

Magnetically assisted fluidized bed bioreactor for bioethanol production

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Ethanol can be produced by fermentation of sugars from agricultural products as wheat and corn, after milling and hydrolysis. The mash after dry mill contains up to 25% reducing sugars, suspended solids and it is very viscous. In this study the effect of a magnetic field on ethanol fermentation with magnetically assisted fluidized bed reactor (MAFBR) was investigated. Magnetically loaded polyurethane foam cubes (3x3x3mm) were used as support material for biofilm formation in a fluidized bed reactor. The magnetite content was 100% wt/wt; density 1050kg/m³ and immobilized biomass 150mg/g dry support. The polyurethane carriers were stable and had high ethanol fermentation activity. The reticulated structure of the polyurethane foam enabled adherence as well as entrapment of biomass. Magnetically assisted fluidised bed experiments were performed in a glass column 50mm ID and total volume of 1 L, surrounded by a pair of Helmholtz coils with 200mm ID. The performance of ethanol fermentation of mash in the MAFBR was affected by dilution rate and magnetic field intensity. The ethanol productivity reached 17 g/L.h at a feed dilution rate of 0.6 h⁻¹ with reducing sugars concentration of 150g/L when the magnetic field intensity was 10kA/m.

Key words: bioethanol, magnetically assisted, fluidization, magnetic support.

INTRODUCTION

Ethanol can be produced by fermentation of sugars from agricultural products or waste plant materials [1]. The batch fermentation process is relatively slow process, and continuous fermentation can increase the rate, even higher rates can be achieved if cell retention is also employed. To eliminate inhibition caused by high concentration of substrate and product as well as enhance ethanol productivity, cell immobilization approaches have been applied to produce ethanol continuously in bioreactors [2, 3]. A continuous fluidized bed reactor with the immobilized cell particles was demonstrated to significantly improve ethanol volumetric productivity as compared with the traditional batch systems and other continuous reactor configurations [4]. Magnetically assisted fluidized bed reactor (MAFBR) offers a number of potential advantages over the conventional fluidized bed reactor, such as elimination of solid mixing, low pressure drop through the bed, ease of solid transportation as well as the possibility of operation at increased fluid velocities [5]. The MAFBR has been used as an efficient system coupled with magnetic immobilized cells and enzymes for process intensification of biocatalysis and biotransformation [4, 5]. One of the major problems associated with application of MAFBR is development of suitable supports with magnetic properties. Taking into account that the magnetically stabilized bed

bioreactors operate at velocities greater the minimum fluidization velocity in absence of a field, the bio-support density is of a principal importance. The higher relative fluid/particle velocity (due to density differences) increases the mass transfer efficiency, but this decreases the liquid residence time in the reactor and requires deep beds or recirculation. The employment of polyurethane foam beads with entrapped cells has performed by many researchers [3, 6, 9]. The main purpose of present study was to investigate the influence of hydrodynamic conditions and magnetic field on ethanol productivity in the MAFBR with immobilized *Saccharomyces cerevisiae* cells, in continuous mode of operation.

MATERIALS AND METHODS

The commercially available wetted polyurethane foam was cut as cubes (3x3x3mm). The magnetite was synthesised by co precipitation of Fe³⁺ and Fe²⁺ salts [7]. Briefly 5,4 g of FeCl₃·6H₂O and 2,78 g FeSO₄·7H₂O were dissolved in 20 ml distilled water. The polyurethane foam support (2g) was soaked and mixed well in a solution and heated for 10 minutes to 80 °C. For co precipitation the support cubes were added drop wise into the NH₄OH (8 M) solution under stirring. After 3 h aging, the magnetic support was collected by external magnet, washed with distilled water to neutral pH and then dried at 60 °C. The procedure was repeated 5 times to obtain magnetic support. The obtained by co precipitation magnetic supports were characterized by XRD, after

