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MICROBIAL SYNGAS CONVERSION BY MESOPHILIC AND THERMOPHILIC ANAEROBIC MIXED-CULTURES

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Syngas, carbon monoxide, anaerobic bioconversion, microbial diversity.

ABSTRACT

Synthesis gas (or syngas) can be produced from the gasification of a variety of recalcitrant or biodegradable wastes. Syngas is a mixture composed of mainly H_2, CO and CO_2 that can be used in a biological process for the production of fuels or usable chemicals. The main goal of this work was to study the physiology and microbial composition of anaerobic cultures able to utilize syngas. The results indicated that the thermophilic sludge inoculum presents a promising carboxydotrophic potential comparing to the mesophilic sludge inoculum. Monitoring of microbial structure of thermophilic enriched cultures by using PCR-DGGE and cloning techniques showed that bacterial community profiles clustered in three different groups.

INTRODUCTION

Syngas (synthesis gas) is produced during the gasification of different materials, e.g. coal, oil and natural gas, tar sand, recalcitrant waste, lignocellulosic biomass. Syngas is a gas mixture mainly composed by H_2 and CO, but also CO_2 and traces of methane, nitrogen and hydrogen sulfide, depending on its origin and on the conditions of gasification process (Sipma et al., 2006). Syngas is nowadays produced from non-renewable sources, such as natural gas and coal, but if produced from biomass (that is also a possibility) syngas represents a renewable source of energy (Hussain et al., 2011). The interest in the biological conversion of syngas relies on the knowledge that some anaerobic microorganisms can effectively use CO and H_2 to produce added-value carbon compounds, such as ethanol, butanol, acetic acid, butyric acid, hydrogen and methane (Basu et al. 1993; Henstra et al. 2007; Worden et al. 1997). Microbiology of syngas bioconversion to biofuels has been recently reviewed (Henstra et al. 2007; Oelgeschlager and Rother 2008; Sokolova et al. 2009). Several mesophilic anaerobic microorganisms, e.g. *Clostridium carboxidivorans* and *Butyribacterium methylotrophicum*, were shown to produce short-chain fatty-acids and alcohols from CO and H_2. Mesophilic and thermophilic carboxydotrophic hydrogenogenic bacteria, e.g. *Rubrivivax gelatinosus*, *Rhodopseudomonas palustris*, *Rhodospirillum rubrum*, *Carboxydothermus hydrogenoformans*, *Carboxydocella thermoautotrophica*, and *Desulfotomaculum carboxydivorans*, can convert CO and H_2O to H_2 and CO_2. Direct conversion of CO to CH_4 can be achieved by a few methanogenic archaea, namely *Methanosarcina barkeri*, *Methanosarcina acetivorans* and *Methanothermobacter thermoautotrophicus*. Syngas fermentation offers several advantages over catalytic conversion. The greater resistance to catalyst poisoning, independence of a fixed H_2:CO ratio, and higher catalytic specificity are generally mentioned (Henstra et al. 2007). The aim of this work was to study the physiology and microbial composition of anaerobic cultures able to utilize syngas.

METHODS

Mesophilic (37 ºC) and thermophilic (55 ºC) enrichment experiments were performed with syngas mixtures as sole carbon and energy sources; CO was supplied to the cultures at different final partial pressures ranging from 5 to 50% (total pressure 1.75×10^5 Pa). Anaerobic suspended sludge from a lab scale reactor (Braga, Portugal) was used as seed sludge for starting the enrichment series under mesophilic conditions. The thermophilic enrichments were inoculated with anaerobic suspended sludge from a thermophilic anaerobic reactor treating organic fraction of municipal solid wastes (Barcelona, Spain). Headspace gas composition was analysed by GC and fatty-acids and alcohols present in the liquid by HPLC. Cultures were subsequently transferred to fresh medium once CO was completely used. Microbial community changes in the enrichment series were monitored by 16S rRNA-based techniques (PCR-DGGE). Microorganisms present in stable enrichment cultures were identified by cloning and sequencing. Sequence similarity for the 16S rRNA gene sequences was analyzed by using the NCBI BLAST search program within the GenBank database.

RESULTS & CONCLUSIONS

Two different enrichment cultures were obtained by successive transfers of active culture (10%) into new medium containing a synthetic mixture of syngas as the sole carbon and energy sources. Incubation was performed at 37 °C and 55 °C. Under mesophilic conditions, CO could not be used at partial pressures higher than 10%. However, thermophilic enrichment cultures could convert CO at partial pressures up to 50%. With these results, two thermophilic enrichments (T1 and T2) were performed and fed with a syngas mixture during the first 4 transfers. After this period of 4 transfers, the substrate continuously given to T1 was the synthetic mixture of syngas, but the substrate given to enrichment T2 was carbon monoxide. During two enrichment series CO concentration was increased until 50%. In both stable enrichment cultures, acetate and CO_2 were the main products formed. It was also observed that the degradation of CO was faster on T1, possibly due to substrate used during the initial transfers was the same as during the entire experiment. The diversity of the microbial community present, checked by DGGE and evaluated as the number of dominant bands in the DGGE profiles, decreased drastically from the inoculum sample, suggesting a fast specialization of microbial community on this type of substrate. Analysis of the microbial composition of stable syngas and CO enrichment cultures showed that predominant microorganisms present both in T1 and T2 were most closely related to *Desulfotomaculum* and *Thermoanaerobacterium* genus, all belonged to Firmicutes phylum. No archaea could be detected. Isolation of these bacteria is ongoing and their physiology regarding CO conversion will be further studied. This study gave insight into the microbiology and physiology of syngas and carbon monoxide conversion by anaerobic mixed cultures.

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