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Methanogenic capacity and robustness of hydrogenotrophic cultures based on closed nutrient recycling via microbial catabolism: Impact of temperature and microbial attachment

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ABSTRACT

A biological methanation system based on nutrient recycling via mixed culture microbial catabolism was investigated at mesophilic (37 °C) and thermophilic (55 °C) temperatures. At mesophilic temperatures, the formation of biofilms on two different types of material was assessed. Results showed that with intense mixing the biofilm reactors presented methanogenic capacities (per working volume) 50% higher than the ones operated with suspended cultures. Gas feeding rates of 200 L/L/d were achieved at a H_2/CO_2 to CH_4 conversion efficiency of above 90% by linking two reactors in series. Furthermore the robustness of the cultures was assessed under a series of inhibitory conditions that simulated possible process interferences at full scale operation. Full recovery after separate intense oxygenation and long starvation periods was observed within 2–5 days.

1. Introduction

The utilization of CO_2 as a precursor for chemicals has recently started to gain growing attention since it can add economic benefits to carbon sequestration (Jajesniak et al., 2014; Styring et al., 2011). In particular, the Power to Methane (PtM) route as described in (Götz et al., 2016) offers the additional advantage of directing a substantial percentage of renewable electricity towards green fuel production. This presents an attractive solution to the storage of excess renewably generated power. The methanation of CO_2 can be performed thermo-chemically via catalytic hydrogenation (Wang et al., 2011), however, through a biological route known as hydrogenotrophic methanogenesis, high quality CH_4 can also be produced at ambient pressures and temperatures and without the need of metal catalysts (Lecker et al., 2017). The process makes use of a distinctive microbial group that uses CO_2 and H_2 as their carbon and energy source respectively. The group consists of a number of archaeal species called hydrogenotrophic methanogens which are capable of working on their own (pure cultures) or in conjunction with other archaeal and bacterial species (mixed cultures) (Liu and Whitman, 2008).

Sources of CO_2 include but are not limited to industrial combustion processes, distilleries, cement production and waste water treatment. Among them, anaerobic digestion (AD) plants are ideal candidates for the initial implementation of the PtM technology as they can produce high quality CO_2 without inhibitory for the microbes contaminants. Furthermore, due to its composition (30–40% CO_2/60–70% CH_4) the biogas output from an anaerobic digester could be directly upgraded to natural gas quality without the need for CO_2 pre-separation (Martin et al., 2013). Sources of H_2 include among others natural gas reforming, gasification, water electrolysis and a number of biological routes (U.S. Department of Energy, 2010). Water electrolysis presents a number of advantages over other technologies namely, extra pure H_2 streams, ease of coupling with renewable electricity streams and the added production of pure O_2 which can be used onsite (e.g. waste water treatment, oxy-combustion) (Global Carbon Capture and Storage Institute, 2012; Patterson et al., 2017).

Significant research has been conducted over the last decade (Bassani et al., 2015; Burkhardt et al., 2015; Guneratnam et al., 2017; Kougias et al., 2017; Luo and Angelidaki, 2012; Martin et al., 2013; Seifert et al., 2014; Strübing et al., 2017; Yun et al., 2017) regarding hydrogenotrophic methanogenesis and its potential as a continuous process. A number of feasibility studies also indicate a good integration of the process within biogas plants (Estermann et al., 2016; O'Shea et al., 2017; Patterson et al., 2017). Nevertheless, commercialization of the process has yet to occur. This can partially be attributed to the implication of the biological factor which adds a degree of uncertainty when it comes to long operational periods and intermittent operation. The biochemical variables that directly affect metabolic activity are

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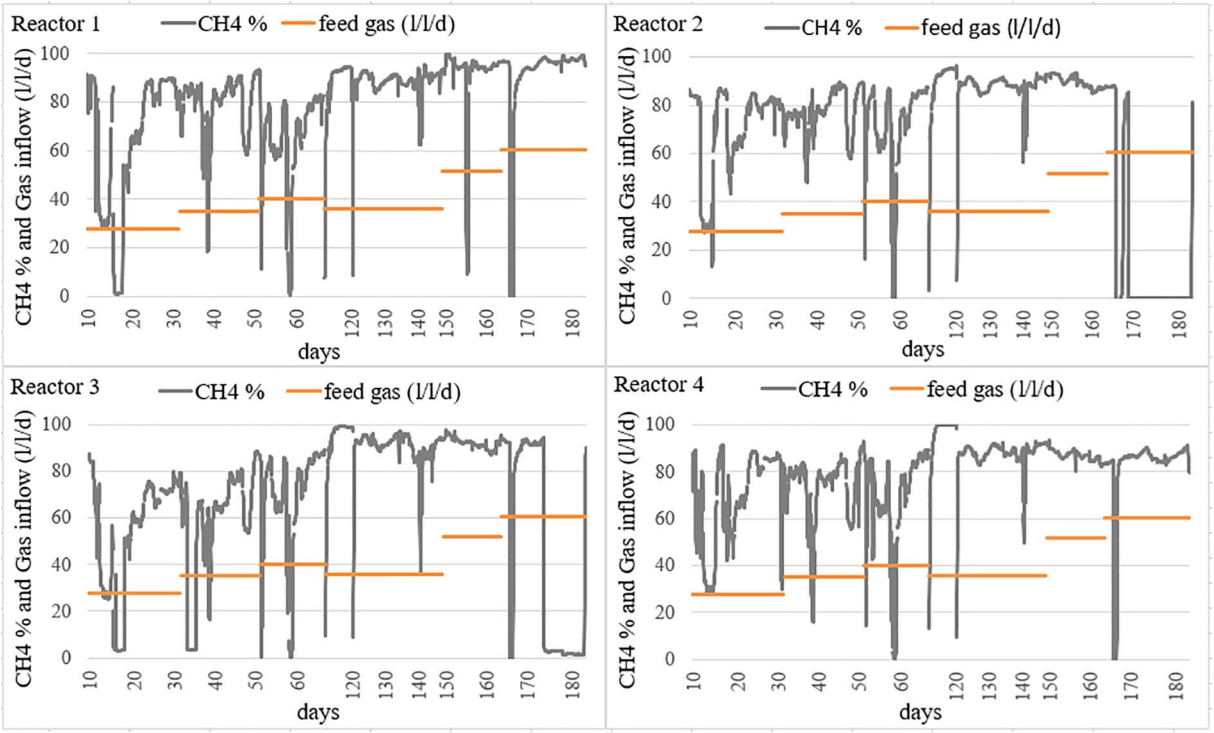

