Anaerobic granular sludge as a microbial platform for the conversion of gaseous substrates at moderate pressures

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Abstract: As the end of fossil fuels-era is approaching, new biotechnological alternatives for biofuels and biochemicals production are emerging. Here we focus on the use of gaseous substrates, namely H_2/CO_2 and $CO/H_2/CO_2$ (syngas) as carbon and energy sources for the production of compounds with economical value, such as biomethane and fatty-acids by anaerobic granular sludge at moderate pressures up to 5×10^5 Pa. It was proven that anaerobic granular sludge can stand increased H_2/CO_2 pressure maintaining a high rate of methane production. On the other hand, the rise of syngas pressure up to 5×10^5 Pa led to a reduction on CO and H_2 consumption rates and to a shift towards the formation of propionate and butyrate. The increase of initial H_2/CO_2 or syngas total pressure resulted in a specialization of the microbial communities. Anaerobic granular sludge was a stable and efficient microbial platform for the conversion of gaseous substrates.

Keywords: Gaseous substrates; Moderate pressure; Granular sludge

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Introduction

The replacement of fossil fuels by renewable energy sources is a worldwide priority. The excessive use of fossil fuels has become a concern related to the emission of carbon dioxide (CO_2), a major anthropogenic greenhouse gas. Bioconversion of gaseous substrates, namely syngas fermentation (CO, H_2 and CO_2) and hydrogenotrophic methanogenesis (microbial reduction of CO_2 with H_2), are examples of bioprocesses that appear to be promising alternatives compared to the existing chemical techniques. Additionally, these bioprocesses might contribute to achieve goals of CO_2 emission reduction and CO_2 sequestration, while producing methane and other alternative fuels and commodity chemicals (short- and medium-fatty acids and alcohols) (Verma et al. 2016; Lee et al. 2012).

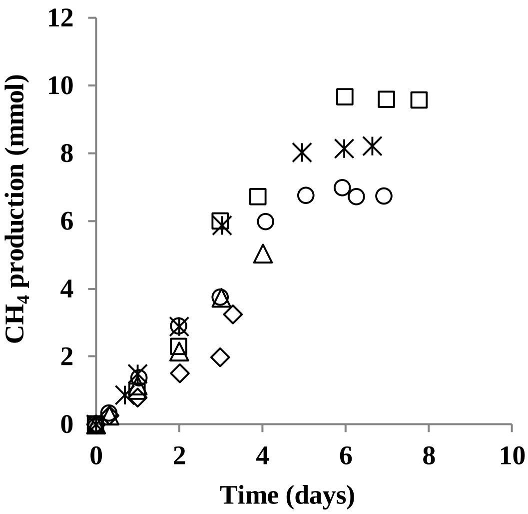
Even though syngas fermentation and hydrogenotrophic methanogenesis are relatively well-known processes, there are still some drawbacks that need to be overcome such as H_2 and CO gas–liquid mass transfer limitations (Xu et al. 2015; Yasin et al., 2015). In this study, different pressurized systems (employing initial H_2/CO_2 or syngas pressures up to 5×10^5 Pa) were used for the bioconversion of gaseous substrates by anaerobic granular sludge, aiming the improvement of gas-liquid mass transfer and consequently the increase of CH_4 and biochemicals production rates. Anaerobic granular sludge was the biocatalyst used in these biological processes, and has proven to be versatile and resilient, giving opportunity to study a more diverse and complex net of reactions and possible pathways.

Material and Methods

Anaerobic granular sludge from a brewery industry, was used as inoculum for all experiments. A mixture of H_2/CO_2 (80 % H_2 and 20 % CO_2, v/v) or a synthetic mixture of syngas (60 % CO, 30 % H_2 and 10 % CO_2, v/v) were used as sole carbon and energy source. A phosphate-buffered mineral salt medium (20 mM, pH 7.0) was used through the all process. Different pressurized bioreactors were used and operated in batch mode at 37 °C. Several experiments under increased initial pressure up to 5×10^5 Pa for both gaseous substrates, were carried out in order to evaluate its effect on methane production, metabolites production and microbial communities structure. Gaseous compounds were analysed by gas chromatography, liquid products were analysed by HPLC and microbial diversity of granular sludge was monitored by 16S r-RNA based techniques (sequencing by using an Illumina MiSeq platform).

Results and Conclusions

Anaerobic granular sludge showed to be resistant and efficient during the conversion of both H_2/CO_2 and $CO/H_2/CO_2$ (syngas) at moderate pressures. Initial CO pressures up to 2×10^5 Pa (Navarro *et al*. 2016; Chang *et al*. 2001; Hurst & Lewis 2010) or $CO/H_2/CO_2$ pressures up to 2.94×10^5 Pa (Younesi *et al*. 2005; Dadak *et al*. 2016; Singla *et al*. 2014) were reported in literature. However, it is worth to notice that this is the first time that such high values of total pressure (5×10^5 Pa) were employed in syngas and H_2/CO_2 bioconversion by anaerobic granular sludge.
Conversion of H_2 into methane was only complete at 1×10^5 Pa and 2×10^5 Pa. The rise of pressure from 2×10^5 Pa to 5×10^5 Pa led to a slight decrease of methane yield from H_2/CO_2, achieving 80 % conversion at 5×10^5 Pa (Figure 1). Though H_2/CO_2 conversion into methane was not complete, no inhibition was observed.



