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# Effect of COD/$SO_4^{2-}$ ratio and Fe(II) under the variable hydraulic retention time (HRT) on fermentative hydrogen production

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ARTICLE INFO

*Article history:*
Received 12 December 2008
Received in revised form
7 April 2009
Accepted 11 April 2009
Published online 24 April 2009

*Keywords:*
Hydrogen producing bacteria
Sulfate reducing bacteria
Fluorescence In Situ Hybridization
COD/$SO_4^{2-}$ ratio
Hydraulic retention time

ABSTRACT

The effect of chemical oxygen demand/sulfate (COD/$SO_4^{2-}$) ratio on fermentative hydrogen production using enriched mixed microflora has been studied. The chemostat system maintained with a substrate (glucose) concentration of 15 g COD $L^{-1}$ exhibited stable $H_2$ production at inlet sulfate concentrations of 0–20 g $L^{-1}$ during 282 days. The tested COD/$SO_4^{2-}$ ratios ranged from 150 to 0.75 (with control) at pH 5.5 with hydraulic retention time (HRT) of 24, 12 and 6 h. The hydrogen production at HRT 6 h and pH 5.5 was not influenced by decreasing the COD/$SO_4^{2-}$ ratio from 150 to 15 (with control) followed by noticeable increase at COD/$SO_4^{2-}$ ratios of 5 and 3, but it was slightly decreased when the COD/$SO_4^{2-}$ ratio further decreased to 1.5 and 0.75. These results indicate that high sulfate concentrations (up to 20,000 mg $L^{-1}$) would not interfere with hydrogen production under the investigated experimental conditions. Maximum hydrogen production was 2.95, 4.60 and 9.40 L $day^{-1}$ with hydrogen yields of 2.0, 1.8 and 1.6 mol $H_2$ $mol^{-1}$ glucose at HRTs of 24, 12 and 6 h, respectively. The volatile fatty acid (VFA) fraction produced during the reaction was in the order of butyrate > acetate > ethanol > propionate in all experiments. Fluorescence In Situ Hybridization (FISH) analysis indicated the presence of *Clostridium spp.*, *Clostridium butyricum*, *Clostridium perfringens* and *Ruminococcus flavefaciens* as hydrogen producing bacteria (HPB) and absence of sulfate reducing bacteria (SRB) in our study.



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doi:10.1016/j.watres.2009.04.023

## 1. Introduction

Hydrogen is a clean and sustainable energy source for various industrial activities with very high energy capacity per unit mass (118.2 kJ/g) (Park et al., 2005). It is non-polluting fuel and can be used in fuel cells for the production of electricity (Lay et al., 1999). Conventional and present sources of hydrogen production (e.g., water electrolysis or chemical cracking of hydrocarbons) require electricity derived from fossil fuels or nuclear fission; thus biohydrogen production is gaining wide attention due to recent concerns over global warming (Dincer, 2002; Hawkes et al., 2002). Hydrogen can be produced biologically through microbes either by photosynthetic bacteria cultured under anaerobic conditions or by anaerobic fermentative bacteria. In contrast to photolytic production of $H_2$, anaerobic fermentative processes have fast production rates, reduced waste generation and no requirement of additional light energy (Das and Verziroglu, 2001). Fermentative hydrogen production from organic substances results in the incomplete decomposition of substrate into organic acids such as acetate and butyrate. Butyrate is more dominant because of its lower Gibbs free energy ($\Delta G = -257.1$ kJ) compared to acetate ($\Delta G = -184.2$ kJ) and its production involves enzyme activity (Nandi and Sengupta, 1998; Zaborsky, 1998).

