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Scaling up of ethanol production from sugar molasses using yeast immobilized with alginate-based MCM-41 mesoporous zeolite composite carrier

Chunming Zheng [a,b], Xiaohong Sun [b], Landong Li [b], Naijia Guan [b,*]

[a] State Key Laboratory of Hollow-fiber Membrane Materials and Membrane Processes, School of Environmental and Chemical Engineering, Tianjin Polytechnic University, Tianjin 300387, PR China
[b] Key Laboratory of Advanced Energy Materials, Ministry of Education, School of Chemistry, Nankai University, Tianjin 300071, PR China

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ABSTRACT

Microporous and mesoporous zeolites, including ZSM-5, H-β, H-Y, and MCM-41, were modified with 3-aminopropyl-triethoxysilane (APTES), then inorganic fillers, such as abovementioned zeolites or mesoporous materials, (α-AlOOH or γ-Al_2O_3), were mixed with alginate embedded with yeast; and finally these carriers were cross-linked through the double oxirane. The alginate-based immobilized yeast with MCM-41 exhibited much shorter fermentation time and higher ethanol concentration than pure alginate and other composite carriers with the highest cell concentration of 4.8×10^9 cells/mL. The composite carrier maintains the highest ethanol productivity of 6.55 g/L/h for 60 days in continuous fermentation process, implying good operational durability for commercial applications. The reason for the higher bio-catalytical function of the immobilized yeast might lay in the uniformly yeast distribution in the bio-reactor and high yeast cell concentration, which contributed by the improved transmission of fermentation media and combined effects of yeast adsorption by MCM-41 and embedment by alginate.

1. Introduction

The oil crisis of the last 30 years has focused research conducted in the scientific area of ethanol fermentation primarily toward increasing the ethanol productivity of bioprocesses and reduction of energy demands. In addition to providing a solution to the environmental issue arising from the disposal of sugar molasses, the production of ethanol as a fuel can also help stabilize the agricultural sector in sugar-producing countries (Kopsahelis et al., 2007; Thomas and Kwong, 2001). The fermentation of sugar molasses has continued to enhance ethanol productivity (Nahvi et al., 2002). To increase ethanol productivity and to reduce labor intensity, aspects such as bioreactor volume, energy consumption in the production of ethanol, and cell immobilization for ethanol production have been extensively studied and reviewed (Kopsahelis et al., 2009; Kourkoutas et al., 2004; Marignetti et al., 1997). Various adjuncts have been proposed by different researchers for yeast and bacteria immobilization for application in ethanol fermentation from various raw materials. These research efforts included the use of inorganic and organic adjuncts in batch and continuous processes, including organic materials such as calcium alginate, polyvinyl alcohol, and polyacrylamide (Kourkoutas et al., 2004) and inorganic materials such as alumina (Loukatos et al., 2000), silica (Gill and Ballesteros, 1998) and zeolite (Hartmann, 2005; Kourkoutas et al., 2004). The reduction of costs involved in ethanol production from bioprocesses employing immobilized cells systems are associated with aspects such as the cost of raw materials, the use of cheap, abundant and stable immobilization adjuncts, the high cell concentration in the bioreactors, the simplicity and low cost of immobilization techniques, the stability of the immobilized biocatalyst in its operational state, ease of regeneration and the design and development of a suitable bioreactor system (Kourkoutas et al., 2004; Wang et al., 2011). Alginate, as a carrier, has a high immobilized yeast concentration; however, its biochemical stability and mechanical strength is poor, and, therefore, the industrial application of organic materials is restricted. The MCM-41, a type of ordered mesoporous inorganic materials, has been applied successfully as a carrier in immobilizing cells processes (Tope et al., 2001). It has good mechanical property, permeability and renewability, all of which increase the mechanical strength of immobilized yeast, and enhance the transportation of fermentation products; however, it has low immobilized yeast concentration for relying on weak physical or chemical adsorption with microorganisms. Therefore, the preparation of organic–inorganic composite materials for yeast immobilization appears very attractive as a prospective method because it combines features of organic and inorganic materials. These organic–inorganic carriers not only utilize their good embedded affinity to organic materials and fast propagation of yeast cells, but also possess good mechanical properties and permeability to inorganic materials; these traits prolong the operating life of carriers, and effectively improve the

* Corresponding author. Tel./fax: +86 022 23500319.
E-mail address: guannj@nankai.edu.cn (N. Guan).

