Enrichment of carbon monoxide utilising microorganisms from methanogenic bioreactor sludge

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Abstract

Conversion of CO is the rate limiting step during anaerobic conversion of syngas (a gaseous mixture mainly composed of CO, CO_2 and H_2). In this work we study the microbial diversity in anaerobic sludge submitted to extended contact to syngas in a multi-orifice baffled bioreactor (MOBB). Methane was the main product resulting from syngas conversion in the MOBB. Enrichment cultures started with this sludge produced methane as final product, but also acetate. 16S rRNA gene analysis revealed a predominance of *Acetobacterium* and *Sporomusa* species in the enrichments. These are homoacetogenic bacteria that might be involved in CO conversion to acetate. Hydrogen was formed as intermediary from CO conversion and likely used by hydrogenotrophs with the formation of methane. Pasteurisation and serial dilutions of stable CO-converting enrichments resulted in a microbial culture dominated by two *Sporomusa* species that are able to use CO as sole substrate.

Keywords

synthesis gas; carbon monoxide; anaerobic; carboxydotrophic microorganisms

INTRODUCTION

Anaerobic microorganisms can be used as catalysts to produce added-value products from renewable sources. A promising route for this is the combination of thermal (e.g. gasification) and biological processes. Cost-effective gasification technology is currently available, and biological conversion of the generated syngas to ethanol is already applied at full-scale (Latif *et al.*, 2014). For a biobased economy it is important to broaden the range of products generated from syngas. Methane and fatty-acids are added-value products that can be derived from syngas by methanogenic and acetogenic microorganisms. In this work, we study the microbiology of anaerobic sludge from a MOBB converting syngas to mainly methane. Anaerobic enrichments on CO were performed and the microbial community was characterised to investigate if novel carboxydotrophic bacteria can be obtained.

MATERIALS AND METHODS

Sludge source and microbial diversity analysis. Granular sludge withdrawn from a 10 L multi-orifice baffled bioreactor (MOBB) continuously fed with syngas mixture composed of 60 % CO, 10 % CO_2, and 30 % H_2 (v/v), as described elsewhere (Pereira, 2014). Microbial diversity of this sludge (sludge **Osc**) was analysed by denaturing gradient gel electrophoresis (DGGE) and cloning and sequencing, using methodologies previously described by Sousa *et al.* (2007).

Enrichment cultures growing on CO. Enrichment cultures were started-up with sludge Osc. Enrichment series **O-CO** was incubated in the presence of CO as sole energy and carbon source. Two additional CO enrichment series were prepared in the presence of vancomycin and erythromycin for inhibiting bacterial activity: **O-CO-v$_{50}$e$_{100}$** (50 μM vancomycin + 100 μM erythromycin) and **O-CO-v$_{100}$e$_{100}$** (100 μM of each antibiotic). Enrichment cultures were prepared in 120-mL serum bottles containing 30 mL bicarbonate-buffered mineral salt medium; the headspace of the bottles was flushed with a mixture of N_2/CO_2 (80:20; 1 bar) and 40% CO (pCO 0.7 bar). CO degrading enrichment cultures were obtained by successive transfers of active cultures. Physiological characterization of stable enrichment cultures was performed by measuring CO, hydrogen, methane and acetate over time; gaseous compounds were measured by gas chromatography and fatty acids by high-performance liquid chromatography (HPLC), as previously described by Alves *et al.* (2013). Additionally, microbial composition was analysed by DGGE and cloning and sequencing.

Isolation techniques Different strategies were used in isolation trials: pasteurization, growth with 100% CO (1.7 bar), rolling tubes and soft agar bottles for colony growth. Pasteurization (80°C for 20 min) was performed for highly enriched cultures after the observation of spore-forming bacteria. Colony growth was performed in soft agar (0.8% noble agar) bottles with bicarbonate-buffered mineral salt medium, and in rolling tubes with the same medium and 2% noble agar. All the incubations were done with CO (pCO 0.7 bar) at 37 °C.

