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Optimal operational conditions for biohydrogen production from sugar refinery wastewater in an ASBR

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ABSTRACT

The performance of biohydrogen production in an anaerobic sequencing batch reactor (ASBR) was evaluated with respect to variations in the key operational parameters – pH, hydraulic retention time HRT, and organic loading rate OLR using sugar refinery wastewater as substrate. Analysis of variance (ANOVA) indicated HRT had less significant influence on hydrogen content and yield in comparison to pH and OLR, whereas OLR has much impact on hydrogen production rate. Taxonomic analysis results showed that diverse bacterial species contributed to hydrogen production and the dominant species in the bioreactor were governed by all operational parameters. Even without pretreatment of the seed sludge, a high proportion of *Clostridium* spp. over the other bacterial species was observed at pH 5.5, and this is compatible with the high hydrogen productivity. Consequently, pH 5.5, HRT 10 h, and OLR 15 kg/m^3 d were delineated as the optimal operational conditions for an ASBR fed with sugar refinery wastewater.

1. Introduction

Hydrogen has been deemed the future energy carrier, due to its high energy content and non-polluting nature upon combustion to release water vapour. At present, supply of hydrogen is achieved through processes such as steam reforming of methane (a non-renewable fossil fuel source), partial oxidation of hydrogen-rich feedstock, and electrolysis of water. Hydrogen production using biological methods and in particular, from the recycling of organic waste and wastewater, has been regarded as a potentially greener process over conventional processes since it does not require high temperature and pressure and hence less energy intensive [1]. Anaerobic fermentation under dark conditions or dark fermentation has proven to be more feasible than photo-fermentation (via photosynthetic bacteria) for practical applications, including integration with fuel cell technologies, because of its much higher hydrogen synthesis rate and efficiency, no requirement of additional light energy, and lower operating cost [2,3].

Experimental studies of hydrogen production using anaerobic fermentation have largely been conducted using continuous stirred tank reactors (CSTRs); the operational conditions are very dependent on the type of substrates and the concentration of carbon sources. Besides, relatively high OLR and short HRT have been applied, and the loss of microorganisms from the reactor is of concern. While a biofilm reactor using attached microbial growth could avoid this loss of microorganisms [4], an ASBR (define this) also has the

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capacity to keep relatively high microbial biomass in the reactor when compared to a CSTR.

A number of studies introduced various techniques (such as heat-shock, acids, alkalis, repeated aeration, and chemicals) for the pretreatment of inoculum or seed sludge in order to exclude hydrogen-consuming bacteria for high hydrogen production [5]. The advantages of inocula pretreatment include the ability to select hydrogen producing bacteria from mixed microbial sources or eliminating hydrogen-consuming bacteria from the sources of mixed microbial communities. However, the major disadvantage lies with the fact that only spore-forming hydrogen producing bacteria such as *Clostridia* are selected, while it blocks other non-spore-forming H_2-producing microbial strains such as *Enterobacter* and *Prevotella* [6]. Moreover, it could not eliminate the H_2-consuming homoacetogens, which are able to convert glucose into acetic acid through both heterotrophic and autotrophic mechanisms [7,8]. According to Ohnishi et al. [9], pretreatment of inoculum may not be desirable in terms of cost-effectiveness and operational control over the long term.

Hydrogen productivity is dependent upon the carbohydrate content of wastewater when anaerobic fermentation is operated under short HRT and high OLR conditions. As an application of ASBR to produce hydrogen from real wastewater, sugar refinery wastewater stream may be a good substrate since its composition was known to be mostly carbon source with some trace minerals. The main objective of this research was, therefore, to determine the optimal levels of the key factors for biohydrogen production from anaerobic digestion of sugar refinery wastewater. In order to achieve the goal, the effects of the key operational parameters – pH, HRT, and OLR were investigated by response surface methodology (RSM). Moreover, the changes of the microbial community were analysed with respect to variations in pH, which is regarded as the most significant factor affecting hydrogen productivity.

