Effect of pH and pressure on syngas fermentation by anaerobic mixed cultures

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Abstract
Syngas fermentation by mesophilic anaerobic mixed cultures was studied, regarding the effect of initial medium pH (5.8, 6.9 and 7.6) and total initial syngas pressure (1.0, 1.75 and 2.5 atm) in methane production and other alternative products (acids and alcohols). Complete consumption of CO and H_2 was achieved in less than 72 h at 1.0 atm syngas, and within 240 h for higher syngas pressures. The highest specific CH_4 production (normalized to the initial syngas concentration, $CO+H_2$) was 0.89 mol CH_4/mol syngas, and was obtained for pH 5.8 and 1.0 atm syngas. Volatile fatty acids (VFA) were produced in methanogenic-inhibited assays, with 0.92 mol VFA/mol syngas as the best conversion yield (at pH 6.9 and 1.0 atm). Only residual concentrations of alcohols (< 0.005 mol alcohols/mol syngas) were produced by methanogenic-inhibited sludge. Syngas pressure had a major effect on conversion yields, which might be related to the susceptibility of microbial communities to the higher CO partial pressures.

Keywords
Syngas fermentation; mixed culture; anaerobic digestion, methane production

INTRODUCTION

Biomass and recalcitrant residues are suitable and available feedstocks for the production of biofuels (Bridgewater, 1995). These materials can be converted to syngas – a gaseous mixture mainly composed by carbon monoxide (CO), hydrogen (H_2), and carbon dioxide (CO_2) –, which can be further converted to biofuels using thermochemical or biotechnological processes. Anaerobic digestion (AD) of syngas can be used for the production of *e.g.* hydrogen, methane, ethanol, butanol, acetic and butyric acid (Henstra *et al.*, 2007). Coupling syngas AD processes to waste treatment facilities can substantially improve energy recovery – for example, it is estimated that the production of biogas from municipal solid wastes can be improved by over 80% if the nonreadily organic fraction is gasified to syngas and further anaerobically converted (Guiot *et al.*, 2011). Products formed during the AD of syngas are dependent on the environmental conditions of the process. The big challenge nowadays is to direct biochemical pathways towards the formation of a specific compound of interest, which can be done by exploiting energy niches within mixed cultures (Rodríguez *et al.*, 2008). It is important to study the effect of environmental parameters, such as media composition, pH, temperature, pressure, mixing properties, and substrate or/and product concentrations in the conversion pathways of syngas (Gaddy, 2000). In this study, syngas fermentation by mesophilic anaerobic mixed cultures was studied in batch assays. The effect of the initial medium pH and the initial syngas pressure was analyzed to evaluate methane production. In parallel, methanogenic-inhibited sludges were used to study the potential of mixed cultures for the production of short-chain fatty acids and alcohols.

MATERIALS & METHODS

Experimental setup

A factorial experiment with 36 (2×3^n, n=2 variables, in duplicate) runs was designed to test the effect of initial medium pH and initial syngas pressure on substrate conversion by anaerobic mixed cultures, at mesophilic conditions (37 ^oC). Two scenarios were evaluated using methanogenic-active and methanogenic-inhibited inocula. Test vials were prepared with 80 mL of liquid medium and equal volume of headspace. The liquid medium was composed by 20 mM of buffer solution (MES, 2-(N-morpholino)ethanesulfonic acid, or HEPES, 4-(2-hydroxyethyl)-1-piperazineethane-sulfonic acid, depending on the required pH), 5 g/L of granular sludge (from a brewing industry), 0.1 M of reducing agent (Na_2S) and 1 mL/L of vitamin solution (Stams *et al.*, 1993). In vials where methanogens were inhibited, 50 mM of BES (2-bromoethanesulphonate) was added. Vials were sealed with rubber stoppers and aluminum caps and the headspace was filled with syngas mixture (60/30/10 % of $CO/H_2/CO_2$) at different initial pressures, 1.0, 1.75 and 2.5 atm (corresponding to CO partial pressures of 0.6, 1.0 and 1.5 atm, respectively). Medium pH was tested at 5.8 (MES), 6.9 and 7.6 (HEPES).

Analytical Methods

Gases (H_2, N_2, CO_2, CH_4, and CO) were analyzed by GC (Chrompack 9001B) equipped with a TCD detector and two columns: Porapak Q and Molsieve 5 Å (2 m × 1/8" × 2 mm SS, 80-100 mesh, Varian). Argon was the carrier gas (10 mL/min). The column, injector, and detector temperatures were 35, 110, and 110 °C, respectively. Volatile fatty acids (VFA) were determined by HPLC (Jasco) equipped with a UV detector (210 nm) and a Chrompack column (6.5 × 30 mm^2) at 60°C. Sulphuric acid (0.01 N) was the mobile phase (0.6 mL/min). Alcohols were determined by GC (Varian 4000) equipped with a FID detector and a TRB-WAX column (30 m × 0.25 mm × 0.25 μm, Teknokroma). Helium was the carrier gas (1 mL/min). The injector and detector temperatures were 250 °C, and column run with a temperature ramp from 50 to 60 °C (rate of 0.5 °C/min) and 200 °C (for 1.5 min).

