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Enrichment of a solventogenic anaerobic sludge converting carbon monoxide and syngas into acids and alcohols

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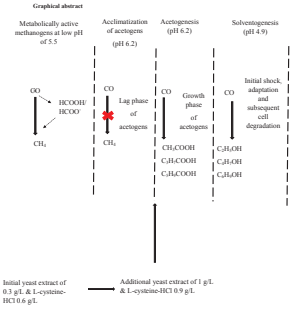
GRAPHICAL ABSTRACT



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ABSTRACT

An anaerobic granular sludge was acclimatized to utilise CO in a continuously gas-fed stirred tank bioreactor by applying operating conditions expected to stimulate solventogenesis, i.e. the production of alcohols, and allowing to enrich for solventogenic populations. A cycle of high (6.2) and low (4.9) pH was applied in order to produce volatile fatty acids first at high pH, followed by their bioconversion into alcohols at low pH. The addition of yeast extract stimulated biomass growth, but not necessarily solventogenesis. The highest concentrations of metabolites achieved were 6.18 g/L acetic acid (30th day), 1.18 g/L butyric acid (28th day), and 0.423 g/L hexanoic acid (32nd day). Subsequently, acids were metabolized at lower pH, producing alcohols at concentrations of 11.1 g/L ethanol (43rd day), 1.8 g/L butanol (41st day) and 1.46 g/L hexanol (42nd day), confirming the successful enrichment strategy. Similarly, the enriched sludge could also convert syngas into acids and alcohols.

1. Introduction

Carbon monoxide (CO) is a major compound found in waste gases from some industries such as steel producing plants and it is also a major component of synthesis gas or syngas (Abubackar et al., 2011). In biorefineries, CO-rich syngas can be obtained from the gasification of biomass, solid waste or other carbonaceous feedstocks. Some of those feedstocks, such as lignocellulosic biomass, can also be hydrolyzed to obtain carbohydrates that can then similarly be fermented to generate high value metabolites, biofuels or platform chemicals (Fillat et al., 2017).

CO can be converted anaerobically by acetogenic bacteria into a spectrum of commercially useful metabolites. This gas-fermentation technology represents an environmentally-friendly alternative to the

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more conventional crude oil-based refineries or other petrochemical processes for the production of fuels or chemicals. Several acetogenic bacteria assimilating CO are also able to metabolize CO_2 and H_2 as well as syngas, e.g. *Clostridium ljungdahlii*, *Clostridium autoethanogenum*, *Clostridium ragsdalei*, *Clostridium carboxidivorans* (Fernández-Naveira et al., 2016a; Huhnke et al., 2010; Philips et al., 2015). Recent research on CO, CO_2/H_2 and syngas fermentation has focused on the production of different end-products, such as ethanol (Van Groenestijn et al., 2013), higher alcohols such as butanol or hexanol (Fernández-Naveira et al., 2017a; Philips et al., 2015), 2,3-butanediol (Köpke et al., 2011), biomethane (Sancho Navarro et al., 2016) or biopolymers, e.g., polyhydroxyalkanoates (Lagoa-Costa et al., 2017). Some biofuels obtained from C1-gas fermentation like biogas or biomethane (López et al., 2012) may need to be upgraded to remove CO_2 or other by-products such as hydrogen sulphide (López et al., 2013). Many studies have been done with available pure cultures and only a few with mixed cultures (Rachbauer et al., 2017; Xu et al., 2015). Metabolic engineering is used as an additional opportunity to broaden the range of potential biocommodities that can be produced through anaerobic gas (CO, syngas and waste gas effluents) fermentation (Bengelsdorf et al., 2013).

Bioalcohols such as ethanol and butanol, among others, are interesting biofuels that can be obtained through anaerobic gas fermentation and are suitable to replace gasoline or to be mixed with it in different ratios (Fernández-Naveira et al., 2017a). So far, only one pure acetogenic strain has unequivocally been described to produce higher alcohols through such gas fermentation, namely *C. carboxidivorans* (Fernández-Naveira et al., 2017a,b; Philips et al., 2015). Other bacteria should potentially be able to perform similar metabolic conversions. Acetogens generally first convert gases into organic acids at relatively high (near-neutral) or slightly acidic pH, e.g. pH 6. The production of these acids induces a pH drop if the latter is uncontrolled. Such acidic conditions will then stimulate solventogenesis and the production of alcohols, i.e. either ethanol or higher alcohols.

Although some studies have been done on the enrichment of ethanol producing communities (Singla et al., 2014), to the best of our knowledge no thorough enrichment studies have been performed for the production of higher alcohols through anaerobic gas fermentation. Therefore, this study aimed to enrich an anaerobic sludge for solventogenic bacteria able to ferment C1 gases, i.e. CO, CO_2 and syngas, to ethanol and higher alcohols, applying specific conditions such as the alternation of high and low pH values of the bioreactor medium or the addition of a specific inhibitor of methanogens (i.e., bromoethane sulphonate, BES) in order to select and facilitate the growth of solventogens instead of methanogens. The effect of yeast extract and L-cyseine–HCl on the metabolism of the solventogenic acetogens was also studied.

