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Study of microbiological and operational parameters in thermophilic syntrophic degradation of volatile fatty acids in an upflow anaerobic sludge blanket reactor

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ABSTRACT

Volatile fatty acids (VFAs) are critical intermediates in anaerobic digestion. Temperature affects thermodynamics of syntrophic digestion of VFAs as well as solubilities and diffusion coefficients of intermediate metabolites. In this research interactive effects of propionic (HPr), butyric (HBu) and acetic (HAc) acids, hydraulic retention time (HRT) and methanogen to acetogen population ratios (M/A) were investigated as major microbiological and operating variables in syntrophic reactions at thermophilic temperature (55 °C). Experiments were carried out in upflow anaerobic sludge blanket (UASB) reactor inoculated with enriched acetogenic and methanogenic cultures. Central composite design (CCD) was used to design experiments and results were analyzed using response surface methodology (RSM). Corresponding to maximum VFA removals and biogas production rate (BPR), optimum conditions were found to be HPr = 1.9 g/L, HBu = 2.2 g/L, HAc = 2.5 g/L, HRT = 22 h and M/A = 2.5. Results of verification experiments and predicted values from fitted correlations were in close agreement at 95% confidence interval. Analysis of the results of thermophilic process showed that trends and interactive effects of different parameters as well as its optimum conditions were very similar and comparable with the previous study at mesophilic temperature (37 °C).

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Introduction

Among the four steps (hydrolysis, acidogenesis, acetogenesis and methanogenesis) of anaerobic digestion process, acetogenesis and methanogenesis from the viewpoint of thermodynamic and microbiology are the critical steps [1]. The acetogens (HPr- and HBu-degrading bacteria) and methanogens (hydrogenotrophs and aceticlasts) in a close relationship form a special interrelated connection called "syntrophic interaction" [2]. Because of the thermodynamic limitations, anaerobic oxidations of HPr and HBu are considered as the rate-limiting steps in the anaerobic digestion [3]. The anaerobic degradation reactions of HPr and HBu to HAc, CO_2 and H_2 is highly endergonic (ΔG°_{HPr} = +62.3 and ΔG°_{HBu} = +37.9 kJ/mol both at 55 °C) and does not occur in nature. Practically the acetogenic reactions can be carried out during the syntrophic collaboration of HPr- and HBu-oxidizing bacteria and H_2/formate-consuming colleagues [2].

Operation of anaerobic digesters usually occurs at mesophilic temperatures; however, anaerobic digestion in a thermophilic range of temperatures offers several potential advantages, such as an increase of reaction rates, an increase of efficiency (fraction of organic solids destroyed), an improvement of solid–liquid separation and an increase of the elimination of pathogenic organisms. One of the major advantages of thermophilic digestion is the ability to operate the digester under autothermal conditions. The biogas produced can be used as a heat source for the digester and to generate power [4]. Moreover, temperature affects the thermodynamics of syntrophic HPr and HBu oxidation [3]. At elevated temperatures, the thermodynamics for acetogenic conversions are more favorable. Using van't Hoff equation it can be calculated that hydrogen formation becomes energetically more favorable at higher temperatures, whereas hydrogen consumption by the methanogens becomes less favorable [3,4]. In addition, temperature affects the flux of hydrogen and formate transferred between the acetogens and the methanogens via diffusion constants and their solubilities. Because diffusion coefficients under thermophilic conditions become higher and the diffusion gradient becomes

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steeper, it can be expected that HPr and HBu conversion rates are higher at elevated temperatures [3]. In the other hand, the yield of microorganisms per unit amount of substrate for thermophilic temperature is also lower. The lower growth yield of thermophilic anaerobes could be due to their increased decay rate, which is double that of mesophilic cultures, because the cells have a tendency to lyse quickly under thermophilic conditions; it may also be due to their higher energy requirement for maintenance or the specific molecular properties of enzymatic reactions at thermophilic temperature [5]. However, there have been few reports done on the anaerobic syntrophic reactions under thermophilic conditions.

Creation of structured ordered microbial agglomerates resembling the granules decreases the distance between acetogenic and methanogenic microorganisms and minimize resistances to efficient mass transfer of metabolites [3]. UASB processes are based on the growth of granules formed by the normal self-immobilization of the anaerobic microorganisms [6]. As well, the very fine construction let close microbial consortia closeness, most favorable interspecies distances for syntrophic metabolite transfer and diffusion limitations supply stability to process shocks and toxins [7].

