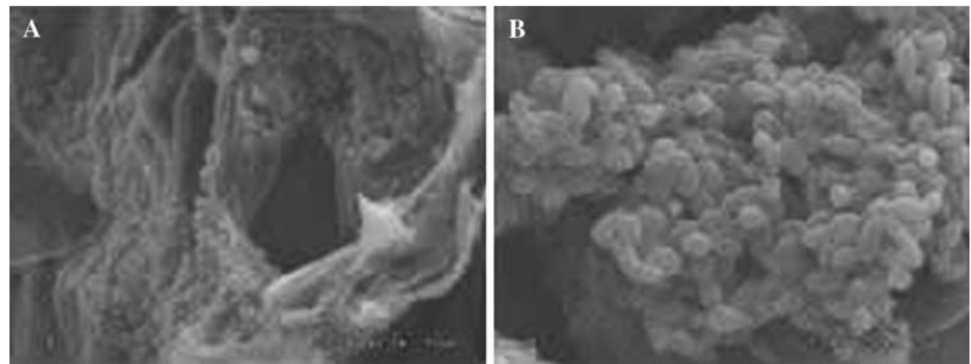

Fig. 3 Scanning electron micrographs of the support's surface during fermentation. **a** The support's surface after immobilization process. **b** Support surface view after ten batch fermentations

one or several polymers (polyacrylamide, alginate, carrageinate, or synthetic resins), or those involving the embedding in membranes, encapsulating them inside microcapsules composed of synthetic polymers. A combination of the three former methods constitutes the last category.

As we known, the adhesion of hydrophilic cells such as *S. cerevisiae* is essentially dependent upon electrostatic interactions between the support and the normally negatively charged cell surface. Based on these immobilization techniques, the sugarcane pieces is believed to immobilize yeast as a result of natural entrapment into the porous structure of sugarcane materials and due to physical adsorption by electrostatic forces between the cell membrane and the carrier. Also, these observations indicate that cell retention is due to the action of capillary forces during the process of immobilization, which pull the cells to approach and keep close contact with the surface and through the channels where they can be entrapped or attached, and multiply. The flow of cells over a porous support causes a pressure differential within the vessels causing the cells to be taken into the vessels. High populations of immobilized yeast cells on the external surface of the support were observed after ten batches of fermentation. This can be explained by the greater nutrient availability (sugars) near the surface of the support. In addition, the greater nutrient availability (sugars) in the cavities of parenchyma cells is also an attraction to yeast and enable yeasts tend to migrate into the inner of parenchyma cells and budding in immobilization process.

Repeated fermentation batch

Yeast cell immobilization on the sugarcane and suitability of the immobilized biocatalysts for alcoholic fermentation was confirmed by satisfactory operational stability during repeated batch fermentations of sugarcane juice.

The variation of viable cell was measured by the number of colony-forming units (cfu) in the fermentation broth (cell leakage) and on the biocatalyst (immobilized cells) during the repeated batch fermentation of sugarcane juice and molasses. According to Fig. 4, enumeration of immobilized viable cells on sugarcane immediately after immobilization indicated that the microbial populations were 10^{10} cells/g of biocatalysts. At the end of the third fermentation batch using sugarcane juice, the immobilized cells increased to 10^{11} cells/g of biocatalyst. It was observed that the number of cells attached to the support increased during the period, while fewer and fewer yeast cells leaked into fermentation broth. This result was also achieved by the scanning electron micrographs of the support's surface during fermentation (Fig. 3). Therefore, the immobilization of the cells is believed to be the time-dependence process. This occurrence is due to two main factors: cell multiplication and the formation of a strong and irreversible adhesion. According to the immobilization theory [19], we think this time-dependence condition might be also influenced by interactions both Vander Waals forces and electrostatic forces.

It was obvious that high densities of immobilized cells were achieved, which keep their viability at stable levels during successive fermentation batches, For comparison, fermentations of sugarcane juice were carried out using free yeast cells, the viable cells in the fermentation broth only about 2–4 × 10^8 cells/ml, far lower than immobilized cells in our biocatalyst system.

