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Bioethanol Production From Sugarcane Bagasse Hemicellulose Hydrolysate by Immobilized *S. shehatae* in a Fluidized Bed Fermenter Under Magnetic Field

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Abstract
Bioethanol production from sugarcane bagasse hemicellulosic hydrolysate using immobilized Scheffersomyces shehatae on magnetic biosupports in a fluidized bed bioreactor assisted by magnetic field has been studied. Fermentations were carried out in two experimental setups operating in a magnetically stabilized bed mode with transversal and axial magnetic field lines at 8 and 12 kA/m, respectively. The best results were attained when experiments were carried out using a fermenter assisted by axial field whose ethanol/substrate yield and ethanol productivity were 0.15 ± 0.8E-3 g/g and 0.055 ± 0.3E-3 g/gh. These values were 12 and 34%, respectively, higher than those observed in fermentations with transversal field lines (Tukey's test, $p < 0.05$). Thus, these results are attractive and can be considered as a technological advance in the bioethanol production from biomass using this unconventional fermentation technology.

Keywords Bioethanol · *Scheffersomyces shehatae* · Sugarcane bagasse · Hemicelluloses hydrolysate · Unconventional bioreactor · Magnetic field

Introduction

The indiscriminate use of fossil fuels is the main cause of increase of the greenhouse gas emissions in the atmosphere, resulting in global warming and problems related to climate changes. Aware of the seriousness of these facts, in the 1970s, Brazil started a program to replace gasoline with ethanol. In this program, sugarcane was chosen as the raw material for the production of ethanol and, consequently, studies on technological and agricultural development were intensified [1, 2], resulting in a well-established technology. However, a significant increase in ethanol production can be possible using biomass as feedstock to convert the polysaccharides present in leaves, straw, and sugarcane bagasse into bioethanol, rather than being burned in most factories to produce electricity by cogeneration [3–5].

As a consequence, innumerable researches have been conducted to improve the pentoses fermentation, using strains from several sources such as *Scheffersomyces stipitis*, *Scheffersomyces shehatae* and *Spathaspora arborariae*, among others, to produce second-generation bioethanol [6–8]. According to scientific literature, different studies using synthetic media and hydrolyzed in Erlenmeyer flasks and small reactors at bench scale have shown promising results with regard to the optimization of several factors such as substrate concentration, cell growth, pH, aeration and agitation, among others [9–14]. In addition, determination of the most appropriate biomass pretreatment method is very important because it is not only related to the type of lignocellulosic material to be used but should also prevent the degradation of cellulose and hemicellulose, as well as the formation of inhibitors low operating and design costs. Therefore, the

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choice of pretreatment is directly associated with the product and its final application. In this context, the most used pretreatments for the hemicellulose extraction from biomass are the steam explosion and the biological and chemical treatment using diluted acids [1, 4]. The acid hydrolysis is usually chosen because the percentage of acid used is low, minimizing the corrosive effects. Furthermore, when compared to the steam blasting process, the energy cost is lower, the operating conditions are smoother and not only solubilize the hemicellulosic fraction but also results in a hydrolysate containing fermentable sugars, i.e., xylose rich [11, 15].

Furthermore, the fermentation process presents difficulties about adjustment of microbial metabolism of pentoses consumption, resulting in low productivity and bioethanol yield, as consequence of the large fermentation times [16]. Therefore, new approaches must be developed for improving the pentoses fermentation using, for example, suitable indigenous strains or genetically modified or still, exploring unconventional fermentation technology such as bioprocess assisted by magnetic fields [17–20], seeking increases in the bioethanol productivity with scale-up potential at industrial level.

Bioreactors assisted by electromagnetic fields of low frequency and intensity are being studied and operated under different configurations [18, 20, 21]. But, given the innovative nature of this technology, basic laboratory scale studies are needed in order to assess the techno-economic advantages of these processes for later implementation at industrial scale. It would result in some cases in advantages as greater efficiency and ease of magnetic biocatalysts recovery, allowing their reuse in several batch cycles or continuous system, ensuring of high flow feed without danger of washout [19, 22]. Thus, the aim of this short communication was to report the experimental results about the bioethanol production by unconventional way, e.g., from sugarcane bagasse hemicellulose hydrolysate using immobilized *Scheffersomyces shehatae* on biosupports with magnetic properties in a fluidized bed bioreactor assisted by magnetic field.

