

## Biohydrogen production with an EGSB reactor using chloroform and 2-bromoethanesulfonate as inhibitors of hydrogen consuming bacteria

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### Introduction

Hydrogen is a CO<sub>2</sub>-neutral energy source with a very promising future as an alternative to fossil fuels for energy production. In a mixed culture system under anaerobic conditions the hydrogen produced by bacteria, such as *Clostridium* or *Enterobacter* is often readily consumed by hydrogen-consuming bacteria. In order to obtain hydrogen production from a mixed culture system, the seed sludge needs to be pretreated in order to suppress as much hydrogen-consuming bacterial activity as possible while still preserving the activity of hydrogen-producing bacteria. Effective pretreatment processes include heat, acidic or alkaline conditions, aeration, chemicals, and electric current. Heat treatment has been most commonly used for the screening of hydrogen producing bacteria (Lay et al., 1999). Although, some studies reported that heat treatment was unable not inhibit the activity for all hydrogen-consuming bacteria (Oh et al., 2003). Oh et al. (2003) found that some homoacetogenic bacteria may survive the heat treatment process and consume hydrogen to produce acetate. In the present study, two chemicals, 2-bromoethanesulfonate (BES) and chloroform were used to compare their efficacy as hydrogen-consuming bacteria inhibitors in a continuous system. Their effect was not only compared on the hydrogen production, but also on the presence of homoacetogenic and the distribution of fermentation intermediates.

### Material and Methods

The experiments were carried out in plexiglas Expanded Granular Sludge Blanket (EGSB) reactors (1.3l). Reactors were operated continuously at a temperature of 37 ± 1 °C. During the first 37 days, the reactors were operated with a HRT of 21 hours in order to acclimatize the biomass to the substrate. Reactor 1 was inoculated with 400ml of anaerobic granular sludge from brewery wastewater treatment plant subjected to a pretreatment with BES (15mM) for 72h at 37°C. Reactor 2 was inoculated with 400ml of the same anaerobic granular sludge used in reactor 1 but subjected to a pretreatment with BES (15mM) and Chloroform (30µM) for 72h at 37°C. The reactors were fed with glucose and L-arabinose (1/1) at a final concentration of 5gCOD l<sup>-1</sup>. Sodium bicarbonate (1g/l) and macronutrients were also added to the reactor. The COD and VSS were determined according to Standard Methods (Standard Methods, 1998). Biogas flow rate was measured by a Ritter Milligascounter. Hydrogen and methane content of biogas was determined by gas chromatography. Volatile fatty acids (VFA), ethanol, L-arabinose, and glucose were determined by HPLC.

### Results and Discussion

In both reactors acetate and n-butyrate were the most prevalent VFA's during the first period of operation, with an organic loading rate (OLR) of 5kgCOD/m<sup>3</sup>/d (Fig.1). Hydrogen production was suppressed when acetate concentrations were ≥1200mg/l in both reactors, suggesting hydrogen consumption by apparent homoacetogenesis. When the OLR was increased to 10kgCOD/m<sup>3</sup>/d, the acetate concentration in R1 increased to a maximum of 2100mg/l at day 60. At day 60, BES (15 mM) was injected into the reactor in order to inhibit hydrogen consumption. Subsequently, acetate

concentrations decreased to approximately 800mg/l at day 75. In addition and at same time (day 75) hydrogen production resumed after an absence of approximately 40 days. In R1 after day 75, a pseudo-steady state of hydrogen production rate of approximately 200mLH<sub>2</sub>/l/d was observed. Hydrogen production rates were observed to increase when the concentrations of acetate and lactic acid decreased and the concentrations of n-butyrate increased. Hydrogen production was lower and more unstable in R2 compared to R1.

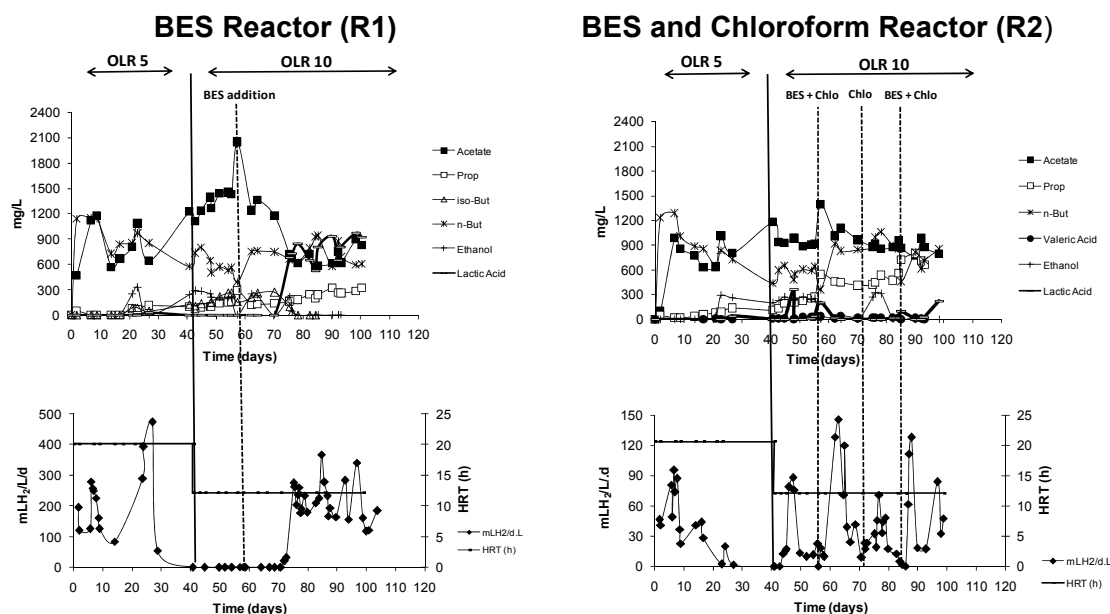


Figure 1 - Time course of reactor of R1 and R2 performance (a) profile of volatile fatty acids and ethanol; (b) hydrogen production rate and hydraulic retention time.

When an OLR was increased to 10kgCOD/m<sup>3</sup>/d, acetate concentrations in R2 were stable at approximately 900 mg/l. However, acetate concentrations increased at day 60 to approximately 1400 mg/l. At this time, the first pulse of BES/Chloroform (BES (15 mM)/Chlo (30 μM)) was added to the reactor. This addition subsequently returned acetate concentrations to their previous level of 900 mg/l. Propionate was present in higher concentrations than in R1 suggesting that some hydrogen was consumed for propionate production. Hydrogen production rates increased for several days after the first BES/Chlo pulse addition. However, the rates were not sustained and they subsequently started decreasing. This same pattern was repeated with the addition of Chloroform only (day 71) and after the second addition of BES/Chlo (day 85). However, higher hydrogen production rates were observed after the addition of BES/Chlo rather than Chloroform only. Small amounts of methane was detected in both reactors (<5mLCH<sub>4</sub>/l/d) during the entire operation. Pretreatment with BES and BES/Chlo and subsequent pulse additions during operation were effective at inhibiting hydrogen consumption by methanogenesis. BES/Chlo pretreatment was more efficient at inhibiting homoacetogenesis as acetate concentrations were lower in R2 than BES pretreatment (R1). The lower hydrogen production rates obtained using pretreatment and pulses of BES/Chlo suggest that chloroform may not only inhibit hydrogen consumption but some hydrogen production as well.

### References

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