RESULTS & DISCUSSION

Methane can be directly produced from syngas components, according to the reactions: (1) $4CO + 2H_2O \rightarrow CH_4 + 3CO_2$; (2) $CO + 3H_2 \rightarrow CH_4 + H_2O$; and (3) $CO_2 + 4H_2 \rightarrow CH_4 + 3H_2O$. Acetate can also be produced directly from CO or CO/CO_2 plus H_2: (4) $4CO + 4H_2O \rightarrow$ Acetate + $3CO_2$; (5) $2CO + 2H_2 \rightarrow$ Acetate; (6) $2CO_2 + 4H_2 \rightarrow$ Acetate + $4H_2O$. In methanogenic systems, acetate can be further converted to biogas. In this work, and for the sake of comparison between the different conditions tested, we report product formation normalized to the initial amounts of CO plus H_2 (referred as mol syngas).

Syngas conversion by methanogenic sludge

Methanogenic mixed cultures were able to completely consume CO and H_2 present in syngas, with the production of mainly CH_4. Carbon monoxide conversion by these cultures, when incubated with 1.0 atm syngas, was achieved in less than 72 h. A longer incubation time, up to 240 h, was necessary for converting CO in assays at higher syngas pressures. Methane production, in assays conducted at different combinations of pH and syngas pressure, is shown in Figure 1. Variation of syngas pressure had a stringent effect in methane production, which is more visible for the pH of 5.8. The highest methane production, i.e. 0.89 mol CH_4/mol syngas, was achieved with pH 5.8 and 1.0 atm syngas. On the other hand, the lowest methane production, 0.21 mol CH_4/mol syngas, was obtained at the same pH and 2.5 atm of syngas. A negative trend between methane production and pH was observed in assays with 1.0 atm syngas, that is, methane production increased with decreasing the pH. The opposite was observed for the highest syngas pressure – in these assays methane production increased with increasing the pH. Residual concentrations of VFA were detected in almost all the conditions tested, corresponding to a production of VFA below 0.01 mol VFA/mol syngas by the end of the assays performed under 1.0 atm syngas. Nevertheless, a VFA production as high as 0.15 mol VFA/mol syngas was obtained for the assay at pH 5.8 and 2.5 atm syngas, the same assay where methane production was the lowest. This seems to indicate that, under these conditions, methanogens activity tends to be inhibited. No alcohols were detected during the experiment time.

The effect of CO partial pressure in methane production has been reported in literature, using either pure or mixed cultures, during syngas fermentation. *Methanosarcina barkeri* could use H_2 to produce methane, but consumption time doubled with an increase in CO partial pressure from 0 to 0.6 atm. This shows that some hydrogen-utilizing organisms require low dissolved CO concentrations (Klasson *et al.*, 1991). In fact, the decrease in syngas conversion with increasing syngas pressure, observed in the present study, might have been caused by the inhibition of methanogenic microorganisms for higher CO partial pressures. Mixed cultures might be more robust than pure cultures, but methanogenic activity from anaerobic sludges seems also to be affected by CO. Guiot *et al.* (2011) tested different CO partial pressures ranging from 0.42 to 0.96 atm, in a continuous gas-lift reactor with a mixed culture. Maximal CO conversion efficiency of 75 % was obtained at 0.6 atm of CO partial pressure combined with gas recirculation (Guiot *et al.*, 2011).