Materials and Methods

Materials

The hemicellulosic hydrolysates were obtained from sugarcane bagasse (Sugar Factory "Costa & Pinto," Piracicaba, SP, Brazil) containing 34.6% cellulose, 18.9% hemicellulose, 27.6% lignin, 8.8% extractives, and 6.4% ashes. *Scheffersomyces shehatae* UFMG HM 52.2 yeast was kindly supplied by Federal University of Minas Gerais, Belo Horizonte, Minas Gerais State, Brazil. All chemicals including standards and culture media used in this work were analytical grade and purchased from Sigma-Aldrich and Merck, respectively.

Inoculum Preparation

Previously for inoculum preparation, *Scheffersomyces shehatae* was cultivated on yeast extract–malt extract agar (YMA (g/L): glucose 10.0, peptone 5.0, yeast extract 3.0, malt extract 3.0 and agar 20) plates at 30 °C for 24–48 h. For inoculum preparation, the yeasts were cultivated on 400 mL YPX culture medium (yeast extract 10 g/L, peptone 20 g/L, and D-xylose 30 g/L) in 1000-mL Erlenmeyer flasks at 30 °C and 200 rpm for 24 h. Cells were recovered by centrifugation at 2.600×*g* for 20 min, washed twice, and resuspended in sterile distilled water.

Cells Immobilization

Scheffersomyces shehatae UFMG HM 52.2 was grown in culture medium at 30 °C and 200 rpm for 24 h. The biomass produced was separated by centrifugation and then added to the sodium alginate solution (2.0%, *w*/*v*) containing 1.0% and 8.0% (*w*/*v*) magnetite powder and then stirred until complete mixture. The magnetic powder was prepared as previously described by the co-precipitation method [23]. Beads containing immobilized cells and 8% *m*/*v* of magnetic particles were prepared by droplets using a Watson-Marlow peristaltic pump with a 3-mm diameter hose in a sterilized $CaCl_2$ solution (0.1 mol/L).

The granulometric distribution of the calcium alginate particles with magnetite incorporation was determined by the laser diffraction method using a SALD-3101 particle analyzer (SHIMADZU) that allows to analyze a wide range of sizes between 0.05 and 3000 μm in diameter. The prepared particles presented a relatively good sphericity and monomodal size distribution of about 97 to 99%, corresponding to average diameter around 3.05 mm. These beads remained in $CaCl_2$ solution for 12 h to allow their complete gelation before being used.

Preparation and Treatment of the Sugarcane Bagasse Hemicelluloses Hydrolysate

Hemicellulose hydrolysate was prepared in a 250-L stainless steel reactor loaded with sugarcane bagasse and sulfuric acid solution (1.84 kg acid/18.4 kg of dry matter). The reactor was operated with a solid/liquid ratio of 1:10 at 121 °C for 20 min [24]. After hydrolysis, the hemicellulosic hydrolysate attained from solid material was removed by filtration, resulting in the following composition (g/L): 14.19 xylose, 1.52 glucose, 1.43 arabinose, 0.85 acetic acid, 0.15 furfural, 0.015 5-hydroxymetylfurfural, and 2.57 total phenols. While, the cellulignin composition (solid fraction) was (weight percentage on dry basis) 50.7% cellulose, 7.3% hemicellulose, 33.2% lignin, and 3.4% ashes [11]. Then, the hemicellulose hydrolysate was concentrated in a 30-L evaporator at 70 ± 5 °C [25]

to obtain a xylose concentration of about 60 g/L, and then detoxified to remove fermentation inhibitors. The pH of the hydrolysate was raised to 7.0 with calcium oxide and reduced to 5.50 with phosphoric acid. Then, 2.5% active charcoal was added to the hydrolysate, agitated at 200 rpm and 30 °C for 1 h [26]. Finally, the hydrolysate was autoclaved at 120 °C, 0.5 atm for 15 min.

Fermentation Procedures

Experimental Setup for Fermentation Assisted by Electromagnetic Field

Fermentations were conducted for 48 h in a 500-mL glass column fermenter (volumetric glass column with a ratio height (H) >> internal diameter (D)) using a working volume of 300 mL assisted by electromagnetic field (Fig. 1). The internal diameter and the height of the bioreactor were 0.04 m and 0.4 m, respectively. The fermentation temperature was controlled at 30 °C by a thermostatic bath and verified through an infrared thermometer, while the initial pH of the culture medium was adjusted to 6.50. The experiments were carried out in triplicate and samples were collected periodically for analysis.

