Enrichment of syngas-converting communities from a multi-orifice baffled bioreactor

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Introduction

Biomass can be biologically converted to fuels and chemicals, but poorly biodegradable biomass (e.g. straw, wood) requires costly chemical or enzymatic hydrolysis prior to microbial fermentation. Gasification of biomass, and subsequent syngas fermentation, is an alternative that can maximize carbon recovery compared to conventional fermentations (Bredwell et al., 1999; Munasinghe and Khanal, 2010; Guiot et al., 2011). Syngas is mainly composed of CO, H2 and CO2, and these compounds can be used by anaerobic microorganisms to produce value-added chemicals. In the absence of external electron acceptors, CO can be converted by hydrogenogenic, acetogenic and methanogenic microorganisms (Diender et al., 2015). The hydrogenogenic conversion of CO results in the formation of H2 and CO2, and is typically performed by thermophilic carboxydotrophs, such as Carboxydotermus hydrogenofomans, Moorella stamsii and Desulfitomaculum nigrificans (Svetличnyi et al., 2001; Parshina et al., 2005; Henstra et al., 2007; Alves et al., 2013a; Visser et al., 2014; Diender et al., 2015). Carboxydotrophic mesophiles, such as Clostridium carboxidivorans, C. ljungdahlii, C. autoethanogenum, Acetobacterium woodii, Sporomusa ovata and Butyribacterium methylotrophicum, have the capability of converting CO into short-chain fatty acids (mainly acetate) and alcohols (ethanol, butanol, 2,3-butanediol) (Henstra et al., 2007; Balk et al., 2010; Köpke et al., 2010, 2011; Munasinghe and Khanal, 2011; Jang et al., 2012; Liu et al., 2014a,b; Diender et al., 2015). In general, CO is a poor substrate for methanogens. Methane production from CO has been reported only for some species of Methanosarcina and Methanothermobacter (Daniels et al., 1977; Rother and Metcalf, 2004; Henstra et al., 2007; Diender et al., 2016). For this reason, the utilization of mixed cultures (sludges) for the production of methane from syngas may be advantageous as normally these systems are more robust and less susceptible to inhibition (Guiot et al., 2011). Although methane production directly from CO is possible, hydrogenotrophic and acetoclastic methanogens can coexist with carboxydotrophic, hydrogenogenic and acetogenic bacteria and use the bacterial products (H2 and acetate) to ultimately produce methane. An additional advantage of using anaerobic sludge for syngas conversion is the possibility of implementing low-cost, open fermentation.

Summary

The substitution of natural gas by renewable biomethane is an interesting option to reduce global carbon footprint. Syngas fermentation has potential in this context, as a diverse range of low-biodegradable materials that can be used. In this study, anaerobic sludge acclimatized to syngas in a multi-orifice baffled bioreactor (MOBB) was used to start enrichments with CO. The main goals were to identify the key players in CO conversion and evaluate potential interspecies metabolic interactions conferring robustness to the process. Anaerobic sludge incubated with 0.7 × 10⁵ Pa CO produced methane and acetate. When the antibiotics vancomycin and/or erythromycin were added, no methane was produced, indicating that direct methanogenesis from CO did not occur. Acetobacterium and Sporomusa were the predominant bacterial species in CO-converting enrichments, together with methanogens from the genera Methanobacterium and Methanospirillum. Subsequently, a highly enriched culture mainly composed of a Sporomusa sp. was obtained that could convert up to 1.7 × 10⁵ Pa CO to hydrogen and acetate. These results attest the role of Sporomusa species in the enrichment as primary CO utilizers and show their importance for methane production as conveyers of hydrogen to methanogens present in the culture.

Received 31 July, 2017; revised 31 August, 2017; accepted 3 September, 2017.
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Microbial Biotechnology (2018) 11(4), 639–646
doi:10.1111/1751-7915.12864

Funding information

Nederlandse Organisatie voor Wetenschappelijk Onderzoek (024.002.002); FP7 Ideas: European Research Council (323009); Norte 2020 - Sistema de Apoio à Investigação Científica e Tecnológica (NORTE-01-0145-FEDER-000004); Fundação para a Ciência e a Tecnologia (PD/BD/128030/2016, SFRH/BPD/104837/2014).