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heating at 500 °C for 30min. The X-ray powder diffraction pattern of the sample was recorded on a Philips PW1050 diffractometer using $\text{CuK}\alpha$ (1.5406Å) radiation at room temperature in the range of 10 to 80° in the 2θ scale, with a scanning speed of 0.02°/s and a step time of 3s. The void volume of support was determined by the weighting of dry and saturated with water samples. The cell adsorption and accumulation was carried out on a shaker at 150 rpm and 30°C for 7 days. The culture medium had

the following composition (g/L): 10 yeast extract, 20 peptone, 20 glucose, 1.5 KH_2PO_4 , 4 $(\text{NH}_4)_2\text{SO}_4$, and 0.5 MgSO_4 . Every 24 hours the medium was replaced and support washed with fresh medium. The study of ethanol production activity of free and immobilized biomass of *Saccharomyces cerevisiae* was carried out with batch fermentation in shake flasks and corn hydrolysate from bioethanol plant (initial reducing sugars concentration 224g/L) was used as substrate.

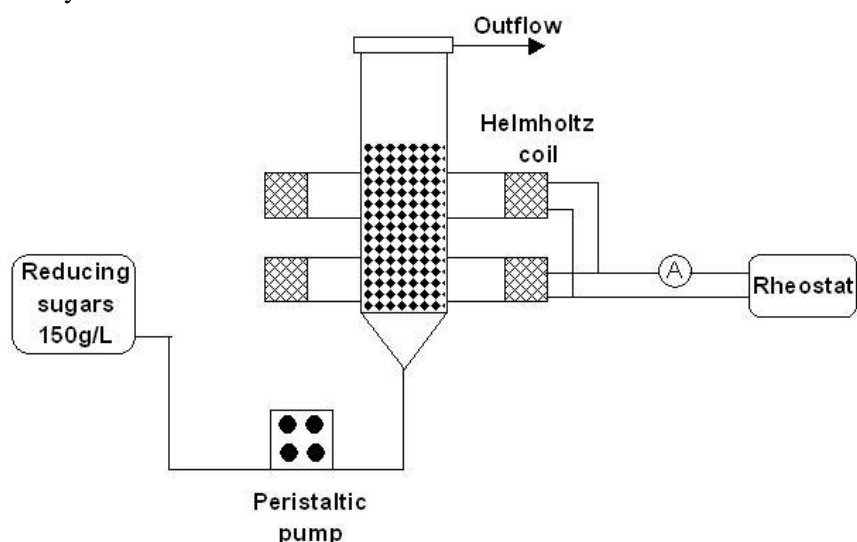


Fig. 1. Experimental set-up for ethanol production in MAFBR.

The magnetically assisted fluidization experiments were performed in a glass column (50mm I.D.) and total volume of 1L. The column was packed with support and substrate with 150g/L glucose was pumped thru the column. In all the experiments a pair of Helmholtz coils was used as a magnetic system. The magnetic field was axially oriented (e.g. parallel to the column axis). The internal coil diameter was 200 mm, distance between symmetry planes - 200 mm. The schematic view of experimental set-up is shown in Fig.1. The concentration of glucose was determined by DNS method. This method tests for the presence of free carbonyl group (C=O), the so-called reducing sugars. This involves the oxidation of the aldehyde functional group. Simultaneously, 3,5-dinitrosalicylic acid (DNS) is reduced under alkaline conditions [3]. The concentration of ethanol was determined by reaction with an excess of potassium dichromate in acid. The reaction was carried out in a special device [8]. The amount of unreacted dichromate is then determined by titration with ferrous ammonium sulphate and the ethanol concentration was calculated as chemical oxygen demand of solution.

RESULTS AND DISCUSSION

Whether the expected Fe_3O_4 particles rather than other iron species were synthesized was confirmed

via XRD pattern. In order to compare the crystalline structure to define the iron species, XRD characterisation of both commercial Fe_3O_4 purchased (Merck) and synthesised were undertaken. In Fig.2, the spectra of synthesized Fe_3O_4 indicate similar characteristic peaks with

