Bioresource Technology Reports 3 (2018) 200–204

Contents lists available at ScienceDirect

Bioresource Technology Reports

journal homepage: www.journals.elsevier.com/bioresource-technology-reports

Ethanol production from coffee mucilage fermentation by *S. cerevisiae* immobilized in calcium-alginate beads

David Orrego[a,b], Arley David Zapata-Zapata[c], Daehwan Kim[d,*]

[a] Laboratory of Renewable Resources Engineering, Purdue University, West Lafayette, IN 47907-2032, United States
[b] Department of Agricultural and Biological Engineering, Purdue University, West Lafayette, IN 47907-2032, United States
[c] Universidad Nacional de Colombia - Sede Medellín, Escuela de Química, Laboratorio de Procesos Biológicos, Carrera 65 # 59A-110, Medellín 050034, Colombia
[d] Department of Biology, Hood College, 401 Rosemont Avenue, Frederick, MD 21701, United States

ARTICLE INFO

Keywords:
Coffee mucilage
Immobilization
Calcium-alginate
Saccharomyces cerevisiae
Ethanol fermentation

ABSTRACT

Soluble sugars in coffee mucilage were directly fermented into ethanol using immobilized *Saccharomyces cerevisiae* cells in calcium alginate beads. Several tests were performed via alginate-entrapped cells in different bead sizes (3 or 7 mm) combined to alginate concentrations (2–4%). The highest yield of 0.33 g ethanol/g sugar was achieved at 18 h using alginate-entrapped cells in 3 mm diameter and 2% (w/v) alginate, which corresponded to 64% of the theoretically achievable yield of ethanol. Tests with 7 mm beads took 6 h longer to reach maximum ethanol productions. The reduction of bead size increased mass transfer of substrates from the liquid to the immobilized cells, accelerating sugar consumption and ethanol production. Entrapment enabled reuse of the cells for up to 3 consecutive batches with stable ethanol production (up to 72 h). This work confirms the reuse of alginate-entrapped cells is feasible for ethanol fermentation of mucilage without supplement of nutrients.

1. Introduction

One of the main challenges in the coffee industry is the generation of residual by-products such as silver-skin, parchment, pulp, pericarp, and mucilage, which are commonly released during coffee bean roasting, preparation, and wet/dry processing (Narasimharao et al., 2008; Mussatto et al., 2012). The green coffee bean is ordinarily extracted for brewed coffee while un-used components are removed and discarded after coffee processing; they account for > 50% of whole coffee fruit weight, estimating a generation of 15 million tons of residues per year, which leads to environmental pollution with toxic chemicals issues (Orrego et al., 2018). With these concerns of rapid rises in coffee consumption, and consequently, waste by-products, research has been devoted to the reuse of coffee waste as a renewable resource for transforming it to value-added molecules and animal feedstock. However, most of industrial by-products are still dumped into ground and water resources owing to an insufficiency of economically attractive and technically suitable methods (Khelil et al., 2016; Murthy and Naidu, 2012).

Coffee mucilage protects the coffee bean with other components of pericarp and silver-skin, and it is mainly derived from coffee fruit and underutilized in the wet process. The abundance of carbohydrates and nitrogen in mucilage, in particular glucose, galactose, and minerals, is thought to be a proper source for hydrogen, lactic acid, and ethanol productions (Hernández et al., 2014; Neu et al., 2016; Pérez-Sariñana et al., 2015a). Our recent study (Orrego et al., 2018) reported an optimization and scale-up of coffee mucilage waste fermentation that enables it to be directly fermented into ethanol, without demanding nutrient supplements and pretreatment processes both in the flasks and 5 L bio-reactors.