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systems. Methane is a direct substitute of natural gas, and infrastructure to distribute methane to industry and households is existing.

Bioreactor technology for open fermentation of syngas is also developing, with special attention to the requisites of high gas–liquid mass transfer rates and cell retention times. Several reactor types have been studied for syngas open fermentations, including gas-lift reactors (Haddad et al., 2014), reverse membrane bioreactors (Youngsukkasem et al., 2015; Westman et al., 2016) and multi-orifice baffled bioreactor (MOBB) (Pereira, 2014). The MOBB described by Pereira (2014) showed methane production rates of about 1.5–2 times higher than reported for the other systems. In this study, we analysed the microbial communities in the MOBB sludge and performed enrichment and microbial diversity studies to get more insight into key players and metabolic networks involved in CO biomethanation. The main aim was to verify whether methane production from CO occurs predominantly via direct methanogenesis or indirectly via bacterial–archaeal associations. Identifying key microbial interactions could explain and justify the higher robustness of mixed cultures for syngas conversion to methane.

Results and discussion

Multi-orifice baffled (bio)reactors (MOBB) are recognized for their excellent performance in gas–liquid mass transfer and therefore suited for the conversion of gaseous substrates (Ni et al., 2003). In a previous work, Pereira (2014) described the application of a MOBB to continuous syngas conversion to methane using open anaerobic mixed cultures (sludge). Although methane was efficiently produced from CO, the microorganisms involved in this conversion and possible metabolic interactions were not disclosed. In this study, we used a combination of enrichment studies and microbial diversity analyses to identify key microbial players and microbial interactions occurring in the MOBB sludge.

**CO to methane conversion by MOBB enrichments is dependent on bacterial–archaeal interactions**

Incubation of MOBB sludge with 40% CO as sole carbon and energy source resulted in the production of methane and acetate. In Fig. 1, substrate consumption and product formation are shown for a stable enrichment culture obtained after 12 successive transfers of the MOBB sludge on CO (CO12): 68.9 mmol L⁻¹ medium CO resulted in the formation of 10.4 mmol L⁻¹ medium methane and 7.9 mmol L⁻¹ medium acetate.

When the MOBB sludge was incubated in the presence of the antibiotics vancomycin and erythromycin (CO-v₅₀e₁₀₀ and O-CO-v₁₀₀e₁₀₀), no CO conversion or methane production was observed over a long incubation period (over 3 months) (data not shown). Vancomycin and erythromycin are bacterial inhibitors and the fact that no CO conversion was observed in their presence indicates that CO was not directly metabolized by carboxydrotrophic methanogens. Carbon monoxide is inhibitory for most methanogens, and only a few species can actually grow on this substrate: Methanothermobacter thermautotrophicus (Daniels et al., 1977), Methanothermobacter marburgensis (Diender et al., 2016), Methanosarcina barkeri (O’Brien et al., 1984; Bott et al., 1986) and Methanosarcina acetivorans (Rother and Metcalf, 2004). Both thermophilic Methanothermobacter species grow significantly slower on CO than on H₂/CO₂ (Daniels et al., 1977; Diender et al., 2016). Methanosarcina acetivorans can withstand higher CO partial pressures than the hydrogenotrophic methanogens, but its methanogenic metabolism shifts towards formation of acetate and formate at increased CO pressures (Rother and Metcalf, 2004).