H_2/CO_2 (80:20% v/v)	Time of incubation (days)	CH_4 conversion (%)
1×10^5 Pa	3.3	100
2×10^5 Pa	4.0	100
3×10^5 Pa	6.9	93
4×10^5 Pa	6.6	86
5×10^5 Pa	7.8	80

Figure 1 – Hydrogenotrophic methane production profile by anaerobic granular sludge in the experiments carried out at (◊) 1×10^5 Pa, (△) 2×10^5 Pa, (○) 3 $\times10^5$ Pa, (✳) 4×10^5Pa and (□) 5×10^5 Pa.

As in H_2/CO_2 cultures, experiments with syngas did not show any inhibition by the use of moderate pressures. H_2 and CO were completely consumed, even at 5×10^5 Pa, proving that granular sludge has a hydrogenotrophic and carboxydotrophic potential. Moreover, no lag phase on gases consumption was observed at 1×10^5 Pa and 3×10^5 Pa (Figure 2). At 5×10^5 Pa, however, the consumption of both gases was slower and approximately 300 h were required to a complete depletion of CO and H_2 from the gas phase (Figure 2).

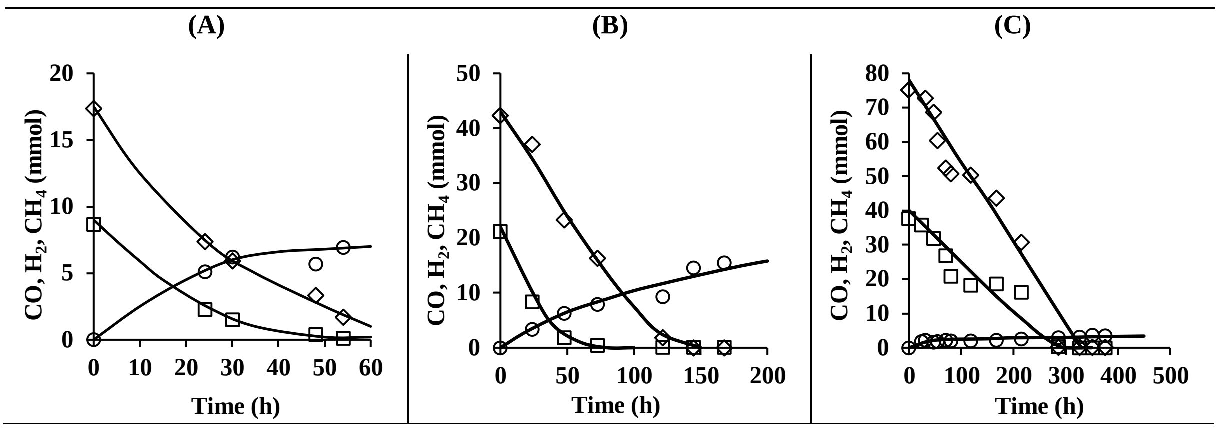


Figure 2 – Profiles of (◊) CO and (□) H_2 consumption and (○) CH_4 production by anaerobic granular sludge at **(A)** 1×10^5 Pa, **(B)** 3×10^5 Pa and **(C)** and 5×10^5 Pa (syngas initial total pressure).

Methane yields reached 100 % of stoichiometric yield in the experiments performed at 1×105 Pa, but decreased drastically when rising the initial syngas pressure. At 5×105 Pa, methane yield was only 12 % of maximum stoichiometric yield. When methanogenic activity was partially inhibited above 1×105 Pa, butyrate and propionate were the liquid products from syngas fermentation (Table 1).

Table 1 – Butyrate and propionate production in the experiments carried out under increased syngas pressure with anaerobic granular sludge

Pressure (Pa)	iso-butyrate (mM)	propionate (mM)
1×10^5 Pa	0	0
3×10^5 Pa	0.48	4.35
5×10^5 Pa	1.15	4.83

The Illumina sequence results showed that organisms related to *Methanobacterium* and *Methanosaeta* genera were predominant in the archaeal community, for both gaseous substrates. However, hydrogenotrophic methanogens from *Methanobacterium* genus increased 2-fold their relative abundance as a result of rising total H_2/CO_2 or syngas pressure in the system. In the case of syngas incubations, the predominant phylotypes in the bacterial community were organisms belonging to *Eubacteriaceae*, *Synergistaceae* and *Syntrophobacteraceae* families. The overall results showed changes in the structure of microbial communities, demonstrating a microbial enrichment and adaptation to the successively higher pressures.

This work showed that increased pressure leads to different metabolic pathways in the conversion of gaseous substrates by anaerobic granular sludge. The results allow us to understand how anaerobic mixed cultures respond with different gaseous substrates at moderate pressures (up to 5×10^5 Pa). Moreover, these results suggest that it is possible to inject pressurized gas streams directly in fermentation reactors for the bioconversion into biogas or biochemicals, which represent an interesting solution for industrial waste gas streams.

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