Cell immobilization, which is considered as a promising technology that could be applied to diverse agricultural biomass and lignocellulosic feedstock, has several advantages over free cell processes, for instance, simple separation of products in the medium, less inhibition by substrate or end product, cost effectiveness, and reductions of cell growth (lag phase) and reaction times (Duarte et al., 2013; Kim, 2018; Razmovski and Vučurović, 2011). Different types of carriers, with their chemical structures/physical properties and advantages, have been investigated and proposed for immobilized yeast cells, which include artificial materials (glass and silicone), gels (pectinates, polyacrylamide, and alginates), and natural sources (cellulose, wood, porous grass, and fruit peel) (Plessas et al., 2007; Santos et al., 2018; Shen et al., 2004; Tara et al., 1999). Calcium alginate is one of the most utilized materials for immobilization of microorganisms that offers simple preparation, low cost, and biocompatibility (Santos et al., 2018). Dissolved sodium alginate is a polysaccharide widely found in the cell

* Corresponding author.
E-mail address: kimd@hood.edu (D. Kim).

https://doi.org/10.1016/j.biteb.2018.08.006
Received 19 July 2018; Accepted 12 August 2018
Available online 20 August 2018

walls of brown seaweed, and it plays a key role in alginate gel formations with the calcium cations. The copolymers of β-D-mannuronate (M) and α-L-guluronate (G) link together in different sequences and help calcium alginate gels to maintain stable in spherical bead structure (Santos et al., 2018; Schäpper et al., 2009). However, to the best of our knowledge there is no literature regarding immobilized cells for serial alcoholic fermentations of coffee mucilage waste. The current manuscript addresses the potential application of cell immobilization supporting practical utilization of industrial waste and contributing toward a comprehensive enhancement of the mucilage ethanol production by recyclable systems. The main objective of this study is to determine the best condition of immobilized yeast cells for continuous fermentations, which are entrapped in beads with different diameters and calcium alginate concentrations. Furthermore, we tested and compared the sustainability of beads and whether stable ethanol production is available from each test. This work expands the knowledge on the effects of sphere diameter and alginate concentration on immobilized cells to effectively convert soluble sugars into ethanol.

2. Materials and methods

2.1. Raw material

Coffee mucilage from the San Rafael farm (Envigado, Colombia) was autoclaved at 121 °C for 15 min and sieved through a 20 mesh screen (0.84 mm, Tyler USA standard testing sieve, VWR, Philadelphia, PA, USA). The sterilized and mesh-sieved liquid was centrifuged at 8000 rpm for 5 min to remove solids, and the pH was adjusted to 4.0 using NaOH. Concentrations of glucose, galactose, lactose, and acetic acid in the prepared mucilage liquid were 37.1 $g\,L^{-1}$, 14.7 $g\,L^{-1}$, 0.8 $g\,L^{-1}$, and 1.2 $g\,L^{-1}$, respectively (Orrego et al., 2018). All chemicals, including sodium alginate and calcium chloride, were purchased from Sigma Aldrich (St. Louis, MO).

2.2. Microorganism and culture condition

S. cerevisiae NRRL Y-1546 was maintained in accordance with methods as described previously (Kim et al., 2016). Briefly, 1 ml of stock solution was inoculated in 250 ml Erlenmeyer flasks containing 100 ml of YEPD medium (10 g/l yeast extract, 20 g/l peptone, 20 g/l glucose) and cultured overnight in a shaking incubator set at 28 °C and 200 rpm. The cells were separated by centrifugation (5000 rpm, 5 min), re-suspended in 10 ml YEP (no glucose) medium, and this cell preparation was used for free cells fermentation and immobilization in calcium-alginate beads (containing 3 g dry cells/l).

2.3. Cells immobilization in calcium alginate beads

Sodium alginate powder was dissolved in distilled water at 2%, 3%, and 4% (w/v), and sterilized at 121 °C for 15 min. The prepared cells described in the previous section were added to 10 ml of each alginate solution and gently mixed for 15 min. The alginate-cell mixture solution was dripped dropwise into a 150 ml of 2% (w/v) calcium chloride solution through pipette tips with 3 or 7 mm diameters (Duarte et al., 2013). Each bead was sealed and hardened in this solution for 15 h, and then washed with distilled water prior to fermentation tests. Immobilization in alginate concentration below 2% (w/v) was technically challenging for continuous operations of bead separation and fermentation process because of the possibility of bead rupture. Thus, a range of 2–4% (w/v) alginate was selected and used for a continuous reuse of entrapped cells.