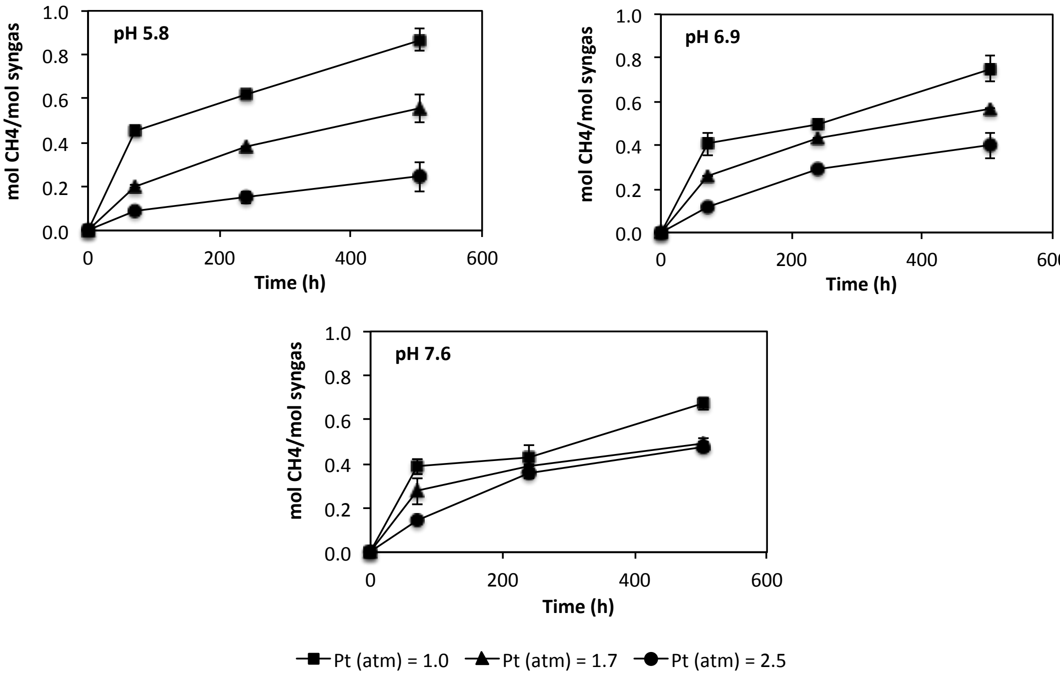

Figure 1. Normalized methane production (mol of CH_4 produced per mol of initial syngas concentration) along time, varying gas pressure at constant pH. Vertical bars represent standard deviation.

Syngas conversion by methanogenic-inhibited sludge

The main products from syngas conversion by methanogenic-inhibited sludge were VFA. Alcohols were detected in small concentrations (maximum, 1.5 mM ethanol and 0.5 mM butanol). Lower syngas pressure lead to the accumulation of higher amounts of VFA. Production of VFA, under the different conditions tested, is shown in Figure 2. Acetate and propionate were VFA detected in higher concentrations, varying in the range of 9.0–18.3 mM and 5.0–10.6 mM, respectively; lower amount of butyrate was also detected (maximum 2.3 mM). The best VFA production occurred at pH 6.9 and 1.0 atm (0.92 mol VFA/mol syngas). Furthermore, the lowest VFA production (0.22 mol VFA/mol syngas) was obtained at the same pH and the 2.5 atm. According to the data, no trend is possible to attribute to pH variation with gas pressure, for VFA production using methanogenic-inhibited sludge. In the present study the final concentrations of VFA have shown to be independent of the pH tested.

The effect of pH and gas pressure in VFA and alcohols production has been reported in literature, using pure cultures, during syngas or only CO fermentation. Enhancement in the production of butyrate over acetate by *Butyribacterium methylotrophicum*, or ethanol over acetate by *Clostridium ljungdahlii*, has been achieved by reducing pH during the process (Worden *et al.*, 1989; Gaddy and Clausen, 1992). Regarding the gas pressure effect, no inhibition was observed in ethanol and acetate production by *Clostridium carboxidivorans* P7T, up to 2.0 atm of CO partial pressure (Hurst and Lewis, 2010). *Peptostreptococcus productus* was able to convert CO to acetate, with gradual increase of the CO partial pressure up to 10 atm. However, this high level of resistance to CO was only possible after promoting cell growth in the absence of CO in order to get very high cell densities (Klasson *et al.*, 1991).

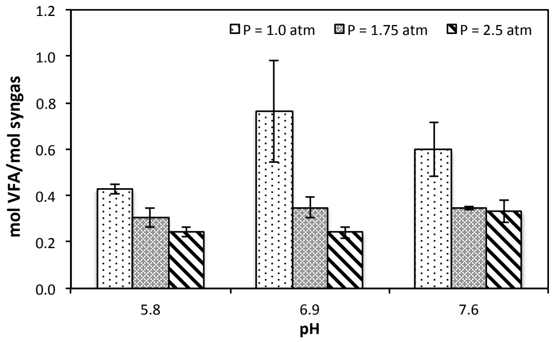

Figure 2. Variation of normalized VFA production (expressed as sum of the total mol of VFA per mol of initial syngas concentration) with pH. Vertical bars represent standard deviation.

CONCLUSIONS

In this study, syngas was converted to methane by methanogenic mixed cultures. Conversion was mainly affected by syngas pressure, being more efficient at low pressure. This is likely due to CO partial pressure, as this compound is known to inhibit methanogens. Further studies are needed to clarify the relation P_{total}/p_{CO} and its influence on methane production. When methanogenesis was inhibited, acetate and butyrate were the main products resulting from syngas fermentation and only residual concentration of alcohols were detected.

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