The homogeneous magnetic field assisting the fermentation was generated by two different coil assemblies (Fig. 1) allowing two orientations of the field line direction with respect to the vertical axis of the fermenter, i.e., transversal (Fig. 1a) and axial (Fig. 1b). The coils are supported by a nonmagnetic (wooden) frame with a center piece supporting the glass column fermenter. The coils were energized by DC current and the desirable magnetic field intensity stablished using a Variac. Thus, the cellular suspension was subjected to magnetic field in a fermenter entirely encircled by the magnetic field strength at 0 kA/m (control experiment, i.e., without magnetic field) and 8 kA/m for transversal system and 12 kA/m for axial system. The culture medium was externally recycled through a fluidized bed bioreactor containing the immobilized yeasts on biosupports (calcium alginate) containing magnetic particles. The fermentation process was carried out during 48 h, withdrawing samples at each 12 h. The magnetic field intensity was monitored by GM08 Gaussmeter from Hirst Magnetic Instruments Ltd. (UK).

Analytical Procedures

The samples were centrifuged and their supernatants analyzed to xylose, glucose, arabinose, xylitol, ethanol, and acetic acid determination by HPLC (Agilent), containing a Bio-Rad Aminex HPX-87H (300 × 7.8 mm) column. Operating at the following conditions: 45 °C, 0.005 M sulfuric acid as eluent at a flow rate of 0.6 mL/min, and sample volume of 20 μL. The fermentation parameters $Y_{P/S}$ (g/g, ethanol-substrate yield), Q_P (g/L/h, ethanol productivity), η (%, fermentation efficiency), and xylose and/or glucose consumption (%) were experimentally determined. The slope of the line through the origin provided the estimate of $Y_{P/S}$. Ethanol productivity (Q_P, g/L/h) was determined by the ratio of ethanol concentration (g/L) to fermentation time (h). Conversion efficiency (η, %) was determined as the ratio between $Y_{P/S}$ (g/g) and the

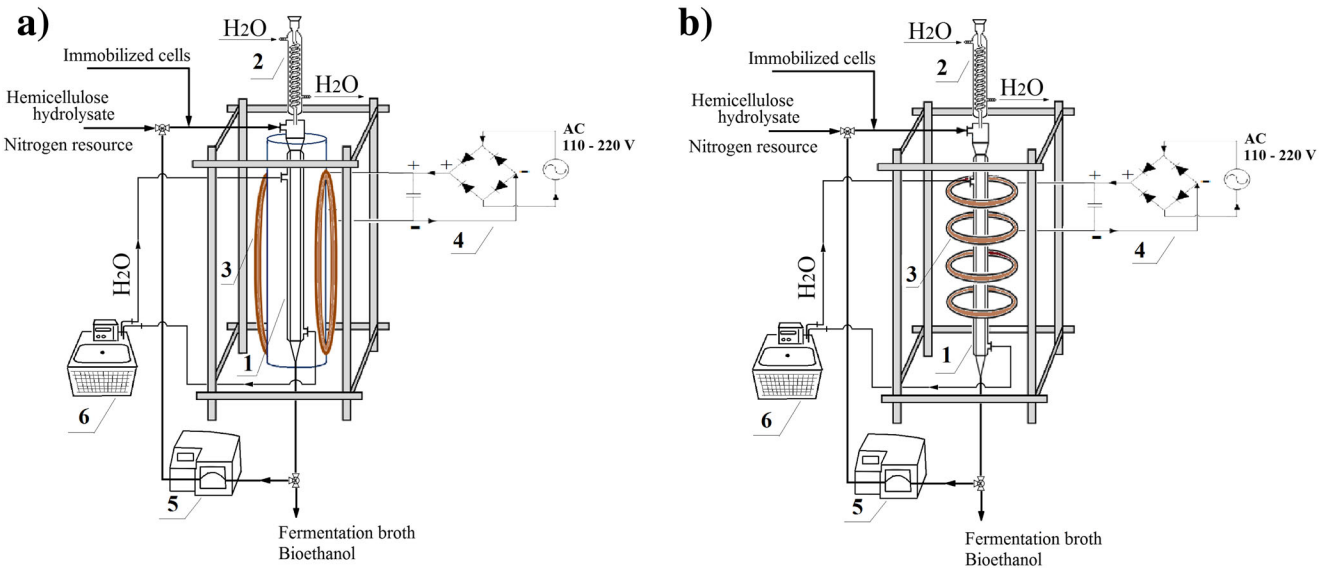

Fig. 1 Experimental setup for fermentation assisted by electromagnetic field. **a** Fermenter under transversal magnetic field lines. **b** Fermenter under axial magnetic field lines. Symbols: (1) bioreactor, (2) condenser, (3) coils, (4) Variac system, (5) peristaltic pump, and (6) thermostatic water bath