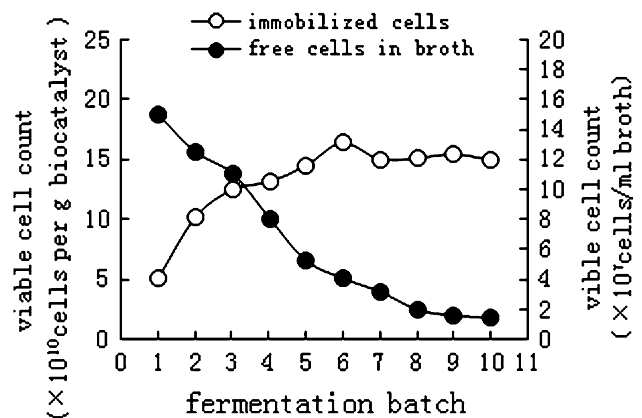

Fig. 4 Viable cell counts of free and immobilized cells during repeated batch fermentations of sugarcane juice. (as $\times 10^{10}$ cfu/g wet sugarcane or $\times 10^{7}$ cfu/ml fermentation broth)

Effective immobilization was also established by the ability of the biocatalyst (after washing to remove free cells) to perform efficiently repeated batch fermentations of sugarcane juice and molasses in the production of bioethanol. The fermentation kinetics was monitored via the weight loss as a result of CO_2 released. As shown in Fig. 5. A delay in the batch fermentation 1 was observed in relation with the rest as a consequence of the adaptation of the immobilized yeasts to the fermentative medium. The maximum fermentation rate of fermentation 1 was higher than that of the subsequent fermentations, this might be due to the decrease of sugar transportation activity and thus lower availability to the yeast cells because higher population immobilized yeasts occupied its channel. They have been successfully reutilized up to six times without any obvious decrease in the yield of CO_2 and the rate of fermentation. Compared to free yeast cells, the immobilized cells used about six hours less to reach the maximum fermentation rate time, and consequently about 10 h shorten in the total fermentation time. However, there is no obvious difference in the value of the max fermentation rate for immobilization fermentations. Once the sixth fermentation batch were completed, the same biocatalysts were reutilized for a medium containing 40% molasses producing about 10% (v/v) ethanol in 4 days. By contrast, free yeast cells were thoroughly depressed in the same medium. This could be due to a higher biomass concentration in yeast sugarcane pieces and/or to an osmotic protection effect of the yeast cell on the outermost layer of the biocatalysts as well as yeast cells in deeper regions. Moreover, the structure of supports was not significantly altered during these fermentation processes.

The kinetic parameters obtained after repeated batch fermentations of sugarcane juice and molasses for ten

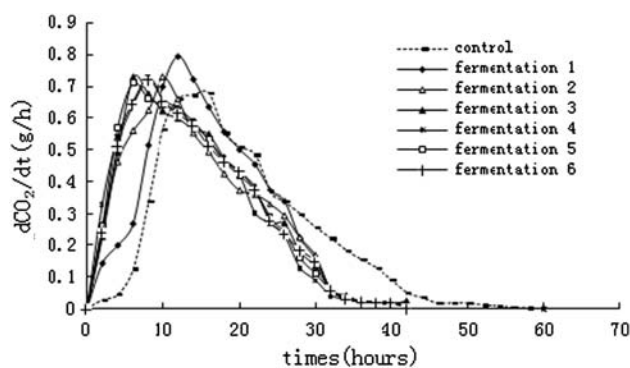

Fig. 5 Fermentation kinetics. Evolution of CO_2 production rate during batch fermentations using sugarcane juice. Control fermentation was carried out by free yeast cells, whereas the batch fermentations were performed by yeast immobilized in sugarcane pieces