Analysis of the archaeal communities in the inoculum and initial CO enrichment cultures by cloning and sequencing (Sanger) revealed the presence of hydrogenotrophic methanogens closely related to Methanobacterium and Methanospirillum species (Fig. 2). However, microorganisms related to known carboxydrotrophic methanogens were not detected, which again supports the hypothesis that methane was not directly produced from CO in these cultures. Methanobacterium and Methanospirillum species are capable of using H₂/CO₂ to produce methane (Whitman et al., 2006). Although H₂ could not be detected in CO enrichments (Fig. 1), microbial H₂ production from CO is possible (water–gas shift reaction, CO + H₂O → H₂ + CO₂) (Diender et al., 2015).

![Fig. 1. Substrate consumption and product formation by stable enrichment CO-degrading cultures: CO(12) (after 12 successive transfers)]. Symbols: (●) carbon monoxide, (○) acetate, (■) methane.
The two predominant bacteria identified in the enrichments were related to *Acetobacterium* and *Sporomusa* species (Fig. 2), which normally have a homoacetogenic metabolism when growing on CO (Genthner and Bryant, 1987; Balk *et al.*, 2010; Diender *et al.*, 2015). However, interspecies H₂ transfer during growth of *Acetobacterium woodii* with different methanogens on sugars has been previously reported (Winter and Wolfe, 1980), indicating that a similar mechanism could take place during CO fermentation. Acetoclastic methanogens were not detected in the enrichment cultures (Fig. 2), and acetate accumulation was observed (Fig. 1). *Methanosarcina* and *Methanosaeta* species are commonly present in anaerobic sludges (Karakashev *et al.*, 2005; Pan *et al.*, 2016; Sancho Navarro *et al.*, 2016), and the reason for their absence in the MOBB sludge is not clear. It might be that these microorganisms suffer from the shear stress caused by the oscillations in the MOBB. In addition, effects of CO toxicity towards acetoclastic methanogens cannot be excluded.

A novel *Sporomusa* *sp.* is present in MOBB sludge that can convert CO to H₂.

From the DGGE profiles of cultures CO(0) and CO(1), it is evident that bacterial communities were dominated by *Acetobacterium* and *Sporomusa* species (Fig. 2). Carbon monoxide metabolism is well studied in *Acetobacterium woodii*. This organism was initially described to grow homoacetogenically on CO as a sole energy source (Genthner and Bryant, 1987), but it has been recently found that it can use CO only in cofermentation with H₂ or formate (Bertsch and Müller, 2015). *Acetobacterium* population in the CO enrichments is most closely related to *Acetobacterium wieringae* (99% 16S rRNA gene identity). Recently, Sancho Navarro *et al.* (2016) reported the presence of bacterial species closely related to *A. wieringae* after incubation of anaerobic sludge at high CO concentrations. While only *A. woodii* is reported as carboxydrotrophic organism, *A. wieringae* genome contains the gene clusters for both carbonyl and methyl branches of the Wood–Ljungdahl pathway, identical to what is found in *A. woodii* (Genthner and Bryant, 1987; Poehlein *et al.*, 2016). *Acetobacterium* population became more predominant during the enrichment process (Fig. S1), and based on MiSeq results, it represented about 82% of the community in enrichment culture CO(12) (Table 1A).

The presence of closely related organisms to *Sporomusa* species in the initial enrichments was rather striking. Carbon monoxide metabolism in *Sporomusa* species is poorly studied, although CO conversion to acetate has been reported for *S. termitida*, *S. ovata* and *Sporomusa* strain An4 (Breznak *et al.*, 1988; Balk *et al.*, 2010).
Sporomusa strains are known to form spores (Möller et al., 1984), and it was clear from microscopic examination that spores were present in early CO enrichment cultures (data not shown). With the aim of enriching/isolating the Sporomusa species in the CO enrichments, we proceeded with the pasteurization of culture CO(4), and later on, a second pasteurization was performed with culture CO-P(4) (see experimental set-up in Fig. 4). Culture CO-P(8) was highly enriched in Sporomusa (97% of the total microorganisms) (Table 1B) and was able to grow under a 100% CO headspace. Culture CO-P(8)-produced H₂ from CO (Fig. 3), which considering the composition of the initial CO enrichment cultures, could benefit growth of both Acetobacterium species and hydrogenotrophic methanogens. H₂ production by Sporomusa strains from CO was not shown before, but these bacteria have an important role in CO conversion in the mixed culture. A remarkable observation is that the Sporomusa species in CO-P(12) can grow with $1.7 \times 10^5$ Pa CO. Growth inhibition of S. termitida was observed for CO partial pressures higher than $0.4 \times 10^5$ Pa (Breznak et al., 1988). Further research is needed to compare the enriched Sporomusa strain with other Sporomusa-type strains.