theoretical value (0.51 g ethanol/g xylose and glucose) of this parameter [27]. Xylose and glucose consumption (%) was determined as a percentage of the initial sugar concentration. The fermentation parameters, sugars consumption, ethanol production, cell growth, YP/S, and QP were analyzed by one-way ANOVA, followed by Tukey's test for post hoc comparison. The level of significance was set at $p < 0.05$. The magnetic characterization of the magnetite and calcium alginate particles containing magnetite was carried out using a SQUID Vibrating-Sample Magnetometer (VSM) (Quantum Design® models MPMS 57, MPMS 7T) [28].

Results and Discussion

Characterization of the Sugarcane Bagasse Hemicelluloses Hydrolysate

The total sugar concentration obtained after biomass hydrolysis was around 17 g/L, resulting in a mass ratio of glucose: xylose:arabinose of approximately 1.1:9.9:1.0, respectively.

The xylose extraction efficiency was defined as the relationship between xylose mass in the hydrolysate and the initial mass of dry matter considering the % hemicellulose in the material [15]. For these conditions, the pretreatment efficiency was around 78%.

Then, the attained sugarcane bagasse hydrolysate was concentrated around fivefold and subjected to a detoxification process to reduce the fermentation inhibitors. In this context, Table 1 shows the composition of the concentrated hydrolysate before and after detoxification step. Thus, the attained results showed a greater effect on the reduction of the phenolic compounds (approximately 75%) that are important inhibitors of the cellular metabolism [29].

Table 1 Results of chemical characterization of the concentrated and detoxified sugarcane bagasse hemicellulose hydrolysate

Component	Hemicellulose hydrolysate (g/L)	
	Concentrated	Detoxified
Xylose	69.65	63.37
Glucose	7.32	7.04
Arabinose	7.08	6.95
Acetic acid	3.15	2.91
Furfural	0.181	0.035
5-HMF	0.02	0.019
Total phenols	5.47	1.33
pH	0.75	5.50

Bioreactor Assisted by Electromagnetic Field

Bioreactor Calibration

Before fermentation, experiments were carried out for calibration purpose and determination of the operational ranges for each parameter in the bioreactor assisted by electromagnetic field. Figure 2 shows the profiles of the electromagnetic field at the geometric center between coils. As can be verified, the center inside the coils at the axial

