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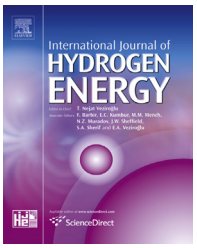

Significance of acclimatization for biohydrogen production from synthetic lignocellulose hydrolysate in continuous-flow systems

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ABSTRACT

This study investigated the response of acclimatized anaerobic hydrogen-producing cultures to feed changes in continuous-flow systems. Two identical integrated biohydrogen reactor clarifier systems (IBRCS) which were used for biohydrogen production from glucose at a concentration of 10 g/L (phase 1) and then switched to a mixture of xylose, arabinose, glucose, and cellobiose at a concentration of 2.5 g/L each (phase 2) prior to reverting to glucose at a concentration of 10 g/L (phase 3) exhibited hydrogen yields in phases 1, 2, and 3 of 2.3, 1.1, and 1.0 mol H_2/mol sugar, respectively. Acetate was the main soluble product in phase 1 while propionate was predominant in phases 2 and 3. The genus *Ethanoligenes*, *Clostridium*, *Bulleidia*, and *Ruminococcus* were dominant in phase 1, while *Coriobacteriaceae* family and genera *Megasphaera* and *Bifidobacterium* were dominant in phases 2 and 3, in addition to *Ethanoligenes* in phase 3 in both reactors.

Introduction

The depletion of fossil fuels and the need for environmentally friendly energy is crucial. Among the recognized alternatives to fossil fuel, hydrogen is considered as a clean energy carrier due to its high energy content (142 kJ g^{-1}) [1]. In addition to its fuel potential, hydrogen can be used in a variety of processes for manufacturing chemicals, semiconductors, and fertilizers [2].

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Lignocellulosic substances are the most plentiful raw materials in nature [3]. Lignocellulosic material residues including pulp-and-paper wastes, food processing wastes, wheat-straw or rice-straw, corn stovers, and sugar cane bagasse [4] are produced at an annual rate of $8.15 * 10^7$ tonnes worldwide [5]. All lignocellulosic materials that basically consist of 35%–45% cellulose (a polymer of glucose), 25%–40% hemicellulose (heteropolymer of hexose and pentose) and 20%–35% lignin (an aromatic organic compound) are often hydrolyzed by acid treatment. The hydrolysate can be utilized for biological hydrogen production [3] as well as bioethanol production through dark anaerobic fermentation [6]. Because lignocellulose hydrolysates contain not only glucose, but also various monosaccharides, such as xylose and arabinose [7], as well as disaccharides, such as cellobiose [8], microorganisms can efficiently ferment these sugars for biohydrogen production. Dark fermentative hydrogen production using carbohydrates [3,9,10] and lignocellulosic wastes [5,11,12] by various pure and mixed cultures has been extensively studied.