### Experimental procedures

#### Source of inoculum and medium composition

Granular sludge obtained from a 10-L MOBB fed with syngas mixture (60% CO, 30% H₂ and 10% CO₂ (v/v)) (Pereira, 2014) was used as inoculum for batch incubations with CO. Cultures were prepared in 120-ml serum bottles containing 30 ml of bicarbonate-buffered mineral salt medium and sealed with butyl rubber stoppers and aluminium crimp caps. Medium was reduced prior to inoculation with 0.8 mM (final concentration) sodium sulphide ($\text{Na}_2\text{S} \times \text{H}_2\text{O}$, $x = 7–9$). Cultures were monitored by measuring CO depletion and methane, hydrogen and acetate production.

#### Batch assays

An overview of the experimental set-up implemented in this study is illustrated in Fig. 4. To test the direct methanogenesis from CO, methanogenic sludge was incubated with the antibiotics vancomycin (v) and erythromycin (e) (bacterial growth inhibitors): CO-v50e100 (50 l M vancomycin + 100 l M erythromycin) and CO-v100e100 (100 l M of each antibiotic). Incubations without

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Table 1. Microbial diversity of enrichments (A) CO(12) and (B) CO-P(8).

| Closest relatives | Amount (%) | Coverage (%) | Identity (%) |
|------------------|------------|--------------|--------------|
| **(A)**  |  |  |  |
| Bacteria | Acetobacterium sp (Acetobacterium sp. enrichment culture isolate DGGE gel band K1-IRE21-Sa 16S ribosomal RNA gene, partial sequence) | 82<sup>a</sup> | 100 | 100 |
| Archaea | Methanospirillum sp (Methanospirillum hungatei strain JF-1 16S ribosomal RNA gene, complete sequence) | 7<sup>a</sup> | 99 | 100 |
| **(B)**  |  |  |  |
| Bacteria | Sporomusa sp (Sporomusa ovata strain DSM 2662 16S ribosomal RNA gene, partial sequence) | 97<sup>b</sup> | 100 | 96 |
| Archaea | Caloramator quimbayensis (Caloramator quimbayensis strain USBA A 16S ribosomal RNA gene, partial sequence) | 2<sup>b</sup> | 98 | 97 |

<sup>a</sup> Percentage calculated based on a total number of counts of 28146.

<sup>b</sup> Percentage calculated based on a total number of counts of 41718.

<sup>c</sup> Results of sequence analysis on NCBI BLAST.
antibiotics were also performed. Bottles' headspace contained 40% CO (pCO = 6.8 \times 10^4 \text{ Pa}) as substrate, and cultures were incubated at 37°C. None of the incubations with antibiotics could use CO or produce methane. Incubations without antibiotics were successively transferred (10% (v/v)) to new bicarbonate-buffered medium and fed with CO for 2 years with 40% CO, resulting in an enrichment series designated as CO(x) (where x stands for number of successive transfers). Since an early enrichment stage, the presence of sporulating cells in the CO cultures was evident (microscopic observation). An aliquot of culture CO(4) was pasteurized and used as inoculum to start the enrichment series CO-P(x) (where P stands for pasteurization; x stands for number of successive transfers). Pasteurization procedure consisted in heating up the culture to 80°C for 20 min. Culture CO-P(1) was transferred four times to fresh medium, and at this time, a second pasteurization was performed (culture CO-P(4)). Pasteurization temperature was now increased to 95°C (for 20 min). In transfers immediately after each pasteurization, yeast extract and ethanol were used to stimulate growth, being removed in posterior transfers. CO was the sole carbon and energy source added (40%; pCO = 6.8 \times 10^4 \text{ Pa}) in the next transfers (CO-P(5) and CO-P(6)). Then, the CO partial pressure in the headspace was raised gradually from 40% (pCO = 6.8 \times 10^4 \text{ Pa}) to 100% CO (pCO = 1.7 \times 10^5 \text{ Pa}) (CO-P(7) to CO-P(12)).

