A novel method for bioethanol production using immobilized yeast cells in calcium-alginate films and hybrid composite pervaporation membrane

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ABSTRACT

Fermentation of sugar for production of ethanol was carried out using *Saccharomyces cerevisiae* cells immobilized in calcium alginate films. Thin films of calcium alginate casted on a microchannel surface were used instead of the typical spherical bead configuration. Yeast immobilized on alginate films produced a higher ethanol yield than free yeast cells under the same fermentation conditions. Also, a silicalite-1/poly dimethyl siloxane composite pervaporation membrane was synthesized for ethanol separation, and characterized with flux and separation factor. The composite membrane synthesized with a 3–1 ratio of silicalite-1 to poly dimethyl siloxane showed promising results, with a flux of 140.6 g/m^2 h $\pm$ 19.3 and a separation factor of 37.52 $\pm$ 3.55. Thus, the performance of both the alginate film with immobilized cells and the customized hybrid membrane suggests they could have an interesting potential application in an integrated reaction-separation device for the production and purification of bioethanol.

1. Introduction

There is an increasing concern for the long-term supply of fossil fuels, and especially on the impacts that burning these fossil fuels have on the environment, as seen by the regulations of the Kyoto Protocol, the Paris Agreement and nationwide initiatives to lower the amount of CO_2 that is released into the atmosphere (Gray et al., 2006). One of the most popular and promising renewable fuel alternatives is bioethanol (Hahn-Hagerdal et al., 2006). Kim and Dale (2004) estimated that production of bioethanol from crop residues could replace 32% of the global gasoline consumption. Although ethanol production by alcoholic fermentation of sugars is a known process that has been used extensively, many improvements must be attained to reach an economically competitive status.

Some of the current trends to improve the production of bioethanol deal with new process design configuration such as cell immobilization, as well as process intensification through reaction-separation integration (Cardona, 2007; Lin and Tanaka, 2006).

Immobilization of microorganisms has proved to be a crucial step to achieve better results in fermentation processes. The immobilization can be done by different techniques, using carrageenan entrapping, collagen casting, chitosan/glutaraldehyde molding, and most notably calcium-alginate gel entrapping, which has showed promising results and is one of the most used techniques for immobilization of cells (Najafpour et al., 2004). Calcium alginate offers several advantages as a support, such as good biocompatibility, low cost, availability, and ease of preparation (Duarte et al., 2013). Calcium alginate gels are formed from aqueous sodium alginate solutions in the presence of calcium cations. Sodium alginate is a linear polysaccharide, normally isolated from marine brown seaweed and algae. The copolymer consists of two uronic acids or polyuronic acid. It is composed primarily of D-mannuronic acid (M) and L-glucuronic acid (G). Typically, calcium alginate matrices have been used for immobilization of cells in spherical bead configuration (Schäpper et al., 2009; Behera et al., 2010).

Productivity of the fermentation process can be markedly improved by continuous selective removal of ethanol (Bai et al., 2008; Shabtai et al., 1991). As the formed ethanol is an inhibitor of the fermentation reaction when it surpasses a critical threshold level, continuous separation helps to maintain noninhibitory concentration of ethanol in the reacting media. This separation of ethanol can be achieved using pervaporation membranes (Peng et al., 2011; Feng and Huang, 1997). Separation of ethanol by pervaporation is usually an energy efficient alternative when compared to distillation or adsorption (Sanchez and Cardona, 2008; Wei et al., 2014). Hydrophobic materials can be used for ethanol recovery from broth by pervaporation in dilute ethanol solutions. The hydrophobic membrane leaves water in the retentate whilst allowing ethanol to pass through. As reported by Wei et al. (2014) there are two main classes of hydrophobic pervaporation membranes: polymeric and inorganic membranes. While polymeric membranes are commonly used today for ethanol recovery, inorganic

membranes made from zeolites have increasingly been the focus of research because of their superior performance (Mori and Inaba, 1990; Fan et al., 2015).

In recent years, a considerable effort has been applied to develop pervaporation membranes with high separation performance. A wide range of materials have been explored including polymers (Ong et al., 2016), zeolites (Xue et al., 2015), and inorganic particles such as metal organic frameworks (MOFs), graphene, and carbon nanotubes (Xue et al., 2016a). Among them, organic-inorganic hybrid materials that combine superior permeability and selectivity of inorganic materials and film forming properties of organic materials are preferred to fabricate the next generation of pervaporation membranes. These novel pervaporation membranes have been combined with liquid extraction, adsorption and gas separation processes recover fermentation products (Xue et al., 2017a,b, 2016b, 2016c).

In this work, fermentation of sugar for production of bioethanol was carried out by *Saccharomyces cerevisiae* cells immobilized in a non-conventional calcium alginate matrix. Instead of the typical spherical beads of calcium alginate, the yeast was immobilized in a thin film gel of alginate on a microchannel surface. This alginate film can be easily installed in a fermentation microreactor device for the continuous production of bioethanol. It is expected that the thin calcium alginate film take up little space and reduce the pressure drop during continuous operation when compared to calcium alginate beads configuration (Tan and Li, 2013).

Ethanol yield in batch fermentation with yeast immobilized on calcium alginate films and free yeast cells was compared under the same batch-fermentation conditions. The effect of sodium alginate concentration and calcium chloride concentration on fermentation yield was also evaluated. Additionally, the calcium alginate films with immobilized cells were subjected to invertase activity studies using sucrose as substrate. Furthermore, a silicalite-1/poly dimethyl siloxane composite pervaporation membrane was synthesized for ethanol separation. The influence of the amount of silicalite-1 zeolite on the flux and separation factor was investigated. Overall, the integrated reaction-separation process where ethanol is produced in the calcium alginate films with immobilized cells and simultaneously separated by pervaporation with zeolite composite membrane may contribute greatly to the efficient production of bioethanol.