Recent studies on anaerobic syntrophic digestion have mainly focused on co-cultivation of acetogens and methanogens [8–10]. In the previous works, interactive effects of operating and microbiological parameters on the syntrophic digestion of VFAs in different cultures (suspended, fixed-bed and granular sludge) at mesophilic conditions were investigated [11–14]. However, thermophilic anaerobic syntrophic digestion of VFAs studies (acetogenesis and methanogenesis) has not yet been reported in the literature and also, key dissimilarities between the performances of anaerobic syntrophic mesophilic and thermophilic temperatures have not still recognized, precisely. Although the performances of the mesophilic and thermophilic anaerobic digestions of difference industrial wastes were studied, previously [15–17], the syntrophic processes have not studied alone, yet. Therefore, the main objective of this research was to investigate, analyze and model the process of VFA syntrophic anaerobic degradation at thermophilic temperature (55 °C) and comparison of the results with the previous work at mesophilic condition (37 °C) [14] in a UASB reactor with granular cultures. This analysis aims to find important parameters in anaerobic digestion of VFAs and identify their interactions at thermophilic temperature.

In this study, the RSM, like the previous work at mesophilic condition in the UASB reactor [14] was used to analyze and model the process with respect to the simultaneous effects of five microbiological and operating variables (HPr, HBu, HAc, M/A and HRT), and four parameters (effluent concentrations of HAc, HPr, HBu and BPR) were assessed as responses. The significant factors and a continuous response surface of the main parameters were developed to yield an optimal region that satisfies the process specifications.

Materials and methods

Inocula

Enriched syntrophic bacteria (propionate- and butyrate-degrading bacteria) and methanogens (hydrogenotrophs) and acetate-oxidizing syntrophs cultures were used as the inocula. Syntrophic bacteria, acetate-oxidizing syntrophs and methanogens were enriched from granular sludge (pH 7.4; volatile suspended solid (VSS), 67.2 g/L; total suspended solid (TSS), 92.4 g/L) from a dairy wastewater UASB reactor. The concentrations of the propionate-degrading bacteria in the enriched cultures were VSS = 68.4 g/L and TSS = 78.2 g/L. For butyrate-degrading bacteria, the concentrations were VSS = 71.2 g/L and TSS = 81.3 g/L; and for acetate-oxidizing syntrophs and hydrogenotrophs, they were VSS = 74.3 g/L and TSS = 84.5 g/L.

Synthetic wastewater

Propionate, butyrate and acetate (99%, Merck, Germany) were diluted in tap water to achieve synthetic wastewater with desired chemical oxygen demand (COD) levels (low, 5.0 mg COD/L to high, 12.0 g COD/L). The COD:N:P ratio was maintained at 100:4:1 by adding $NaNO_3$ and KH_2PO_4 as nitrogen and phosphorus sources, respectively. Oxygen was removed by N_2 sparging for 10 min before feeding to the bioreactor and a balloon containing the N_2 gas was placed on the feed reservoir to prevent oxygen entering into the feed vessel. The pH of the feed and within the reactor was not regulated throughout the experiment and was 4.5–5.5, corresponding to high and low loads, respectively. To increase the alkalinity, 4 g of $NaHCO_3$ was added per 1 L of feed.

The UASB reactor and operating conditions

The UASB reactor was a glass cylinder with a diameter of 100 mm, a height of 250 mm, and 2 L working volume (Fig. 1). At start up, the reactor was seeded by adding 400 mL of the enriched methanogenic and acetogenic cultures, which was taken as their volatile suspended solid (VSS) concentrations (with defined M/A ratio; 1–3 g VSS of enriched methanogenic sludge to g VSS of enriched acetogenic sludge). During the experiments temperature was maintained at 55 ± 1 °C. After addition of the mixture of enriched cultures (inoculation), acclimation and growth of microorganisms in the reactor were accomplished and granules were formed gradually. Following about 6 weeks in each M/A ratio granules completely formed and the experiments were started. All other operating conditions such as primary factors, process responses, method of design of experiments (central composite design (CCD)) were similar to the mesophilic process [14]. The levels of the factors are shown in Table 1.

Analytical methods

CH_4 content in biogas were determined with a model TGS 2611 methane sensor (FIGARO, USA). Analyses of liquid reactor samples were conducted after centrifugation at $12{,}000 \times g$ for 15 min and for acidification of the supernatant 500 μL of 1.0 N HCl was added to the samples. Propionate, acetate and butyrate were quantified using a model 7890 gas chromatograph (Agilent, USA) equipped with an auto-injector (7683 B series), a flame ionization detector (FID; H2 flow rate: 35 mL/min, airflow rate: 350 mL/min) and a Chrompack Cp-Wax 52 CB fused-silica column (25 m × 0.32 mm i.d. and 0.2 μm film thickness). The injector and detector temperatures were maintained at 240 and 280 °C, respectively. Helium (He) was used as the carrier gas at a flow rate of 3 mL/min and makeup flow rate of 5 mL/min. The oven temperature was programmed at 40 °C for 4 min, raised to 180 °C at 30 °C/min, and then held at 180 °C for 1 min. Determinations of VSS, TSS, COD and pH were made using standard methods [18]. pH was measured using a model 620 pH meter (Metrohm, Germany).

Results and discussion

Statistical analysis

The variable ranges for all experiments and acquired responses for thermophilic anaerobic syntrophic reactions are presented in Table 2. Forty-seven experiments were designed using CCD. The predicted responses were determined by adequate quadratic

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