**Analytical methods**

Gas samples were analysed by gas chromatography with a Bruker Scion 456-GC (Billerica, MA, USA) with a thermal conductivity detector and equipped with two columns: a BR-QPLOT column (30 m length, 0.53 mm internal diameter; film thickness, 20 µm) and a Molsieve packed column (13 x 80/100, 2 m length, 2.1 mm internal diameter). The Molsieve column was used to measure CO, H₂ and CH₄, and argon was used as carrier gas at a flow rate of 30 ml min⁻¹; temperatures in the injector, column and detector were 100, 35 and 130°C respectively. Volatile fatty acids (VFA), such as acetate, were determined by high-performance liquid chromatography using an HPLC (Jasco, Tokyo, Japan) with a Phenomenex Rezex ROA – organic acid H⁺ (8%) column (300 x 7.8 mm). The mobile phase used was sulfuric acid (0.005 N) at a flow rate of 0.6 ml min⁻¹. Column temperature was set at 60°C. Detection of VFA was made sequentially with an UV detector at 210 nm.
**DNA extraction and amplification**

Five millilitres of well-homogenized MOBB sludge, CO(x) and CO-P(x) cultures was stored at \(-20^\circ\text{C}\). DNA extraction from these samples was performed using the FastDNA SPIN kit for soil (MP Biomedicals, Solon, OH) according to the manufacturer’s protocol. The microbial 16S rRNA genes were amplified by PCR using a Taq DNA polymerase kit (Invitrogen, Carlsbad, CA). PCR programs and reactions mixtures used were as described elsewhere (Sousa et al., 2007), and all primers set were synthesized by Invitrogen. For 16S rRNA gene amplification for denaturing gradient gel electrophoresis (DGGE), primers set 1401r/968-GCf was used for bacteria and 515-GCr/A109(T)f for archaea (Sousa et al., 1994). The yield and size of PCR products were assessed by electrophoresis in 1% agarose gel (wt/vol), using a 1 kb extended DNA ladder (ThermoScientific, Waltham, MA, USA) and a green safe staining.

**DGGE analysis**

DGGE analysis was performed using a DCode system (Bio-Rad, Hercules, CA). For the purpose, gels of 8% (wt/vol) polyacrylamide (37:5:1 acrylamide/bis-acrylamide) were used with a denaturing gradient of 30% to 50% for archaea and 30% to 60% for bacteria, with 100% of denaturant corresponding to 7 M urea and 40% (v/v) formamide. Electrophoresis ran in a 0.5 TAE buffer at 60°C for 16 h at 85 V. Posteriorly gels were stained with silver nitrate (Sanguinetti et al., 1994) and scanned in an Epson Perfection V750 PRO (Epson, Long Beach, CA, USA).

**16S rRNA gene sequencing**

Cloning and Sanger sequencing were performed using the methodologies previously described by Sousa et al. (2007). Similarity searches were performed using the NCBI BLAST search program within the GenBank database (http://www.ncbi.nlm.nih.gov/blast/) (Altschul et al., 1990). Illumina MiSeq platform sequencing was performed at the Research and Testing Laboratory – RTLGenomics (Lubbock, TX). The MiSeq method used was the Illumina two-step using universal primers for bacteria and archaea, 515f and 806r developed by Caporaso et al. (2011). After sequencing, the data were processed using the data analysis pipeline from RTL, which consists in two major steps, the denoizing and chimera detection step and the microbial diversity analysis step, as described in the company procedures.