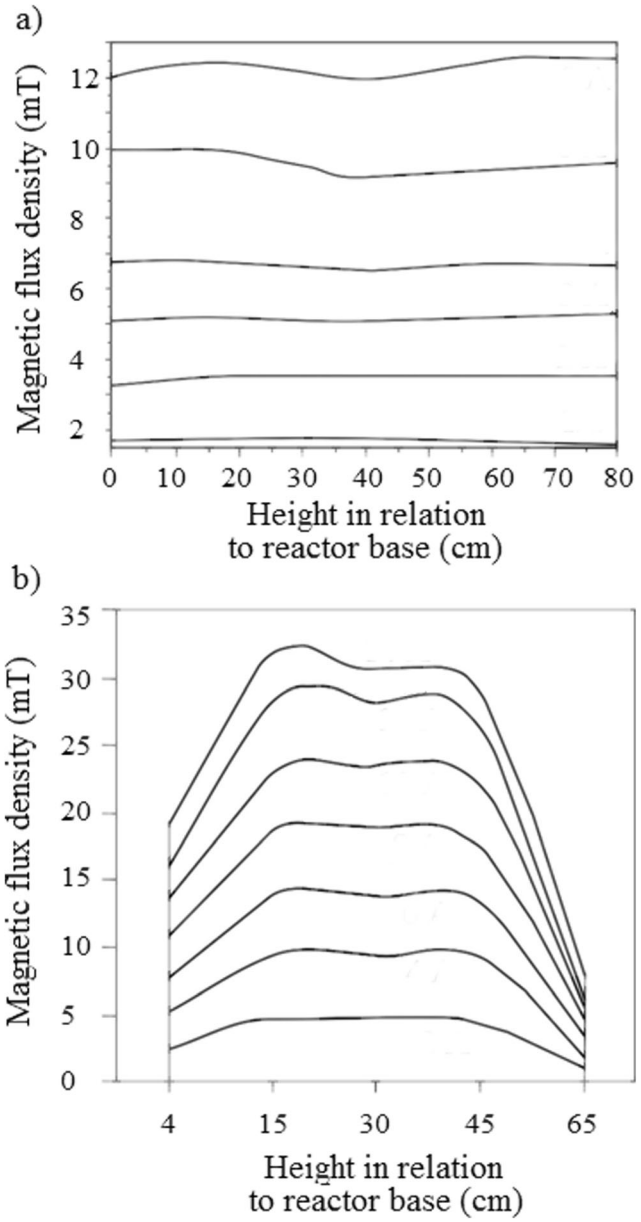

Fig. 2 Profiles of magnetic flux density (B) at the center of the bioreactor assisted by electromagnetic fields as a function of direct current expressed in amperes (A) imposed to the system and the position of the measured along the axial direction, whereas two systems for generating electromagnetic field: (**a**) field lines in the transverse direction and (**b**) field lines in axial direction

Table 2 Effect of the magnetic field lines on the parameters attained during fermentation of sugarcane bagasse hemicellulose hydrolysate by immobilized *S. shehatae* on magnetic support (alginate (2% wt/*v*) plus 8% Fe_3O_4) at 96 h fermentation time

Experimental conditions			Parameters attained after fermentation		
Test no.	Bioreactor	Magnetic field intensity (kA/m)	Ethanol/substrate yield (g/g)	Sugars consumption† (%)	Ethanol productivity (g/L/h)
1	Transversal*	0 (control)	0.11 ± 1.3E-3	25.94 ± 0.9	0.034 ± 0.7E-3
2		8	0.13 ± 2.8E-3	27.11 ± 0.2	0.041 ± 1.2E-3
3	Axial**	0 (control)	0.12 ± 1.0E-3	25.37 ± 0.1	0.037 ± 2.8E-3
4		12	0.15 ± 0.8E-3	30.24 ± 0.6	0.055 ± 0.3E-3

*Bioreactor assisted by electromagnetic field with transversal field lines

**Bioreactor assisted by electromagnetic field with axial field lines

† Sugars: xylose and glucose

length is the most important position because it corresponds to the location of the glass column bioreactor inside of a magnetic field generator, for both magnetic field lines, transversal (Fig. 2a) and axial (Fig. 2b) in the adopted experimental setups.

An analysis of the distribution behavior of these lines suggests that magnetic field is uniform at the center points of the bioreactor. In fact, this field is more intense at the center than at the extremes of the coils. Thus, by placing a cylindrical fermenter at the center of the magnetic field generator, it is possible to guarantee that there will be a uniform distribution of magnetic field focusing on the culture medium and the cellular suspension when free or immobilized cells on calcium alginate with magnetic properties are used.

Ethanol Fermentation of Sugarcane Bagasse Hemicellulose Hydrolysate in a Fluidized Bed Reactor Assisted by Electromagnetic Field

As described above, the fermentations to evaluate the effect of electromagnetic field on the bioethanol production were carried out in two versatile experimental systems as illustrated in Fig. 1, allowing the study the effect of magnetic field according to the incidence of the direction of these field lines on the glass bioreactor, i.e., varying the field lines from transversal to axial. Then, Table 2 and Fig. 3 show the results of fermentations under magnetic field.

In general, despite of the diversity of published papers and the controversies about biological effects of electromagnetic fields [17–21, 30, 31], in the present study, the attained results