2. Materials and methods

2.1. Biomass and medium composition

The anaerobic sludge (120 mL) was obtained from Biothane Systems International BV (Delft, The Netherlands) and it originated from an anaerobic digester producing biogas from industrial wastewater (Van Lier et al., 2015). The sludge was pregrown on CO (in triplicates in 40 mL bottles) for 14 days at uncontrolled pH (initial pH of 5.5 to favour solventogenesis) at a shaking speed of 120 rpm. The acclimatized sludge of 120 mL was used as inoculum in the reactor. A modified basal anaerobic unautoclaved medium was used as described elsewhere (Fernández-Naveira et al., 2016a) with a variation in the initial concentrations of yeast extract (YE – 0.3 g/L) and L-cysteine–HCl (0.6 g/L). No vitamin solution was added to the bioreactor, contrary to other studies focusing on solventogenic clostridia (Fernández-Naveira et al., 2017b,c) as some CO fermenting cultures have been shown to be able to metabolize gases in the absence of vitamins (Abubackar et al., 2015, 2016).

2.1.1. Set-up and operation of the continuous gas-fed bioreactor

Bioreactor experiments were performed in a 2 L BIOFLO 110 bioreactor (New Brunswick Scientific, Edison, NJ, USA) containing 1.2 L medium (working volume). A microsparger was introduced inside the reactor for sparging CO (100%) as the sole gaseous substrate, fed continuously at a rate of 10 mL/min using a mass flow controller (Aalborg GFC 17, Müllheim, Germany). The temperature of the bioreactor was maintained at 33 °C by means of a heating water jacket. Four baffles were symmetrically aligned to avoid vortex formation of the liquid medium and to enhance mixing. The bioreactor was supplied with a DO probe connected to a bio-flow controller and the dissolved oxygen in the bioreactor was less than 0.5% (Fernández-Naveira et al., 2017b,c,d). The agitation speed maintained thoroughout the experiment was 120 rpm. The bioreactor was operated at a constant pH of 5.5 from days 0 to 6 to inhibit methanogenesis and induce acetogenesis. On the 7th day, 2 mM of BES was added to inhibit methanogenesis and a pH of 6.2 was maintained to induce acetogenesis. On the 21st day of bioreactor operation, 1 g/L of YE and 0.9 g/L of L-cysteine–HCl were added for inducing growth of biomass, as it was low originally and thus allowing to reach a redox potential of −189 mV which was found to be suitable for CO metabolizing biomass to grow and simultaneously produce acids. On the 31st day, the pH was changed to 4.9 to stimulate solventogenesis.

2.1.2. Batch studies on syngas utilisation by the enriched sludge

The enriched sludge and dissolved biomass collected from the reactor at the end of the experiment (46th day) were studied in the presence of syngas (mixture of CO, CO_2, H_2 and N_2 in the ratio 20:20:10:50) to check the production of metabolites at initial pH 6.2, a temperature of 33 °C and 120 rpm. pH was not controlled in the batch bottles, but the initial pH was used as it is favourable for biomass growth and production of acids. The experiments were carried out in three 100 mL bottles with 40 mL medium and 10% inoculum. After inoculation, the bottles were sparged with syngas. The overhead pressure was 1 bar. The composition of the medium was described earlier (Fernández-Naveira et al., 2016a).

2.2. Analytical methods

2.2.1. Gas-phase CO and CO_2 concentrations

Gas samples (1 mL) were taken from the outlet sampling ports of the bioreactor to monitor the CO, CO_2 and CH_4 concentrations. A HP 6890 gas chromatograph (GC, Agilent Technologies, Madrid, Spain) equipped with a thermal conductivity detector (TCD) was used for measuring the gas-phase concentrations. The GC was fitted with a 15-m HP-PLOT Molecular Sieve 5A column (ID – 0.53 mm; film thickness – 50 µm). The initial oven temperature was kept constant at 50 °C, for 5 min, and then raised by 20 °C/min for 2 min, to reach a final temperature of 90 °C. The temperature of the injection port and the detector were maintained constant at 150 °C. Helium was used as the carrier gas at a flow rate of 2 mL/min. Another HP 5890 gas chromatograph (GC, Agilent Technologies, Madrid, Spain) connected with a TCD was used for measuring CO_2 and CH_4. The injection, oven, and detection temperatures were maintained at 90, 25, and 100 °C, respectively. The area obtained from the GC was correlated with the concentration of the gases.

2.2.2. Fermentation products

The water-soluble products, “acetic acid, butyric acid, lactic acid, propionic acid, hexanoic acid, ethanol, butanol and hexanol”, were analysed from liquid samples (1 mL) taken about every 24 h from the bioreactor medium using an HPLC HP1100 (Agilent Technologies, Madrid, Spain) equipped with a Supelcogel C610 column and a UV detector at a wavelength of 210 nm. The mobile phase was a 0.1% *ortho*-phosphoric acid solution fed at a flow rate of 0.5 mL/min. The column temperature was set at 30 °C. Before analysing the