**Nucleotide sequence accession numbers**

The 16S rRNA gene sequences obtained in this study have been deposited in the European Nucleotide Archive (ENA) under the accession numbers LT671598–LT671603 (Sanger sequencing) and ERS1422865–ERS1422866 (Illumina MiSeq platform); these sequences can be viewed by following URL http://www.ebi.ac.uk/ena/data/view/PRJEB16760).

**Acknowledgements**

The authors would like to acknowledge Nuno Reis and Filipa Pereira for providing the anaerobic granular sludge used in this research. This study was supported by BioTecNorte operation (NORTE-01-0145-FEDER-000004) funded by the European Regional Development Fund under the scope of Norte2020 – Programa Operacional Regional do Norte. The financial support from FCT and European Social Fund (POPH-OREN) through SFRH/BD/104837/2014 and PD/BD/128030/2016 grants given to JA and ALA are gratefully acknowledged. The financial support from ERC Grant (project 323009) and Gravitation Grant (project 024.002.002) of the Netherlands Ministry of Education, Culture and Science and the Netherlands Science Foundation (NWO) and Wageningen Institute for Environment and Climate Research (WIMEK) is also gratefully acknowledged.

**Conflict of interest**

None declared.

**References**

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., and Lipman, D.J. (1990) Basic local alignment search tool. J Mol Biol 215: 403–410.

Alves, J.I., van Gelder, A.H., Alves, M.M., Sousa, D.Z., and Pluggge, C.M. (2013a) Moorella stamsii sp. nov., a new anaerobic thermophilic hydrogenogenic carboxydotothroph isolated from digester sludge. Int J Syst Evol Microbiol 63: 4072–4076.

Alves, J.I., Stams, A.J.M., Pluggge, C.M., Alves, M.M., and Sousa, D.Z. (2013b) Enrichment of anaerobic syngas-converting bacteria from thermophilic bioreactor sludge. FEMS Microbiol Ecol 86: 590–597.

Balk, M., Mehoob, F., Van Gelder, A.H., Rijpstra, W.I.C., Damsté, J.S.S., and Stams, A.J.M. (2010) (Per)chlorate reduction by an acetogenetic bacterium, Sporomusa sp., isolated from an underground gas storage. Appl Microbiol Biotechnol 88: 595–603.

Bertsch, J., and Müller, V. (2015) Bioenergetic constraints for conversion of syngas to biofuels in acetogenic bacteria. Biotechnol Biofuels 8: 210.

Bott, M., Eikmanns, B., and Thauer, R.K. (1986) Coupling of carbon monoxide oxidation to CO₂ and H₂ with the phosphorilation of ADP in acetate-grown Methanosarcina barkeri. Eur J Biochem 159: 393–398.

Bredwell, M.D., Srivastava, P., and Worden, R.M. (1999) Reactor design issues for synthesis gas fermentations. Biotechnol Prog 15: 834–844.
Breznak, J.A., Switzer, J.M., and Setz, H.-J. (1988) Sporomusa temitida sp. nov., an H₂/CO₂-utilizing acetogen isolated from termites. Arch Microbiol 150: 282–288.

Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Lozupone, C.A., Turnbaugh, P.J., et al. (2011) Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proc Natl Acad Sci USA 108 (Suppl. 1): 4516–4522.

Daniels, L., Fuchs, G., Thauer, R.K., and Zeikus, J.G. (1977) Carbon monoxide oxidation by methanogenic bacteria. J Bacteriol 132: 118–126.

Diender, M., Stams, A.J.M., and Sousa, D.Z. (2015) Pathways and bioenergetics of anaerobic carbon monoxide fermentation. Front Microbiol 6: 1–18.

Diender, M., Pereira, R., Wessels, H.J.C.T., Stams, A.J.M., and Sousa, D.Z. (2016) Proteomic analysis of the hydrogen and carbon monoxide metabolism of Methanothermobacter marburgensis. Front Microbiol 7: 1–10.