2.4. Sequential batch fermentation of coffee mucilage

For the fermentation tests, 100 ml of mucilage medium obtained from the previous section was inoculated with alginate-entrapped cells at an initial concentration of 3 g dry cells/l in 250 ml flasks that were covered with plastic stoppers, allowing a micro-aerobic condition. The prepared flasks were moved to an orbital shaker at 28 °C with agitation of 150 rpm, and incubated for 24 h. Control runs were performed with free cells at the same experimental concentrations. To investigate the reuse of immobilized cells, the beads were recovered through a vacuum filter, washed with sterile distilled water, and were immediately added to a fresh mucilage medium for a consecutive fermentation. The continuous batch tests were reiterated 3 times for a total fermentation time of 72 h. Samples were taken at time intervals during fermentations and stored at −4 °C for later analysis. All tests were repeated in triplicate.

2.5. Analysis

The concentrations of sugars, acetate, and ethanol in mucilage medium prior to fermentation and collected samples during fermentation were analyzed by high-performance liquid chromatography as described in previous works (Cao et al., 2015; Kim et al., 2017) using a HPLC system equipped with an Aminex HPX-87H ion exchange column (Bio-Rad Laboratories Inc., Hercules, CA). Sugar consumption (glucose plus galactose) was evaluated as a first order correlation (Michelin et al., 2016): $\ln(S_c/S_{co}) = -t$ where S_{co} = initial sugar concentration at $t = 0$; S_c = sugar concentration after an elapsed fermentation time, t; and t is time in hours. The cell concentration was determined before immobilization and after fermentation performance by spectrophotometric assay at 600 nm. The final cell density after fermentation was determined in the liquid phase for both free and immobilized cells. Statistical analysis of the *t*-test was conducted by the Minitab 16 software program, with 95% significance differences, for sugar consumption of different bead sizes and alginate concentrations, respectively.

3. Results and discussion

In order to follow up on our previous work with mucilage fermentation at the optimal condition (Orrego et al., 2018), with the aim to convert sugars to ethanol in recyclable systems with alginate-entrapped cells, all fermentation runs were consecutively carried out at 28 °C (pH 4.0) with an initial cell concentration of 3.0 dry cell $g\,L^{-1}$ in a shaking incubator. The extensive data analyses of sugar consumption, ethanol productivity, and appropriate times for beads' reuse also were evaluated and compared to the results from free cells or with different conditions of alginate beads.

3.1. Effect of sphere size and alginate concentration on mucilage fermentation

Entrapped cells of different sphere diameters and alginate concentrations have impacts on the mucilage fermentations. The reduction of alginate concentration from 4% (w/v) to 2% (w/v) was slightly effective for sugar utilization in the immobilized cells both in 3 and 7 mm beads; however, the use of a smaller size gave a dominant effect on sugar consumption (Fig. 1A). As shown in Fig. 1B–D, 3 mm beads size clearly presented a faster sugar consumption than those results from 7 mm. The highest consumption was observed with 2% (w/v) alginate (Fig. 1A and B), which was totally completed in 18 h while only 75% of sugars were utilized with 7 mm and 4% (w/v) alginate beads that delayed completion time for 6 h (Fig. 1B and D). It might be assumed that the smaller bead size could be closely filled with yeast cells due to the volume capacity and sphere diameter that are capable of increasing accessibilities of immobilized cells into the substrates during the fermentation. Moreover, the thin layer alginate films provide the high porosity with the surface structure and densely packed cells near the surface of alginate beads, and thus contribute toward the rapid substrates' depletion. Similar studies showed that using yeast cells immobilized in maize stem ground tissue walls, thickly alginate-entrapped cells in beads (3 mm average diameter), were able to homogeneously