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New generation biofuel from whey: Successive acidogenesis and alcoholic fermentation using immobilized cultures on γ-alumina

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ABSTRACT

Cheese whey exploitation in a biorefinery manner is proposed involving anaerobic acidogenesis by a UASB mixed anaerobic culture and alcoholic fermentation by kefir. Both cultures were immobilized on γ-alumina. The produced organic acids (OAs) and ethanol could be esterified to obtain a novel ester-based biofuel similar to biodiesel. During acidogenesis, lactic acid-type fermentation occurred leading to 12 g L^{-1} total OAs and 0.2 g L^{-1} ethanol. The fermented substrate was subsequently supplied to a second bioreactor with immobilized kefir, which increased the OAs content (15 g L^{-1}), especially lactic acid, and slightly the ethanol concentration (0.3–0.4 g L^{-1}). To further increase ethanol concentration, a second experiment was conducted supplying whey firstly to the immobilized kefir bioreactor and then pumping the effluent into the acidogenesis bioreactor, resulting in 40% increase of OAs and 10-fold higher ethanol content. The residual sugar was ~50% of the initial whey lactose; consequently, future research could result to further increase of ethanol and OAs.

1. Introduction

Numerous studies have proposed ways for treatment prior to disposal or exploitation of whey, the highly polluting liquid waste of the dairy industries. Most of them have focused on the production of added value chemicals such as ethanol [1], butanol [2], glycerol [3], hydrogen, methane and biogas [4–6], and lactic acid [7,8]. Furthermore, whey has been used as substrate for food production such as probiotic starter cultures [9], whey cheeses [10], SCP as livestock feed [11], and baker's yeast [12]. However, large volumes of whey remain untreated, and are usually discarded into the environment.

Recent research efforts have also focused on the production of organic acids (OAs) through acidogenic bioprocesses. Ren et al. [13] reported that the acidogenesis of glucose using mixed anaerobic bacteria cultures, can lead to simultaneous production of small chain OAs and ethanol. These molecules could be subsequently esterified to obtain a new generation biofuel, or alternatively, OAs can be used for other chemical applications. Therefore, the simultaneous OAs and ethanol production approach could reduce the problem of cheese whey disposal. The use of such esters, produced by esterification of OAs with ethanol or another alcohol, in a homogeneous charge compression ignition engine gave promising results for the use of such alternative liquid biofuels [14]. In recent studies the use of γ-alumina, as culture immobilization carrier promoted the anaerobic acidogenic fermentation of glucose, leading to increased concentrations and productivities of ethanol and OAs [15,16]. γ-Alumina was also found to act as promoter in methane [17] and alcoholic [18] fermentations. The ethanol produced during the process is important since it can be used as reagent for the esterification of OAs into ethyl esters for use as fuel molecules. Although, during acidogenesis of glucose ethanol-type fermentation predominated [13] and the ethanol concentration in the fermented medium was high, using vinasse (alcohol distillery waste) as substrate, the ethanol formation dropped significantly [19,20]. Based on the above observations, the aim of this investigation was to increase the ethanol produced simultaneously with the OAs by combined acidogenic and alcoholic fermentations of whey.

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2. Materials and methods

2.1. Culture and media

Mixed bacteria anaerobic culture (sludge) was obtained from an UASB reactor and was grown at 37 °C in lactose media containing: 40 g L^{-1} lactose, NH_3 in aqueous solution and 0.5 kg L^{-1} H_3PO_4 at a COD:N:P ratio of 100:5:1, 4 g L^{-1} $NaHCO_3$, and 4 g L^{-1} yeast extract, without pH adjustment [15,16].

The cheese whey used for all fermentation experiments was obtained from the local dairy industry AVIGAL S.A. (Valmantoura, Achaia, Greece), and was the liquid that remained after the production of Feta cheese and after removal of whey proteins by thermal coagulation for whey cheese production. It contained about 50 g kg^{-1} lactose, 8 g kg^{-1} proteins, and had pH 6.5.

For kefir growth, 20 mL of commercial kefir beverage were inoculated in 400 mL of pasteurized full cream milk containing 8 g yeast extract and 2 g urea. The milk mixture was incubated at 37 °C in a water bath for 24 h. After incubation, 5 mL of the milk mixture was inoculated in 1 L lactose medium (40 g L^{-1}). All media were sterilized by autoclaving at 120 °C for 10 min.

The produced cultures were harvested at 5000 rpm for 8–10 min on a Sigma 3K12 centrifuge (Bioblock Scientific) and were immobilized on γ-alumina (Al_2O_3), which is a porous cylindrical material having a surface area of 1.40 m^2 g^{-1} [15,17], as described below.