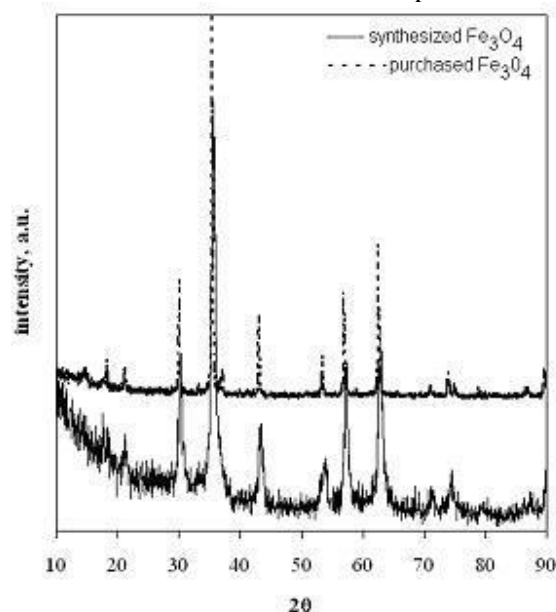


Fig. 2. The X-ray powder diffraction pattern of the synthesized and commercial samples.

commercial Fe₃O₄ particles at 2θ (Brag angle) values of 30.1, 35.5, 43.2, 53.5, 57.1, 62.6°, which correspond to the typical peaks of Fe₃O₄ particles [7]. So, there is no doubt that Fe₃O₄ particles are synthesized successfully.

The main criterion for applicability of obtained support in magnetically assisted bioreactors is magnetic phase content. In order to test the magnetic properties constant magnetic field was applied. The separation of support from substrate under constant magnetic field is shown in Fig.3.



Fig. 3. Magnetic separation of magnetic polyurethane foam particles under an external constant magnetic field

The properties of obtained support and used substrate are summarized in Table 1. The data showed that there are not significant differences between densities of wet biosupport and substrate. The low-density supports allow reduced liquid flow rates and increased substrate residence time in the reactor.

Table 1. Support and substrate properties

Support				
Density dry kg/m ³	Density wet kg/m ³	Fe ₃ O ₄ content kg/kg	Size mm	
100	1050	0,98	3x3x3	Cubes
Liquid phase (substrate)				
Density, kg/m ³	Reducing sugars, g/l		Suspended solids, g/l	
1030	224		126	

The results from biomass adsorption on magnetic and nonmagnetic polyurethane foam supports are shown in Fig.4. The better results were obtained with magnetite containing polyurethane foam support and

up to 0,15g/g yeast biomass was immobilized for 7 days. A comparison of the experimental results for the batch process using immobilized and free biomass is shown in Fig. 5. The figure shows that there is not difference between activities of free and immobilized cells. The time required for full conversion of hydrolysate to ethanol was 48 hours. Both the free and immobilized cells showed similar properties and this is the evidence that there are not any diffusional limitation, probably due the open pore structure of used support.

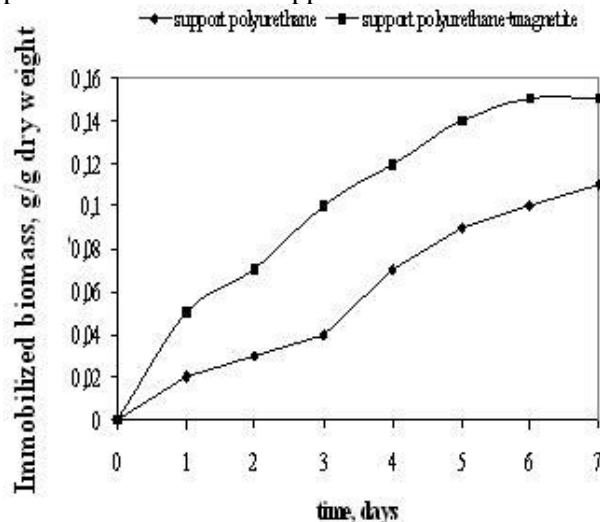


Fig. 4. Effect of incubation time on yeast immobilization.