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fermentation efficiency of immobilized yeast in production of ethanol as a fuel. The published literature (Chen et al., 2007) reports mesopores and macropores as being among carriers those are vital to the high density proliferation of the yeast as well as transportation of substances and products between carriers and the medium.

In this study, micro- and mesoporous zeolites such as ZSM-5, H-β, H-Y, and MCM-41 were modified with 3-aminopropyl-triethoxysilane (APTES); inorganic fillers, including the abovementioned zeolites and other mesoporous materials such as α-AlOOH and γ-Al_2O_3, were mixed with alginate embedded with yeast and then cross-linked through the double oxirane. The alginate-based immobilized yeast with MCM-41 has the highest biology capacity of 4.8×10^9 cells/mL, and can be utilized repeatedly in batch and continuous fermentation applications. The aim of this study was to evaluate and compare the suitability of the alginate-based MCM-41 composite carrier, for utilization in yeast immobilization when applied in ethanol fermentation of sugar molasses, with regard to operational stability, ethanol productivity, and cost-effectiveness of the proposed process. Validated by the scanning electron microscopy (SEM) images and associated fermentation experiments, the possible mechanism underlying the higher biocatalytic function was also studied.

2. Methods

2.1. Microorganism and media

Dry yeast cells (*Saccharomyces cerevisiae*, provided by the Danbaoli Brewery, Guangdong, PR China) were initially cultivated in a sterile growth medium at 30 °C in shaken flasks. Cells were harvested in the early exponential phase by centrifugation at 8000 rpm. The chemical reagents utilized in this study include sodium alginate, sucrose, glucose, calcium chloride, monopotassium phosphate, ammonium sulfate, magnesium sulfate and 3-aminopropyl-triethoxysilane (APTES), a cationic surfactant. All chemical reagents were of analytical grade and were obtained from Sigma–Aldrich (China). ZSM-5 (SiO_2:Al_2O_3 = 100), H-β (SiO_2:Al_2O_3 = 12.5), and H-Y (SiO_2:Al_2O_3 = 3) were provided by the Fuchen Catalyst Co. Ltd., Tianjin. Pseudo-boehmite (short for α-AlOOH, average pore diameter of 10 nm, BET surface area is 380 m^2/g) and γ-Al_2O_3 (average pore diameter of 4 nm and BET surface area of 313 m^2/g) were provided by Tianjin Chemical Research and Design Institute. Sugar molasses were obtained from the Yunxin Sugar Factory (Yunnan, China).

The growth medium for immobilized yeast comprised sucrose 150 g/L, $(NH_4)_2SO_4$ 10 g/L, KH_2PO_4 10 g/L, $MgSO_4$ 5 g/L, and yeast extract 10 g/L (pH 4.8). The fermentation medium comprised molasses of 12 °Bx or 30 °Brix densities (corresponding to sugar content of approximately 70 or 170 g/L, respectively), $(NH_4)_2SO_4$ 10 g/L, and KH_2PO_4 10 g/L (pH 5.6). All media were sterilized by autoclaving at 116 °C for 20 min prior to use.

2.2. Reactor and immobilization

Taken hexadecylpyridinium bromide as a template agent, MCM-41 zeolite was synthesized in HCl medium, and the molar ratio was TEOS:CPTE:CPBr:HCl:H_2O = 1:0.1:0.3:6:120. The mixture was crystallized at a temperature of 50 °C for 24 h, and following filtration, the solid was refluxed in EtOH/HCl (150 mL/g solid) for 2 h. This process was repeated 3 times, and the solid was vacuumly dried at 80 °C for 12 h; the product obtained was designated as MCM-41 zeolite.