Gentheren, B.R.S., and Bryant, M.P. (1987) Additional characteristics of one-carbon-compound utilization by Eubacterium limosum and Acetobacterium woodii. Appl Environ Microbiol 53: 471–476.

Guiot, S.R., Cimpoia, R., and Carayon, G. (2011) Potential of wastewater-treating anaerobic granules for biomethanation of synthesis gas. Environ Sci Technol 45: 2006–2012.

Haddad, M., Cimpoia, R., and Guiot, S.R. (2014) Performance of Carboxydotermus hydrogenoformans in a gas-lift reactor for syngas upgrading into hydrogen. Int J Hydrogen Energy 39: 2543–2548.

Henstra, A.M., Sipma, J., Rinzena, A., and Stams, A.J. (2007) Microbiology of synthesis gas fermentation for biofuel production. Curr Opin Biotechnol 18: 200–206.

Jang, Y.-S., Malaviya, A., Cho, C., Lee, J., and Lee, S.Y. (2012) Butanol production from renewable biomass by clostridia. Bioreseour Technol 123: 653–663.

Karakashev, D., Batstone, D.J., and Angelidakis, I. (2005) Influence of environmental conditions on methanogenic compositions in anaerobic biogas reactors. Appl Environ Microbiol 71: 331–338.

Köpke, M., Held, C., Hujer, S., Liesegang, H., Wiezer, A., Wollherr, A., et al. (2010) Clostridium ljungdahlii represents a microbrial production platform based on syngas. Proc Natl Acad Sci USA 107: 13087–13092.

Köpke, M., Mihalcea, C., Liew, F., Tizard, J.H., Ali, M.S., Conolly, J.J., et al. (2011) 2,3-Butanediol production by aceticogenic bacteria, an alternative route to chemical synthesis, using industrial waste gas. Appl Environ Microbiol 77: 5467–5475.

Liu, K., Atiyeh, H.K., Stevenson, B.S., Tanner, R.S., Wilkins, M.R., and Huhnke, R.L. (2014a) Continuous syngas fermentation for the production of ethanol, n-propanol and n-butanol. Bioreseour Technol 151: 69–77.

Liu, K., Atiyeh, H.K., Stevenson, B.S., Tanner, R.S., Wilkins, M.R., and Huhnke, R.L. (2014b) Mixed culture syngas fermentation and conversion of carboxylic acids into alcohols. Bioreseour Technol 152: 337–346.

Müller, B., Oijler, R., Howard, B.H., Gottschalk, G., and Hippe, H. (1984) Sporomusa, a new genus of gram-negative anaerobic bacteria including Sporomusa sphaeroides spec. nov. and Sporomusa ovata spec. nov. Arch Microbiol 139: 388–396.

Munasinghe, P.C., and Khanal, S.K. (2010) Syngas fermentation to biofuel: evaluation of carbon monoxide mass transfer coefficient (kLa) in different reactor configurations. Biotechnol Prog 26: 1616–1621.

Munasinghe, P.C., and Khanal, S.K. (2011) Biomass-derived syngas fermentation into biofuels. Biofuels 101: 79–98.

Ni, X., Mackley, M.R., Harvey, A.P., Stonestreet, P., Baird, M.H.I., and Rama Rao, N.V. (2003) Mixing through oscillations and pulsations—a guide to achieving process enhancements in the chemical and process industries. Chem Eng Res Des 81: 373–383.

O’Brien, J.M., Wolkin, R.H., Moench, T.T., Morgan, J.B., and Zeikus, J.G. (1984) Association of hydrogen metabolism with unitrophic or mixotrophic growth of Methanosaolina barkeri on carbon monoxide. J Bacteriol 158: 375–378.

Pan, X., Angelidakis, I., Alvarado-Morales, M., Liu, H., Liu, Y., Huang, X., and Zhu, G. (2016) Methane production from formate, acetate and H₂/CO₂: focusing on kinetics and microbial characterization. Bioreseour Technol 218: 796–806.