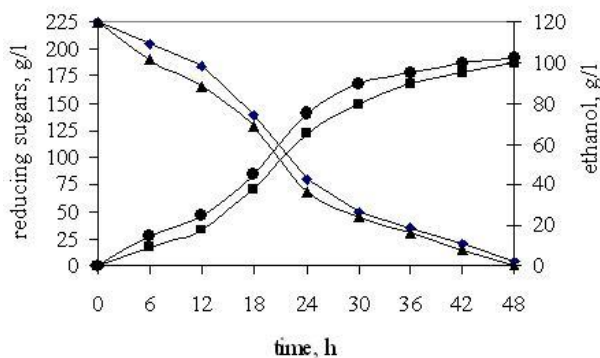


Fig. 5. Ethanol production by batch process with free and immobilized biomass.

In order to determine the hydrodynamic properties of bed the influence of fluid flow speed and magnetic field intensity on fluidisation velocity was examined. The phase diagram of the bed is shown in Fig. 6. It is seen than the fluidisation occurs at fluid velocity over 0,05 mm/s. The bioreactor can be operated as MAFBR at fluid velocities up to 0,2 mm/s and magnetic field intensity 10 kA/m.

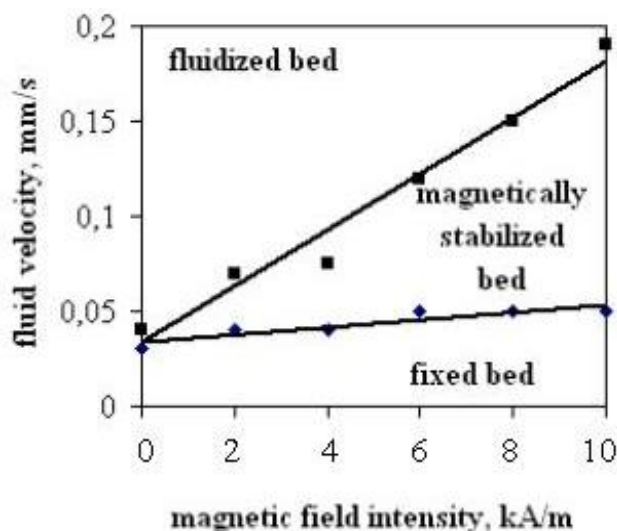


Fig. 6. Phase diagram of the MAFBR

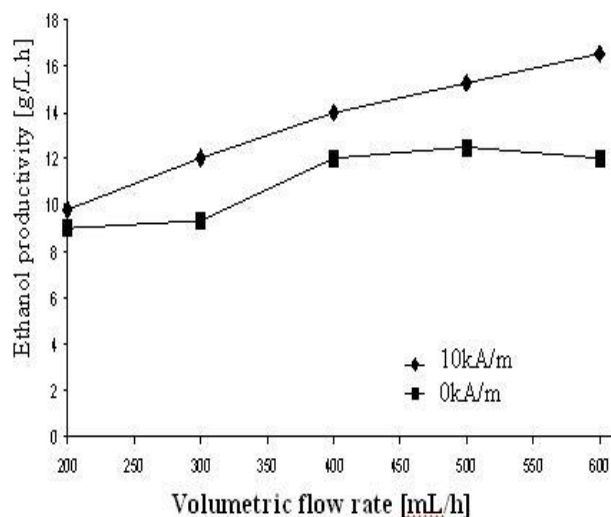


Fig. 7. Ethanol productivity as a function of fluid flow rate.

The results from ethanol production in continuous-flow column reactor are shown in fig. 7. The reactor was run in continuous mode at 25°C, and flow volume speeds from 200ml/h to 600ml/h. The column experiments show that performance of bioreactor increases when magnetic field was applied. The ethanol productivity reached 17 g/L.h, with reducing sugars concentration of 150 g/L when the magnetic field intensity was 10kA/m. The result of this study demonstrated that in MAFBR higher than fluidized bed reactor ethanol productivity can be achieved.

CONCLUSION

The yeast cells of *S. cerevisiae* were immobilized by adsorption onto magnetite coated polyurethane foam support. An increase in the efficiency of the immobilization was observed in the presence of a magnetite (Fe_3O_4 nanoparticles) embedded in a matrix.