RESULTS AND DISCUSSION

Enriched CO-degrading cultures (series **O-CO)** were obtained after several successive transfers to fresh medium with CO as sole energy and carbon source. Carbon monoxide consumption was monitored over time (Figure 1). Methane and acetate were the ultimate products formed by the enrichment cultures, and hydrogen was detected as intermediate.

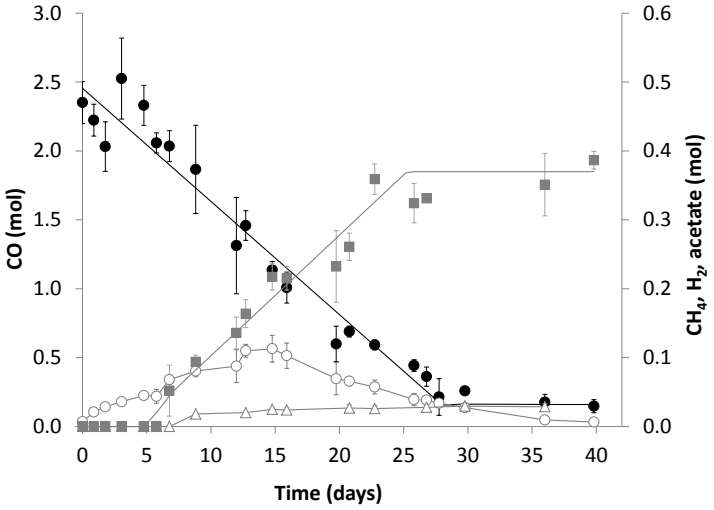

Figure 1: Substrate consumption and product formation by stable enriched CO-degrading culture (methanogenic). Symbols: (●) carbon monoxide, (○) hydrogen, (△) acetate, and (■) methane.

Enrichment series **O-CO-v$_{50}$e$_{100}$** and **O-CO-v$_{100}$e$_{100}$** showed no growth, even not after long-term incubation (over 3 months). Since vancomycin and erythromycin are bacterial inhibitors, the aim of setting up enrichments with these antibiotics was to obtain methanogenic CO-utilizers, which seemingly were not abundantly present in sludge Osc. Thus far, four methanogens are known to use CO: *Methanobrevibacter arboriphilicus*, *Methanosarcina barkeri*, *Methanosarcina acetivorans*, and *Methanothermobacter thermoautotrophicum* (Henstra *et al.*, 2007). Both *M. thermoautotrophicum* and *M. barkeri* grow slowly on CO (doubling time > 200 and 65h, respectively) and growth ceases with increasing levels of CO (Daniels et al. 1977; O'Brien et al. 1984). From a biotechnological point of view it would be interesting to find methanogens with improved CO conversion rates and able to resist to relatively high CO partial pressure.

Microbial diversity in sludge Osc and in stable methanogenic enrichment cultures was monitored by DGGE fingerprinting, and predominant microorganisms were identified by cloning and sequencing (Figure 2).