cycles using our immobilization system were shown in Table 1. These results showed that fermentation times for all the media investigated were lower than 34 h and stable. Fermentation times were low even during the first batch, indicating that no significant period was needed for adaptation of the biocatalyst in the fermentation environment. Ethanol concentrations (about 89.73–77.13 g/l in average value), and ethanol productivities (about 59.53–62.79 g/l d in average value) were high and stable, and residual sugar concentrations were low in all fermentations (0.34–3.60 g/l) with conversions ranging from 97.67 to 99.80%, showing efficiency (90.11–94.28%) and operational stability of the biocatalyst for ethanol fermentation. It is known that from each kilogram of glucose consumed, 0.51 kg of ethanol can be produced. However, as some of the carbon sources is used for biomass and volatile by—products generation, the actual ethanol yield is about 90–95% (~0.46 g ethanol/g sugar) of the theoretical one [20, 21]. In most of the distilleries in Mexico [20], the yield of ethanol from molasses ranged from 0.33 up to 0.38 g ethanol/g reducing sugars. In the case of the fermentation of sugarcane juice (173.85 g of initial sugar/l) and molasses (154.07 g of initial sugar/l) by the biocatalyst that yeast immobilized on sugarcane pieces, the actual ethanol yields of this work were 91.72% (0.47) and 88.94% (0.45) of the theoretical one, respectively, which are considered as acceptable values. Compared with those obtained with free cells, similar concentrations of ethanol were obtained, however, the main difference observed was the higher fermentation rate of immobilized cells, showed higher ethanol productivity and fermentation efficiency obtained with immobilized cells. It is known that yeast cells can also be entrapped in calcium alginate and the resulting immobilized yeast can be used for fast fermentations. Numerous literature references describe this technique as it is applied usually in laboratory scale. Some efforts, however, have also been made to commercialize this technique. One of the best known is probably that of Kyowa Hakko in Japan [22], where growing cells of *S. cerevisiae* immobilized in calcium alginate gel beads were employed in fluidized bed reactors for continuous ethanol fermentation from cane molasses and other sugar sources. According to the report, the ethanol productivity was more than 50 g ethanol/l gel h and prolonged life stability for more than one-half year. Cell concentration in the carrier was estimated over 250 g dry cell/l gel. As a result, it was confirmed that 8–10% (v/v) ethanol-containing broth was continuously produced from nonsterilized diluted cane molasses for over one-half year. The productivity of ethanol was calculated as 0.6 l ethanol per litre of reactor volume one day with a 95% conversion yield versus the maximum theoretical yield for the case of 8.5% (v/v) ethanol broth. The results of this work were generally comparable with these of the previous studies in the

Table 1 kinetic parameters of the anaerobic repeated batch fermentations of molasses and sugarcane juice with *Saccharomyces cerevisiae* AS2.1190 immobilized on sugarcane pieces at 30 °C

Media	RFB	Fermentation time (h)	Initial Sugar (g l^{-1})	Residual sugar (g l^{-1})	Conversion (%)	Ethanol production (g l^{-1})	Ethanol productivity (g l^{-1} d^{-1})	Fermentation efficiency (%)	Yield (%)
Sugarcane juice	1	34	172.31	0.83	99.52	88.05	56.35	91.28	90.84
	2	33	174.54	0.64	99.64	89.88	59.27	91.95	91.62
	3	32	173.08	1.46	99.16	88.68	60.30	91.79	91.02
	4	34	174.24	0.83	99.52	88.84	56.86	91. 03	90.59
	5	32	173.52	0.34	99.80	91.76	62.39	94.14	93.85
	6	32	175.41	1.20	99.32	91.18	62.01	93.02	92.39
Molasses	1	28	154.2	2.83	98.17	77.08	59.90	90.44	88.78
	2	27	153.62	1.51	99.02	77.41	62.38	90.50	89.61
	3	26	154.23	3.60	97.67	76.53	64.05	90.18	88.08
	4	26	154.16	1.44	99.07	77.48	64.84	90.11	89.27
Sugarcane juice	–[a]	44	176.25	2.61	98.52	88.24	48.13	90.36	89.02

RFB repeated fermentation batch

[a] Fermentation with free yeast cells

Table 2 Fermentation parameters (average value) obtained in batch fermentation with *Saccharomyces cerevisiae,* immobilized on various carriers, at 30 °C