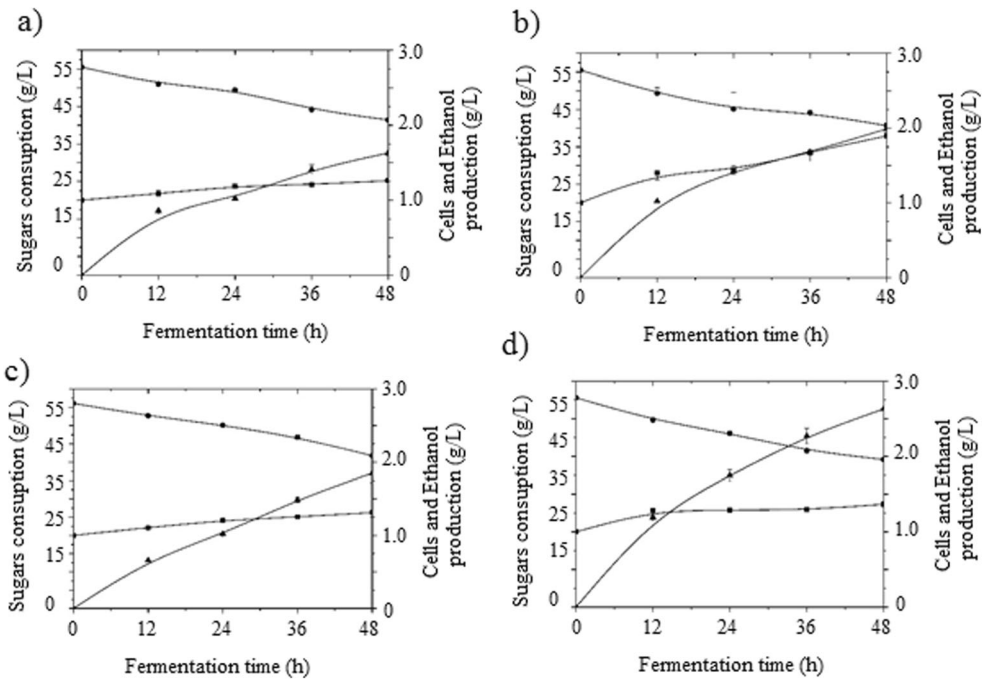

Fig. 3 Fermentation kinetic of bioethanol production in fluidized bed bioreactor assisted by electromagnetic field using sugarcane bagasse hemicellulose hydrolysate by immobilized *S. shehatae* on alginate with magnetic properties. **a** Control experiment in transversal system at 0 kA/m. **b** Transversal system at 8 kA/m. **c** Control experiment in axial system at 0 kA/m. **d** Axial system at 12 kA/m. Symbols: (●) sugars concentration, (■) cells growth, and (▲) bioethanol production

showed a positive effect on the ethanol production using sugarcane bagasse hemicellulose hydrolysate as substrate and immobilized yeast on supports with magnetic properties. Basically, in Fig. 3 and in Table 2, differences between the cases with and without magnetic field for both systems can be observed. The attained results using supports with magnetic properties, for both axial and transversal systems, favored the ethanol fermentation process under electromagnetic field application, noting an increase in the consumption of substrate, ethanol production, and cell growth when the systems were compared with the control of 1.2-, 1.5-, and 1.3-folds, respectively, for fermentation under axial magnetic field and 1.1, 1.2, and 3.4, respectively, for fermentation under transversal magnetic field (Tukey's test, $p < 0.05$). As observed in this study, for all experiments, *S. shehatae* showed a gradual increment in cell viability, reaching concentrations in the range of 1.3 and 1.9 g/L at 48 h. In addition, the ethanol yield and productivity in the bioreactor assisted by axial electromagnetic field (Fig. 3d) were 12% and 34%, respectively, higher than those observed in the bioreactor assisted by transversal electromagnetic field (Fig. 3b). Probably, these results can be explained due to the more stability of the biocatalysts in reactor bed under axial magnetic field lines. While, for the cellular growth, a reduction of 28% was observed when axial system was used. On the other hand, under evaluated fermentation conditions, all experiments showed acetic acid partial consumption (approximately 37%), glycerol concentration around 0.03 g/L and were not observed arabinose consumption and xylitol production.

In addition, when compared with the fluidodynamic behaviors of immobilized cells on the particles with magnetic properties, for both transverse and axial systems, it was possible to verify distinct distributions of biocatalysts stabilized magnetically. This is possible by establishment of correlations well described in the literature among the flow of the cellular suspension, the minimum fluidizing velocity, and the variation of magnetic field intensity required to stabilize magnetically the particles bed [32–34].

Figure 4 shows the magnetization curves for both magnetite (Fig. 4a) and calcium alginate with magnetite (Fig. 4b). According to these results, it was verified that the particles presented behavior superparamagnetic and that the magnetite was well dispersed within the alginate beads. The saturation magnetization was 60.4 emu/g and 10.08 emu/g at 300 K for magnetite and calcium alginate with Fe_3O_4 particles, respectively. After biosupports preparation, the magnetization was reduced around sixfolds. However, at this condition, it is possible to guarantee magnetically stabilized particles bed in the bioreactor during fermentation and thus, heat and mass transfer problems through the biocatalysts and consequently diffusional limitation commonly observed in a fixed bed bioreactor with immobilized cells or high cellular densities can be avoided. Also, particles can be easily separated from the culture medium at the end of the fermentation process for reuse in several cycles.