Comparison with our previous research [8] showed that on closed pore polyurethane foam up to 0,04g/g dry weight yeast could be immobilized by crosslinking, while up to 0,15g/g yeast biomass was immobilized in open pore polyurethane foam support by biofilm formation. In addition, a slight increase in the performance was observed in non-continuous mode and ethanol productivity reached 7,1 g/L.h. The results obtained in denitrification study [9] showed lower degree of conversion as a result of the small liquid residence time in the bed. Our investigation showed that the use of a MAFBR allow a control of the fermentation process. The main advantage is the ability to decrease the

diffusional mass transfer resistance around the support. However, this effect is limited in particular velocity range. The further increase of the liquid flow rate and magnetic field intensity leads to lower degree of conversion. Other researchers also observed a similar effects [4,5,11].

REFERENCES

1. S. Ingale, S. J. Joshi, A. Gupte, *Brazilian J. Microbiol.*, **45**, 885 (2014).
2. G. Najafpour, H. Younesi, K. S. K. Ismail, *Bioresource Technol.*, **92**, 251(2004).
3. C. M. S. G. Baptista, J. M. A. Coias, A. C. M. Oliveira, J. M. S. Rocha, M. J. Dempsey, K. C. Lannigan, P. S. Benson, *Enzyme Microbial Technol.*, **40**, 127 (2006)
4. C. Liu, F. Wang, F. Ou-Yang, *Bioresource Technol.*, **100**, 878 (2009).
5. C. Webb, H-K. Kang, G. Moffat, R. A. Williams, A. M. Estevez, J. Cuellar, E. Jaraiz, M. A. Galan, *Chem. Eng. J./Biochem.Eng. J.*, **61**, 241 (2006).
6. N. Abdehagh, M. T. Namini, S. M. Heydarian, B. Bonakdarpour, D. Zare, *Iran. J. Environ. Health. Sci. Eng.*, **8**, 245 (2011)
7. T. Sulistyaningsih, S. J. Santosa, D. Siswanta, B. Rusdiarso, *Int. J. Materials, Mechanics, Manufacturing*, **5**, 16 (2017)
8. A. Caputi, M. Ueda, T. Brown, *Am J Enol Vitic.*, **19**, 160 (1968)
9. T. Ivanov, J. Hristov, S.Petkova, *1st South-East European Symposium on Fluidized Beds in Energy Production, Chemical and Process Engineering and Ecology, Ohrid, Republic of Macedonia*, p. 379, (1997)
10. T. Ivanov, I. Lalov, L. Yotova, *Scientific Works of University of Food Technologies*, **LXII**, 536 (2015)
11. V. Ivanova, P. Petrova, J. Hristov, *Int. Rev. Chem. Eng.*, **3**, 289 (2011).

ПОЛУЧАВАНЕ НА БИОЕТАНОЛ В БИОРЕАКТОР С МАГНИТНО АСИСТИРАН ФЛУИДИЗИРАН СЛОЙ

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(Резюме)

Биоетанол може да бъде получен от редица селскостопански продукти, като жито и царевица. При този процес е необходима предварителна хидролиза на суровините. Полученият хидролизат се характеризира с високо съдържание на редуциращи вещества, до 25%, суспендирани частици и е много вискозен. Целта на настоящата работа е да се изследва ефекта на магнитното поле върху алкохолната ферментация в реактор с магнитно асистиран слой.

Като носител за имобилизиране на биомасата е използван пенополиуретан 3x3x3 mm, съдържащ магнетит. Съдържанието на магнетит в носителя е 100%, плътността 1050 kg/m³, като имобилизираната биомаса е 150 mg/g сух носител. Получените носители са стабилни и притежават висока ферментационна активност. Структурата на полиуретановата пяна позволява задържане на биомаса в целия обем на частицата. За биореактор с магнитно асистиран слой е използвана колона с диаметър 50mm и общ обем 1L, поставена в центъра на намотка на Хелмхолц с вътрешен диаметър 200 mm. Установено е, че върху производителността на апарата влияние оказват скоростта на разреждане и интензитета на магнитното поле. Производителността по отношение на етанол достига до 17 g/L.h, при скорост на разреждане 0,6 h⁻¹ и интензитет на магнитното поле 10 kA/m.