2.2. Continuous acidogenic fermentation of whey followed by alcoholic fermentation

A 1.2 L glass tower bioreactor was filled with 640 g of γ-alumina pellets and 640 mL of cheese whey containing 20 g of the mixed anaerobic culture. The system was incubated at 37 °C to allow cell immobilization to occur by natural adsorption and entrapment [21]. A second bioreactor of 1 L was filled with 500 g of γ-alumina pellets and 500 mL of lactose synthetic medium containing 17 g of dispersed kefir biomass and the mixture was incubated at 37 °C for 24 h for cell immobilization.

The whole system (two bioreactors connected in series) was operated in continuous mode at 37 °C (Fig. 1A) for 50 days. Specifically, whey was firstly supplied into the 1st bioreactor containing the immobilized mixed anaerobic culture and the effluent was supplied to the 2nd bioreactor containing the immobilized kefir cells. The flow rate of the influent in both bioreactors was adjusted so that a total volume of 1 L was pumped in 5 days.

2.3. Continuous alcoholic fermentation of whey followed by acidogenic fermentation

The experiment was set up as described in Section 2.2., but the bioreactors were used in reverse order (Fig. 1B). Whey was initially supplied into the immobilized kefir bioreactor and then the fermented effluent was pumped into the acidogenesis bioreactor.

2.4. Ethanol determination

Ethanol was determined by gas chromatography on a Shimadzu GC-8A system equipped with a Teknokroma HAYE SEP Q 80/100 column, a C-R6A Chromatopack integrator, He as carrier gas (40 mL min^{-1}), and a FID detector. Injection port and detector temperature was 210 °C. The column temperature was 130 °C. Samples of 2 μL were injected directly into the column. Determinations were done by means of standard curves.

2.5. OAs and residual sugar determination

Residual sugar was determined by HPLC on a Shimadzu chromatograph with a SCR-101N stainless steel column, a LC-9A pump, a CTO-10A oven at 30 °C and a RID- 6A detector. The mobile phase was 0.784 kg mL^{-1} H_2SO_4 at a flow rate of 0.8 mL min^{-1} and propanol-1 was used as internal standard. A volume of 0.25 mL of sample and 0.625 mL of a 10 mL L^{-1} solution of propanol-1 were diluted to 25 mL. Then, 60 μL of the final solution were injected directly into the column. Residual sugar concentrations were calculated using standard curves. The sugar conversion after each fermentation process was calculated by the following equation: Sugar conversion % = (initial sugar conc. – residual sugar conc.)/initial sugar conc. × 100.

OAs were also determined by HPLC on a Jasco LC-2000 Plus chromatograph with a CrestPak C 185 column (150 mm × 46 mm i.d.), a PU-2080 pump, a Jasco CO-2060 Plus oven set at 50 °C, a UV/VIS detector model UV-2075 and an autosampler AS-2057. The mobile phase was 0.784 kg mL^{-1} H_2SO_4 at a flow rate of 0.6 mL min^{-1}. The samples were filtered by a membrane filter of 0.45 nm pore size. All data were processed with ChromNav program. The OAs yield factors were calculated as g of OA produced per g of sugar utilized (g g^{-1}).

3. Results and discussion

3.1. Process rationale and experimental set-up

Research efforts are currently in progress for the production of OAs simultaneously with ethanol. These products could be used as

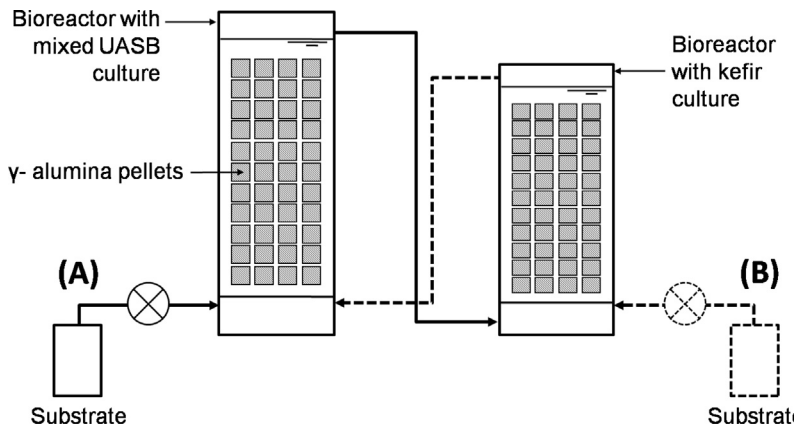

Fig. 1. Continuous process (A) for acidogenesis of whey followed by alcoholic fermentation, and (B) for alcoholic fermentation of whey followed by acidogenesis.