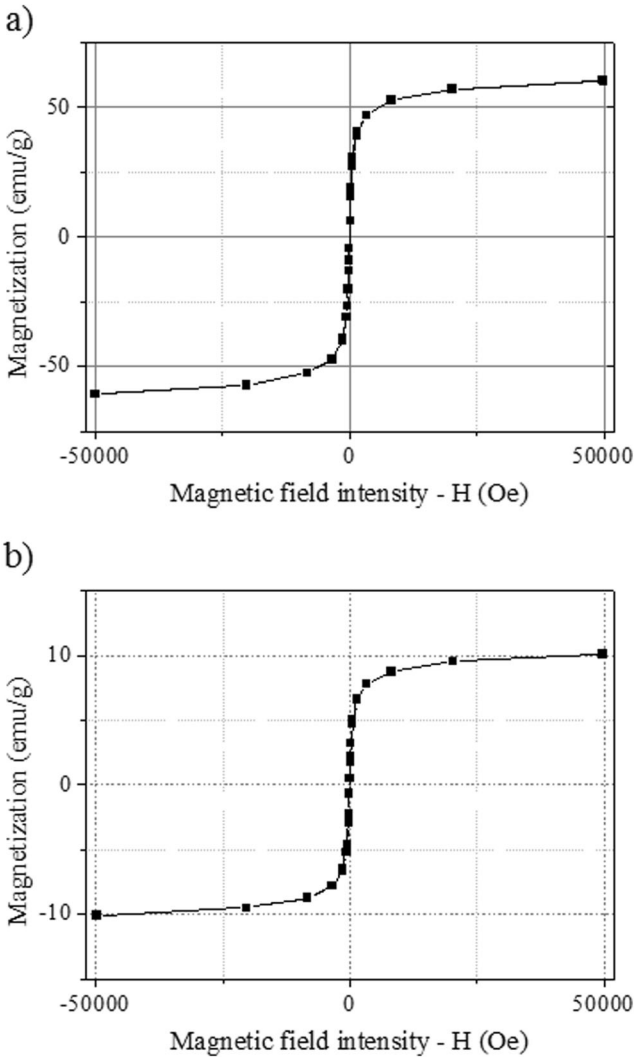

Fig. 4 VSM magnetization curves of **a** magnetite and **b** calcium alginate beads prepared with magnetite and immobilized yeasts

According to Westrin and Axelsson [35], the effect of electromagnetic field in fermentation processes in fluidized bed bioreactors is related to the fact that the bed can be fixed without having problems with larger streams of fluidization. Similar results were observed in previous reports using glucose as carbon source, immobilized *S. cerevisiae* cells, different types of bioreactors, and magnetic field intensities but with the same aim, i.e., evaluate the influence of magnetic field in the performance of ethanol production. In this context, Table 3 shows some published papers about bioethanol production (first generation) by cells immobilized with magnetic properties in bioreactor assisted by magnetic field for comparative purpose and whose attained results reinforce the idea of the positive effect of the magnetic field in these fermentation processes. For example, Ivanova et al. [36] analyzed a continuous

Table 3 Bioethanol production by immobilized cells on particles with magnetic properties

Yeast cells	Carbon source	Cells: supports particles	Bioethanol fermentation System	Ethanol yield	Ref.
S. cerevisiae	Glucose	Cells immobilized on biosupports.	Jacketed glass column bioreactor (1 L) operated without field for 48 h and after under 10 to 33 kA/m to attain magnetically stabilized bed.	Ethanol production increased 1.5 times.	[36]
S. cerevisiae	Glucose	Polyurethane foam cubes (3 × 3 × 3 mm) with magnetite: immobilized biomass 150 mg/g dry support.	Bioreactor glass column 50 mm inner diameter and total volume of 1 L, surrounded by a pair of Helmholtz coils (10 kA/m).	Ethanol productivity reached 17 g/L/h	[37]
S. cerevisiae	Glucose	4.2% dry yeast was immobilized in alginate (2%) with iron power (50%).	Bioreactor of glass column (47 mm in height, 9.6 mm inner diameter) with magnetic field application (400 Oe).	Increased of 12% of ethanol with immobilized yeast.	[38]
S. cerevisiae GT4608	Glucose	Yeast culture ($\geq 10^8$ cell/mL) was mixed with alginate (3% *w*/*v*) and 5.0% (*w*/*v*) Mn–Zn ferrite powder.	Tubular column reactor, diameter of 5 cm and 85 cm length, with magnetic field application (85–120 Oe).	26.7 g/L/h and 95.3%.	[22]
Kluyveromyces marxianus IMB3	Lactose	Yeast culture was mixed into alginate solution (4% *w*/*v*) with 3% Fe_3O_4. The particles were formed with $CaCI_2$ solution (50 mM).	Fed-batch bioreactor.	Increased ethanol of 12 g/L compare with control.	[39]
Scheffersomyces shehatae	Hemicellulose hydrolysate from sugarcane bagasse	Yeast cell: calcium alginate, with magnetite (1–8%), ratio of 1:10.	Bioreactors fluidized bed assisted by electromagnetic field, with transversal and axial magnetic field lines at 8 and 12 kA/m, respectively.	Increase around 1.5 times compare with control.	This work