Biological hydrogen production utilizes organic wastewater or other wastes as raw materials which contain a variety of organic substrates (Lin and Chen, 2006). The high sulfate content in wastes produced from pulp/paper, sea-food processing and alcohol fermentation industries (Chen et al., 2008) has been found to adversely affect the anaerobic digestion (Bitton, 1994). Treatment of sulfate containing wastewater by anaerobic fermentation results in SRB proliferation. In previous reports, most of the acidogenic procedures showed decreased hydrogen and methane gas production in sulfate rich wastewater at pH 6–7 (Li et al., 1996; Mizuno et al., 1998; Esposito et al., 2003). Mizuno et al. (1998) investigated the effects of COD/$SO_4^{2-}$ ratio and HRT in acidogenic phase and clearly suggested that sulfate reducing bacteria can adversely influence on the pathway of sucrose degradation leading to lower hydrogen production. However, improved $H_2$ production was observed at lower pH conditions (e.g., pH 5.5), irrespective of variation in sulfate concentrations up to 3000 mg $L^{-1}$ (Lin and Chen, 2006). Iron is also known to be beneficial to microbial hydrogen production (Lee et al., 2009). Moreover, the effect of COD/$SO_4^{2-}$ ratios on hydrogen fermentation in conjunction with varying HRTs has not been systemically investigated.

The objective of this study was to investigate the effects of COD/$SO_4^{2-}$ ratio and Fe(II) under the variable hydraulic retention time (HRT) on fermentative hydrogen production in a continuous reactor. The distribution of hydrogen producing bacteria (HPB) and sulfate reducing bacteria (SRB) was analyzed by Fluorescence In Situ Hybridization (FISH) analysis.

## 2. Materials and methods

### 2.1. Operation of chemostat reactor systems

Three anaerobic reactors of 2.0 L capacity with 1.0 L working volume were used in this study. The reactor was filled with mixed liquor and continuously stirred by biogas re-circulated with a vacuum pump (Iwaki, max vacuum 34.66 kPa) at a flow rate of 5 L $min^{-1}$. The volume of biogas produced was measured by connecting the reactor to a biogas collection cylinder placed in an acidic (2% $H_2SO_4$) and saturated NaCl solution. The chemostat reactors were installed in a temperature controlled chamber maintained at $35 \pm 1$ °C by a fan heater (WH-601F). The pH in the reactors was adjusted to 5.5 using 3N KOH. The substrate (glucose) was prepared daily and stored in a substrate reservoir maintained at $4 \pm 1$ °C. Substrate was continuously added into the reactor with a micro-tube pump (EYELA, MP-3). Each chemostat reactor system was maintained at HRTs of 6, 12 and 24 h for 282 days.

### 2.2. Seed sludge and substrate

Sludge was obtained from the Wonju Water Supply and Drainage Center (Wonju, South Korea). The pH, carbohydrate

**Table 1 – Characteristics of the 16S rRNA-directed oligonucleotide probes used for FISH analysis.**

| Probe | Specificity | Probe sequence (5′-3′) | Dye |
|---|---|---|---|
| KO226 | *Clostridium butyricum* | GCTGTACCATGCGGTACTACA | 6-FAM |
| Csac67 | *Clostridium spp.* | CTCGGACATTACTGCCCGCG | Cy-3 |
| Rbro730 | *Clostridium leptum* | TAAAGCCCAGYAGGCCGC | Cy-3 |
| RFL1176 | *Ruminococcus flavefaciens* | AACGGCAGTCCCTTTAG | 6-FAM |
| ENT183 | *Enterobacteriaceae* | CTCTTTGGTCTTGCGACG | Cy-3 |
| CP1 | *Clostridium perfringens* | AATCCATTTCCCGAAGAAAC | 6-FAM |
| Enc145 | *Enterococcus spp.* | GGGATAACACTTGGAAAC | 6-FAM |
| SRB687 | *Desulfovibrio spp.* | TACGGATTTCACTCCT | 6-FAM |
| SRB660 | *Desulfobulbus spp.* | GAATTCCACTTTCCCCTCTG | 6-FAM |
| SRB129 | *Desulfobacterium spp.* | TGCGCGGACTCATCTTCAAA | 6-FAM |
| SRB221 | *Desulfobacter spp.* | CAGGCTTGAAGGCAGATT | 6-FAM |
| EUB338 I | Bacteria | GCTGCCTCCCGTAGGAGT | FITC or Cy-3 |
| EUB338 II | Bacteria not covered by EUB338 I and EUB338 III | GCAGCCACCCGTAGGTGT | FITC or Cy-3 |
| EUB338 III | Bacteria not covered by EUB338 I and EUB338 II | GCTGCCACCCGTAGGTGT | FITC or Cy-3 |