2. Materials and methods

2.1. Seed sludge and substrate

Seed sludge was obtained from the Department of Civil Engineering's biological nutrients removal (BNR) pilot plant for sewage treatment at the University of British Columbia. It was picked up from the anaerobic zone as mixed liquor and screened with 1 mm pore mesh and stored at 4 °C prior to use in the experiments. There was no pretreatment of the seed sludge.

Samples of sugar refinery wastewater were collected from Roger Sugar's refining facility in Vancouver, BC, Canada. Before each run, a new batch of wastewater was characterized. As indicated in Table 1, when the wastewater had a relatively low chemical oxygen demand (COD), it did not possess sufficiently high strength for use in the experiment; hence, supplementary sugar (sucrose) was mixed with the wastewater in order to establish a target substrate concentration. Nitrogen and phosphate were added in the form of NH_4Cl, $(NH_4)_2SO_4$, K_2HPO_4, KH_2PO_4, and Na_2HPO_4 to generate a C:N:P ratio of 200:5:1 and the concentrations [mg/L] of other trace minerals are as follows [$MgCl_2 \cdot 6H_2O$, 10; $NiCl_2 \cdot 6H_2O$, 1; $ZnSO_4$, 1; $FeCl_2$, 181; $CuSO_4 \cdot 5H_2O$, 5; $CaCl_2 \cdot 6H_2O$, 10; $Na_2MoO_4 \cdot 2H_2O$, 1].

Table 1 – Characteristics of sugar refinery wastewater.

Parameters	Value[a]
Colour	Dark/light brown
pH	4.7–5.2
TS	3840–5780
TSS	30–170
TDS	3610–5210
VS	560–6470
COD	572–6612
NH_4^+–N	3.7–10.1
P	2.0–4.0
Cu	0.02–0.04
Ni	0.01–0.03
Ca	107–226

a Units in [mg/L] except for pH and colour.

2.2. Experimental apparatus

An anaerobic sequencing batch reactor (ASBR) was operated, which was a lab-scale with 5 L working volume (New Brunswick Scientific Inc., Model BioFlo 3000 fermenter, NJ, USA). ASBR has its own advantages over other types of reactors; i.e. the requirement of a single vessel for reaction and liquid/solid separation, relative ease of operation, and flexibility with respect to the change in organic loading.

At the beginning of the experiment, the reactor was sparged with nitrogen gas for 20 min to induce anaerobic condition and nitrogen gas was also used in every cycle when the effluent was decanted as displacement gas in order to equalize pressure inside of the reactor. The reactor was perfectly sealed and the only biogas produced was released from the reactor and collected in a vessel using the water displacement method. The temperature was maintained at 31 °C using water jacket and pH was monitored and automatically adjusted to the setpoints by addition of 3 M NaOH and HCl. All pumps, solenoid valves, and an agitation motor were powered through a digital timer (XT Series Timer, ChronTrol Corporation, USA).

2.3. Analytical methods

A gas chromatography (GC, Varian Inc., CA, USA Model CP-3800) was used to measure the content of biogas produced during the fermentation. It has two-channel thermal conductivity detector (TCD) with the columns. For H_2, Hayesep Q (80/100 mesh; 1.0 m × 3.2 mm) and Molesieve 5A (60/80 mesh; 1.0 m × 3.2 mm) columns were used with argon as a carrier gas. Poraplot Q (50 m × 0.32 mm) column performed to detect CO_2, CH_4, and N_2 with helium as a carrier gas. The temperature setup of injector, oven, and detector was at 80, 50, 150 °C, respectively. The carrier gas flow rate was 40 mL/min. Within the same GC, a flame ionization detector (FID) was used for the analysis of volatile fatty acids (VFAs; acetic, propionic, and butyric acid) and alcohols (ethanol and butanol). The