Parshina, S.N., Sipma, J., Nakashimada, Y., Henstra, A.M., Smidt, H., Lysenko, A.M., et al. (2005) Desulfotomaculum carboxydivorans sp. nov., a novel sulfate-reducing bacterium capable of growth at 100% CO. Int J Syst Evol Microbiol 55: 2159–2165.

Pereira, F.M. (2014) Intensified bioprocess for the anaerobic conversion of syngas to biofuels. PhD Thesis. University of Minho. URL http://repositorium.sdu.muninho.pt/handle/1822/34834.

Poehlein, A., Bengelsdorf, F.R., Schiel-Bengelsdorf, B., Daniel, R., and Dürr, P. (2016) Genome sequence of the acetogenic bacterium Acetobacterium wieringae DSM 1911T. Genome Announc 4: 2015–2016.

Rother, M., and Metcalf, W.W. (2004) Anaerobic growth of Methanosarcina acetivorans C2A on carbon monoxide: an unusual way of life for a methanogenic archaeon. Proc Natl Acad Sci USA 101: 16929–16934.

Sancho Navarro, S., Cimpoia, R., Bruant, G., and Guiot, S.R. (2016) Biomethanation of syngas using anaerobic sludge: shift in the catabolic routes with the CO partial pressure Increase. Front Microbiol 7: 1–13.

Sanguinetti, C.J., Neto, E.D., and Simpson, A.J.G. (1994) Rapid silver staining and recovery of PCR products separated on polyacrylamide gels. Biotechniques 17: 914–921.

Sousa, D.Z., Alcina Pereira, M., Stams, A.J.M., Alves, M.M., and Smidt, H. (2007) Microbial communities involved in anaerobic degradation of unsaturated or saturated long-chain fatty acids. Appl Environ Microbiol 73: 1054–1064.

Stams, A.J.M., Van Dijk, J.B., Dijkema, C., and Plugge, C.M. (1993) Growth of syntrophic propionate-oxidizing bacteria with fumarate in the absence of methanogenic bacteria. Appl Environ Microbiol 59: 1114–1119.

Svetlichnyi, V., Peschel, C., Acker, G., and Meyer, O. (2001) Two membrane-associated NiFeS-carbon monoxide dehydrogenases from the anaerobic carbon-monoxide-utilizing eubacterium Carboxydothermus hydrogenoformans. J Bacteriol 183: 5134–5144.
Visser, M., Parshina, S.N., Alves, J.I., Sousa, D.Z., Pereira, I.A.C., Muyzer, G., et al. (2014) Genome analyses of the carboxydotrophic sulfate-reducers Desulfotomaculum nigricans and Desulfotomaculum carboxydivorans and reclassification of Desulfotomaculum caboxydivorans as a later synonym of Desulfotomaculum nigricans. Stand Genomic Sci 9: 655–675.

Westman, S., Chandolias, K., and Taherzadeh, M. (2016) Syngas biomethanation in a semi-continuous reverse membrane bioreactor (RMBR). Fermentation 2: 8.

Whitman, W.B., Bowen, T.L., and Boone, D.R. (2006) The methanogenic bacteria. In The Prokaryotes. Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K.H. and Stackebrandt, E. (eds). New York, NY: Springer, pp. 165–207.

Winter, J.U., and Wolfe, R.S. (1980) Methane formation from fructose by syntrophic associations of Acetobacterium woodii and different strains of methanogens. Arch Microbiol 124: 73–79.

Youngsukkasem, S., Chandolias, K., and Taherzadeh, M.J. (2015) Rapid bio-methanation of syngas in a reverse membrane bioreactor: membrane encased microorganisms. Bioresour Technol 178: 334–340.

Supporting information

Additional Supporting Information may be found online in the supporting information tab for this article:

Fig. S1. Bacterial and archaeal DGGE profiles of the enrichments CO(x) and CO-P(x), where (x) corresponds to number of successive transfers (nomenclature in Fig. 4).