Fig. 1. Methanation efficiency of the four reactors relative to the gaseous feed (H_2/CO_2) input flowrate; (non-operational periods have been omitted).

influenced by the flowrate and composition of the gas entering the system (Leonzio, 2016) and therefore the ability of hydrogenotrophic populations to deal with variable feeds and inconsistent gas ratios is still disputable.

In a previous study (Savvas et al., 2017b) an ex-situ hydrogenotrophic reactor based on a self-regenerating mixed microbiome under nutrient closed conditions was tested with results showing that conversion efficiencies close to 100% were achievable at gas feeding rates of up to 60 v/v/d. To the authors' knowledge this type of microbiome has not been replicated elsewhere apart from (Savvas et al., 2017a) where it was used to create biofilms in a plug-flow hydrogenotrophic reactor. The present study went a step further by assessing the robustness of such microbiome and its behaviour under a series of destabilising conditions that can occur during operation of full scale plants. These were sudden changes in the gas feeding rates, periods of carbon/energy starvation and oxygenation. Additionally the evolution of the same inoculum under mesophilic (37 °C) and thermophilic (55 °C) conditions was evaluated as well as its ability to form biofilms under conditions of intense agitation.

With the exception of (Savvas et al., 2017a), hydrogenotrophic biofilms have so far only been assessed in trickle-bed type arrangements with results showing a lesser degree of gas conversion rates to systems that depend on intense agitation (e.g. Continuously Stirred Tank Reactors (CSTRs)) (Lecker et al., 2017). The difference is directly linked to the different gas-liquid mass transfer rates that can be achieved by each system. Conversely, biofilms could potentially add to the stability and robustness of the microbial catalyst as they have protective for the microbes properties (Watnick and Kolter, 2000). The reactor type used in the present study utilised intense gas-liquid mixing through liquid recirculation as a way to enhance gas diffusion but also offered the possibility for the integration of biofilms thus creating a hybrid system.

2. Materials and methods

2.1. Reactors and inoculum

Four identical reactors were operated in parallel; one was kept at thermophilic conditions (55 ± 0.5 °C) and three at mesophilic (37 ± 0.5 °C). No biofilm attachment media was used in one of the mesophilic reactors (**Reactor 1**) or in the thermophilic reactor (**Reactor 2**). Biofilm attachment media was used in the other two mesophilic reactors, Kaldnes K1 (polyethylene wheels) in **Reactor 3** and LECA (Light Expanded Clay Aggregate balls) in **Reactor 4**. The two types of attachment media used in the present study had been previously assessed in denitrification tests (Andersson et al., 2008) and were chosen based on their biofilm formation performance among 20 different materials. The geometry, technical aspects as well as the control and data acquisition parameters of the reactors were identical to the ones described in (Savvas et al., 2017b).

All four reactors were filled with anaerobically digested mesophilic sewage sludge collected from Cog Moors Wastewater Treatment Plant in Cardiff, South Wales, UK. Prior to inoculation the sludge was filtered through a 125 μm stainless steel sieve. After inoculation there was no addition of any solid or liquid feedstock for the whole of the operational period. Thermophilic adaptation in **Reactor 2** proceeded in one step as it had been previously found to be advantageous to multi-step adaptation (Bouškova et al., 2005).

2.2. Analytical methods

Gas composition was measured in real time with infra-red sensors (Premier Series 0–100% Vol CO_2/CH_4 Voltage output 0.4–2.0 V, Dynament Ltd) and by in-line hydrogen solid-state sensors (H2Scan HY-OPTIMA 740, 0–100% Vol H_2, 4–20 mA output). The reliability of the gas sensors was also periodically checked by analysis of the gas with a gas chromatograph (Varian Inc., CP-4900) equipped with two columns, one for CO_2 (Porapack Q, Varian – 10 m × 0.15 mm) and one for CH_4, H_2, N_2 and O_2 (Molsieve 5A Plot, Varian – 10 m × 0.32 mm). The carrier gas used was Ar. Gas flow rates were measured by custom made tip-meters and logged in LabVIEW™ (National Instruments, UK).

Volatile Fatty Acids (VFAs) were determined according to (Cruwys et al., 2002) using a head space autosampler gas chromatograph (Perkin Elmer, AutosystemXL) fitted with a flame ionization detector and a Supelco Ltd. column (30 m × 0.32 mm). The carrier gas was N_2. pH was