To functionalize micro- and mesoporous zeolites such as ZSM-5, H-β, H-Y and MCM-41 with an amino-functional group, 5 g of a single type of zeolite, any of ZSM-5, H-β, H-Y or MCM-41, was heated for 3 h at 250 °C in vacuum. This calcined and activated solid was placed in 200 mL of anhydrous toluene and stirred for 30 min; then, a suspension of APTES (4.56 g) and calcined zeolite was heated to reflux with stirring in an inert atmosphere for 24 h. After cooling to 25–30 °C and filtering with dry toluene and diethyl ether, the resulting mass was subjected to Soxhlet extraction with dry dichloromethane for 24 h. Finally, the resulting solids were dried at 50–55 °C in vacuum for 8 h. This processed zeolite was marked as H_2NZSM-5, H_2NH-β, H_2NH-Y or H_2NMCM-41, separately, based on the type of zeolite used initially.

Then 2 g of alginate was dissolved in 100 mL of sterile water in which 1.2 g of one type of inorganic filler had been added. The mixture was stirred and blended with 4 g of dry yeast cells, which were initially cultivated over a sterile growth medium at 30 °C in shaken flasks. Sterile water was added to the mixture to make the volume up to 200 mL. Then, 0.01% of the total volume of double oxirane was added for cross-linking, and the mixture thus obtained was trickled down into 4% calcium chloride solution with continuous stirring (80 rpm) for 4 h to induce granulation. The immobilized yeast of alginate-based H_2NMCM-41 mesoporous zeolite compound carrier was marked by the abbreviated term 'alginate-based MCM-41 immobilized yeast'. Immobilized yeasts prepared from other inorganic fillers were denoted by the same method.

2.3. Pre-cultivation and ethanol production from sugar molasses by various methods

The growth of immobilized yeast was investigated by cultivating yeast cells in 500 mL shaken flasks, which contained 200 mL of growth medium and 25 mL of either immobilized yeast or yeast suspension (control sample), on an orbital shaker at 115 rpm and a temperature of 30 °C. The abovementioned systems were allowed to ferment for 10–50 h until the density of the fermented liquids was reduced to a stable value (0–0.5 °Bx). The liquids were then washed twice with sterilized molasses medium and were used for repeated fermentation batches. The initial overall cell concentration in the free cells system was approximately 1×10^7 cells/mL. The immobilized yeast and medium were sampled at timed intervals, and analyzed for cell viability and concentration.

A series of batch fermentations was performed in 500 mL flasks, which contained 200 mL of fermentation medium (12 °Bx) and 25 mL of immobilized yeast with various carriers, on an orbital shaker at 115 rpm and 30 °C for 2–4 days each. The fermented medium was decanted at the end of each batch, and the carriers were washed with fresh molasses medium; then, a fresh medium was added for the next fermentation batch. Samples of the fermented medium were collected and analyzed for ethanol and residual sugar. The initial concentration of each kind of immobilized yeast was approximately 2×10^8 cells/mL. These experiments were carried out in triplicate.

Continuous fermentation was performed in a water-jacketed bioreactor with a working volume of 30 L (including the inoculum). The bioreactor comprised of a plug flow plexiglass tubular column (300 mm i.d., 800 mm height, 5 mm wall thickness) and contained 3.6 L of alginate-based MCM-41 immobilized yeast and 26.4 L of fermentation medium without nutrients (packing ratio is 12 v/v%). The above medium was continuously fed into the lower unit of the bioreactor and fermented at 30 °C. The exhaust gas and effluent solution were removed via an outlet located at the top of the bioreactor. Oxygen was introduced through a glass sparger at the bottom of the reactor every 5 min. The ethanol productivity was calculated as the grams of ethanol per liter of liquid volume produced per hour (g/L/h). The initial concentration of alginate-based MCM-41 immobilized yeast was 2×10^8 cells/mL.