fermentation process with immobilized *S. cerevisiae* in a magnetically stabilized bed reactor using an external magnetic field (generated by two saddle coils) transverse to the flow of the culture medium in the bioreactor. The explored magnetic field intensities were from 10 to 33 kA/m and the fermentation medium was glucose. However, their best results were observed when using field intensity from 10.4 to 27.7 kA/m that resulted in ethanol production increase around 1.5 times and glucose uptake rate in 115% higher than in the control experiment. In the same way, Liu et al. [22] investigated the continuous ethanol production from glucose in a magnetically fluidized bed reactor with immobilized *S. cerevisiae* in magnetic particles using coils that produced a maximum magnetic field intensity of 500 Oe. They analyzed the influence of particle loading rates and demonstrated that the ethanol productivity was increased in presence of magnetic field. At the better condition, loading rate 41% and feed dilution rate 0.4 h^{-1} obtained ethanol concentration of 60 g/L using initial glucose concentration of 150 g/L and magnetic field intensity of 85–120 Oe. Also, Velichkova et al. (2017) evaluated the effect of magnetic field on ethanol fermentation, but, using corn hydrolysate, in magnetically fluidized bed reactor. In this case, *S. cerevisiae* yeasts were immobilized on the polyurethane foam cubes (3 × 3 × 3 mm) containing Fe_3O_4 and the performance of ethanol fermentation in bioreactor assisted by magnetic field was affected by both dilution rate and magnetic field intensity. Thus, the ethanol productivity was increased from 12 g/Lh (control experiment) to 17 g/Lh (increase around 30%) when the feed dilution rate and magnetic field intensity were 0.6 h^{-1} and 10 kA/m, respectively.

In general way, the biophysical mechanisms that constitute the basis of the interaction between magnetic fields and yeasts are very complex since the influence of magnetic fields on biological processes depends on several factors such as culture medium composition, temperature, microbial concentration, field strength, variation of intensity over time, exposure time under magnetic field and magnetic field configuration, among others.

Some reports in the literature have postulated that one of the biological effects more likely is due to the change in the permeability of cellular membranes and therefore the changes in cellular metabolism [17, 20]. More recently, change in the cellular activity under magnetic field was attributed to phenomenon known micro-level dynamo, which considers micro mixing of the substrate at the vicinity of the yeast/culture medium interfaces resulting in enhanced transport of nutrients, corroborating by this way, the biological effects observed at macroscopic level, such as, biomass growth and increasing metabolites production [18, 19]. In anyway, since yeast are immobilized on biosupports with magnetic properties, in this work, the fluidodynamic conditions imposed to the system, by the action of the field, probably were more favorable to the bioreactor performance during the fermentation process than the biological effect on free cells.

Thus, the attained results using immobilized yeasts on magnetic supports during sugarcane bagasse hemicellulosic hydrolysate fermentation, in a bioreactor assisted by electromagnetic field, are very attractive since the bioethanol production from biomass (second generation) still demands enormous challenges for its implementation of this technology at industrial scale. In addition, by this technology can be mitigated by some technological drawbacks commonly found in the conventional batch fermentation or in continuous processes for bioethanol production, where the cells are separated and treated for reuse using expensive procedures.

Conclusions

The attained results in this work revealed that the bioethanol production from hemicellulosic hydrolysate by *S. shehatae* immobilized on magnetic particles, in the fermenter under magnetic field, was favored by both axial and transversal magnetic fields. However, the best results were attained when axial magnetic field was used because the ethanol yield and productivity were 12% and 34%, respectively, higher than those observed in the bioreactor assisted by transversal magnetic field. These results can be considered a technological advance for bioethanol production from biomass hydrolysate (second generation) and consequently, further studies should be carried out to verify the challenges to scale up this technology at industrial scale.

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