Acclimatization of anaerobic digester sludge is essential to increase the biohydrogen production potential [9,13]. Furthermore, in continuous-flow systems, aversion of washout of hydrogen producing bacteria is crucial for sustained successful operation. In continuous-flow systems, with pH control, the microorganisms that adapt to the ambient conditions can continue to proliferate in the reactor, whereas other microbial groups with an insufficient growth rate at the ambient operational conditions i.e. hydraulic retention time (HRT), temperature, pH, and substrate concentrations in the bioreactor, would be washed out [12]. The impact of acclimatization for biohydrogen production is scantly discussed in the literature. For example, Kim & Kim [14] studied thermophilic fermentative hydrogen production using acclimatized mixed anaerobic culture. Acclimatization was conducted using mesophilic anaerobic digester sludge in a CSTR operated in a batch mode to avert biomass washout at a pH of 5.5 and 60 °C for 5 days using glucose as a substrate at a concentration of 10 g COD/L, after which the system was switched to a continuous-flow mode using acid-hydrolysed tofu (soybeans) processing wastewater at a concentration of 11.5 g sugar/L. A maximum hydrogen yield of 1.78 mol H_2/mol sugar added and hydrogen production rate of 5.1 L H_2/L/d was obtained. Hafez et al. [15] studied biohydrogen production in a continuous-flow system using acclimatized mesophilic sludge enriched primarily on glucose, prior to switching to corn-syrup waste, achieving a hydrogen production yield of 2.86 mol H_2/mol sugar, in close agreement with the 2.8–3.1 mol H_2/mol sugar observed with glucose. Temudo et al. [4] studied hydrogen production using a mesophilic anaerobic mixed culture as inoculum, consisting of a mixture of two types of biomass, obtained from two different sources, a distillery wastewater treatment plant and a sludge from a potato starch processing in a CSTR at a pH of 8 and 30 °C and initial xylose concentration of 4 g/L, and obtained a yield of 0.65 mol H_2/mol xylose; however the aforementioned microbial culture exhibited a sharp drop in performance during co-fermentation of xylose and glucose at a concentration of 2 g/L each. The aforementioned authors attributed the marked deterioration in substrate conversion to the sensitivity of the selected population to the substrate concentration and/or increased product concentration [4]. One possible explanation is that the unexpected changes in the operating parameters such as an alteration of substrate and substrate concentration variation [11,16] may have resulted in an imbalance in the fermentation process. Another explanation is that the other hydrogen producers may have been washed out during enrichment of the culture using xylose in the first phase. On the other hand, Haroun et al. [17] studied biohydrogen production from xylose and glucose individually in continuous-flow systems at an HRT of 8 h and an influent concentration of 10 g/L at mesophilic temperature and a pH of 5.5 using acclimatized anaerobic digester sludge with gradual increase in influent furfural concentrations of 0, 0.25, 0.5, 1, 2, and 4 g/L. The aforementioned authors reported that the acclimatized culture was not inhibited by furfural concentrations up to 1 g/L.

Notwithstanding the sparsity of the literature studies, the novelty of this work stems directly from the lack of research on the impact of feed changes on acclimatized microbial cultures for biohydrogen production in continuous-flow systems, with a focus on detailed microbial characterization to delineate microbial community changes. Previous studies lack information on the impact of the alteration of the influent readily biodegradable substrates on acclimatized anaerobic mixed culture in continuous-flow system for biohydrogen production, and focused only on non-acclimatized ones in batches. The focus of the very limited continuous-flow biohydrogen studies in the literature [4,11,14,17] as expected has been on steady-state conditions, with very superficial discussion of acclimatization.

Due to the significance of acclimatization in continuous-flow systems, the main objective of the present study was to evaluate the impact of feed changes to the acclimatized sludge on hydrogen production rate, yield, and soluble by-products. Additionally, detailed microbial characterization was undertaken to delineate microbial community changes.

Materials and methods

Seed sludge

Anaerobic digester sludge (ADS) was collected from St. Mary's wastewater treatment plant (St. Mary's, Ontario, Canada) and preheated at 70 °C for 30 min [17,18] prior to inoculation of the integrated bioreactor clarifier system (IBRCS). The total suspended solids (TSS) and volatile suspended solids (VSS) concentrations of the ADS were 13.4 and 8.9 g/L, respectively.

Systems set up and operation

Two IBRCSs (Fig. 1), R1 and R2, were operated as duplicates for biological hydrogen production at 37 °C for 40 days, at organic loading rates (OLR) of 32.1 gCOD/L/d and hydraulic retention time (HRT) of 8 h (Table 1). The IBRCSs comprised a continuously stirred reactor (CSTR) for biological hydrogen production (7 L working volume), followed by an uncovered gravity settler (volume 8 L) for the decoupling of solids retention time (SRTs) from the HRT. Water was recirculated through a water jacket to maintain a constant temperature of 37 ± 1 °C. Nitrogen gas was initially purged in the head space for 4–5 min at 65 psi in

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