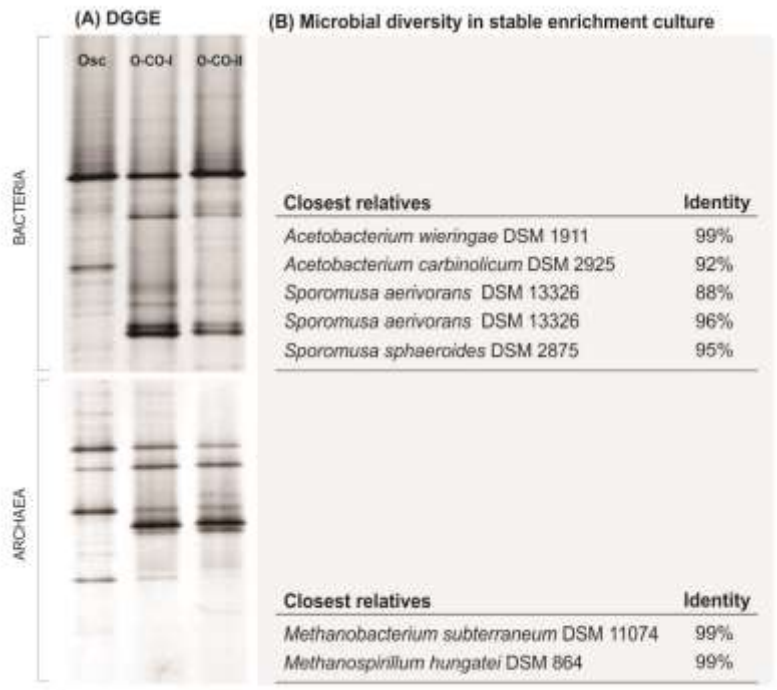

Figure 2: Microbial diversity in CO-converting anaerobic enrichments: (A) bacterial and archaeal DGGE profiles, and (B) closely relative microorganisms of predominant clones obtained from the enrichment cultures. Osc – inoculum sludge withdrawn from a MOBB fed with syngas; O-CO-I and O-CO-II – enrichment cultures incubated with CO as sole carbon and energy source.

Hydrogenotrophic methanogens closely relative to *Methanobacterium* and *Methanospirillum* species were predominant in enrichments O-CO. Acetoclastic archaea were not detected, which is coherent with the observed acetate accumulation. Bacterial communities were dominated by *Acetobacterium* and *Sporomusa* species. These homoacetogens are known to use the Wood-Ljungdahl pathway to synthesize acetyl-CoA from H_2+CO_2; some homoacetogens can also use CO (Schiel-Bengelsdorf and Dürre 2012). *Acetobacterium woodii* is known to utilise CO (Genthner and Bryant 1987). *S. ovata* and *S. termitida* utilise CO as well (Balk *et al.*, 2010; Breznak *et al.*, 1988). Nevertheless, most of the *Sporomusa* species were never tested for CO utilisation.
To unravel the role of the predominant groups present in stable O-CO enrichments, isolation efforts have been performed. Through pasteurisation and sequential dilution series we were able to obtain a culture with two predominant *Sporomusa* species closely related to *S. aerivorans* and *S. sphaeroides* (95% 16S rRNA gene identity). We are now attempting to separate these two species by using soft-agar and roll tubes techniques. This culture is able to grow on CO (pCO 0.70 bar) as sole substrate. Previously described *Sporomusa* species able to grow on CO were inhibited at CO partial pressures higher than 0.25 bar (Breznak et al., 1988).

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REFERENCES

Alves JI, Stams AJ, Plugge CM, Alves MM, Sousa DZ (2013) FEMS Microbiol Ecol 86:590-597.

Balk M, Mehboob FH, van Gelder A, Rijpstra WIC, Damsté JSS, Stams AJM (2010) Appl Environ Microbiol 88:595-603.

Breznak JA, Switzer JM, Seitz H-J (1988) Arch Microbiol 150:282-288.

Daniels L, Fuchs G, Thauer RK, Zeikus JG (1977) J Bacteriol 132:118-126.

Genthner BRS, Bryant MP (1987) Appl Environ Microbiol 53:471-476.

Henstra AM, Sipma J, Rinzema A, Stams AJM (2007) Curr Opin Biotechnol 18: 200-206.

Latif AA, Nielsen AT, Zengler K (2014) Curr Opin Biotechnol 27:79-87.

O'Brien JM, Wolkin RH, Moench TT, Morgan JB, Zeikus JG (1984) J Bacteriol 158:373-375.

Pereira FM (2014) PhD thesis, University of Minho, Portugal.

Schiel-Bengelsdorf B, Dürre P (2012) FEBS Lett 586:2191-2198.

Sousa DZ, Pereira MA, Stams AJM, Alves MM, Smidt H (2007) Appl Environ Microbiol 73:1054-1064.