2. Materials and methods

2.1. Materials and chemicals

D-glucose (99.5%), sucrose, 3,5-dinitrosalicylic acid (DNSA), sodium alginate (99%), ethanol, tetrapropylammonium hydroxide, silica fumed powder (SiO_2), 0.007 µm, and polydimethylsiloxane (PDMS) Sylgard 184® consisting of two components (prepolymer and crosslinker) were purchased from Sigma-Aldrich Co, St Louis, MO, USA. Isooctane (chromatography grade) was from Merck. Meat peptone was obtained from BD Bioxon, Mexico. Yeast extract was from Solbiosa, Monterrey, Mexico. Potato Dextrose Agar (PDA), and *Saccharomyces cerevisiae* cells (Nevada™) were kindly donated by Tecnológico de Monterrey. Potassium phosphate monobasic (KH_2PO_4), ammonium sulphate ($(NH_4)_2SO_4$), and magnesium sulfate heptahydrate $MgSO_4\,7H_2O$, were obtained from Fermont, Monterrey, México. Calcium chloride (96%) was purchased from CTR Scientific, Monterrey, México.

2.2. Preparation of culture medium

Saccharomyces cerevisiae cells were grown in a yeast extract, peptone, and D-glucose media (YPD) containing 1 g of yeast extract, 2 g of meat peptone, and 2 g of D-glucose in 100 mL distilled water. The media was sterilized at 121 °C for 15 min in a Yamato SQ-500 autoclave. At 30 °C, 0.05 g of lyophilized yeast powder was added under sterile conditions. The seed culture of *Saccharomyces cerevisiae* was

grown in a shaking incubator at 30 °C and 150 rpm for 20 h.

2.3. Preparation of calcium alginate films with immobilized Saccharomyces cerevisiae

Aqueous solutions of sodium alginate, and calcium chloride were prepared and autoclaved at 121 °C for 15 min. A culture of *Saccharomyces cerevisiae* was harvested at the exponential growth phase and mixed with sodium alginate solutions. The mixture was dropped into calcium chloride solutions, casted in an acrylic plate with carved microchannels (1 mm deep), and left to harden for 30 min to form calcium alginate films. All the procedures were carried out inside a laminar flow chamber to prevent contamination. The acrylic plate was previously sterilized by submerging in 70% w/w ethanol solution for 15 min, and then placed under UV light.

2.4. Determination of biological activity of cell-enclosing alginate film using sucrose hydrolysis

One gram of the *Saccharomyces cerevisiae* immobilized film was mixed with 5 ml of 2.5% w/w sucrose solution, and placed in a 15-ml Falcon tube. Samples (400 µl) from the Falcon tube were taken into five Eppendorf tubes at 0, 30, 60, 90, and 120 min. Also, 400 µl of DNSA were added to the Eppendorf tube, and placed into boiling water for 5 min, then placed on ice, and finally 1 ml of cold water was added to each tube. All experiments were triplicated. UV–Vis spectrophotometry (DR-5000 from Hach, Colorado, USA) was utilized for measuring the concentration of glucose at 540 nm. Michaelis constant (K_M) of the enzyme was estimated by fitting the experimental results to the Michaelis-Menton model.

2.5. Ethanol fermentation with free and immobilized cells

The films with immobilized cells were added to the fermenters (50 mL Falcon™ tubes, Corning, USA), and mixed with 10 mL of fermentation medium containing 120 g/l of glucose, 2 g/l of potassium phosphate, 1 g/l of ammonium sulphate, and 1 g/l of magnesium sulfate, as carbon, phosphorus, nitrogen and magnesium sources, respectively. Fermenters with free yeast cells (non-immobilized) were prepared to be used as control. All experiments were triplicated, and the agitation rate in the fermenters was 150 rpm. Samples were collected after 1, 2, and 3 h to determine the amount of ethanol produced by fermentation. Ethanol concentration was measured by gas chromatography (Agilent 7820A GC, Agilent Technologies, Santa Clara, CA. USA) equipped with a 122–7032 Agilent Life Sciences DB-WAX column (30 m, 0.25 mm i.d., 0.25 µm film thickness), and a flame ionization detector. Isopropanol was used as an internal standard to measure ethanol content from each fermented sample. Helium was used as carrier gas, with ramping temperature up to 245 °C.

2.6. Preparation of pervaporation membrane

2.6.1. Synthesis of zeolite silicalite-1

Zeolite silicalite-1 was synthesized in our laboratory. The synthesis was based on the work by Jia et al. (1992). Fumed silica (SiO_2), sodium hydroxide, tetrapropylammonium hydroxide (TPAOH), and distilled water were used for the synthesis. A mixture with the molar composition 1/0.244/0.17/11.4 SiO_2/TPAOH/NaOH/H_2O was prepared by mixing TPAOH, water, and sodium hydroxide at room temperature, and then slowly adding SiO_2 powder to the solution under vigorous stirring. The homogeneous dispersion was heated at 80 °C until it became a clear solution. Finally, the clear solution was further heated at 95 °C for 72 h for crystallization to occur. The silicalite-1 crystals were separated from the mother liquor by repeated centrifugation and decantation.