Carrier	Medium	Initial sugar (g/l)	Ferm.time (h)	Residual sugar (g/l)	Ethanol (g/l)	Ethanol productivity (g/l d)	Conversion (%)
Apple pieces [8]	Grape must	206.00	80	30.80	85.00	26.00	85.00
Dried figs [23]	Glucose	120.00	45	1.40	45.00	24.00	98.00
Spent grains [14]	Molasses	187.00	30	8.80	51.40	42.70	95.30
Orange peel [12]	Glucose	125.00	9	4.00	51.40	128.30	96.80
	Molasses	128.00	14	2.00	58.90	100.10	98.40
	Raisin extract	124.00	12	2.30	55.30	110.40	98.10
Sugarcane pieces (present study)	Molasses	154.07	27	2.35	77.13	62.79	98.48
	Sugarcane juice	173.85	32	0.88	89.73	59.53	99.49

fermentation rates, yields and efficiencies, and contributed to an improved quality of the distilled due to the 10–11.5% (v/v) higher ethanol content. In other hand, there are several weaknesses of alginate immobilized yeast for commercial scale operations. A major difficulty with alginate entrapment is the manner in which the particles are formed. It must be carried out at the production site where a yeast slurry and a solution of sodium alginate are mixed together. When this mixture is then fed into a calcium-salt solution, the alginate precipitates and at the same time occludes the yeast cells within the precipitated particles. The particles usually are in the form of droplets/beads. A process plant that utilizes alginate entrapped yeast must have specially designed equipment just to produce these beads. Furthermore, there is a potential risk for contaminating the yeast with wild microorganisms. A second major difficulty for alginate beads used on a commercial scale is in the physical strength of the beads. The beads are soft and easily compressible. Operating large fermentation columns can be a problem and fast down flow process streams are difficult to handle. A third difficulty with entrapment is the diffusion limitations which slow down the accessibility of the substrate in contact with the yeast inside the bead. Finally, if the system becomes contaminated or otherwise disturbed so that a continuous operation must be discontinued, the whole lot of column material (alginate together with the yeast) must be discarded. No reuse is possible. However, the spent sugarcane immobilized supports can be used as protein-enriched (SCP production) animal feed for reusing.

Therefore, Comparison with Ca-alginate entrapped yeast, this sugarcane immobilized biocatalysts is believed to be competitive for an industrial process.

The biocatalysts prepared by natural materials for the production of alcohol have also been extensively studied, such as apple pieces, orange peel, dried figs, etc. (Table 2). Most of the fermentation batches resulted in glucose consumption of 98.00–99.00% for the juice containing 134.00–187.00 g of sugar/l, and stable ethanol production with ethanol productivity ranging from 26.00 to 110.40 g/l d at 30 °C. Anyway, the results presented in this paper, according to initial concentration of sugars in the must, showed that the sugarcane supported biocatalyst was equally efficient to that described in the literature for ethanol fermentation.

Conclusion

Based on the results obtained in the analysis of this work, the following conclusions can be deduced. The results of this study mainly demonstrated the potential applications of the sugarcane and provide a good describing in depth the immobilization of yeast cells. Sugarcane pieces were found suitable as support for yeast cell immobilization in ethanol industry. The sugarcane immobilized biocatalysts showed high fermentation activity. Although ethanol fermentations have been only carried out on sterilized sugarcane juice, the fermentation technologies presented above could be applied in fresh juice as well. The immobilized yeast would dominate in the fermentation broth due to its high populations and lower fermentation time, therefore, the development of other wild yeast and bacteria that may exist in the fresh broth would be inhibited [11]. That in relation with low price of the support and its abundance in nature, reuse availability make this biocatalyst attractive in the ethanol production as well as in wine making and beer production. Also, the particles form of the support give the possibility for fermentations using feed batch bioreactors and separation of biocatalyst employing centrifuge separators or separation after removal of the supernatant liquid. Third, the immobilization method was cheap, simple and easy. The immobilization and fermentation technologies described in the present study could be also applied in other industrial fermentations, since preparation of new biocatalyst with other microorganisms, to carry out different bioprocesses. Further investigation of specific food applications using this biocatalyst would be interesting.

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