



Biological methanation of CO₂ in a novel biofilm plug-flow reactor: A high rate and low parasitic energy process



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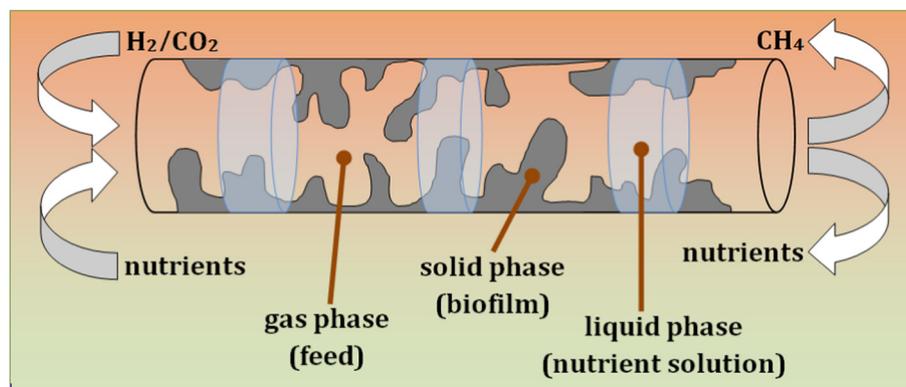
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HIGHLIGHTS

- A novel bio-methanation reactor was designed and evaluated.
- A biofilm consisting of mixed anaerobic consortia served as the biocatalyst.
- High rate methanogenesis was observed without gas-liquid agitation.
- Gas conversion was successfully decoupled from energy consumption.

GRAPHICAL ABSTRACT



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ABSTRACT

The performance of a novel biofilm plug flow reactor containing a mixed anaerobic microbial culture was investigated for the conversion of CO₂/H₂ to CH₄. Unlike conventional gas-liquid contactors that depend on agitation, gas diffusion was decoupled from power consumption for mixing by increasing the gas phase inside the reaction space whilst increasing the gas residence time. The mixed mesophilic culture exhibited good biofilm formation and metabolic activity. Within 82 days of operation, 99% and 90% CH₄ conversion efficiencies were achieved at total gas throughputs of 100 and 150 v/v/d, respectively. At a gas input rate of 230 v/v/d, methane evolution rates reached 40 v/v/d, which are the highest to date achieved by fixed film biomethanation systems. Significant gas transfer related parasitic energy savings can be achieved when using the novel plug flow design as compared to a CSTR. The results and modelling parameters of the study can aid the development of high rate, low parasitic energy biological methanation technologies for biogas upgrading and renewable power conversion and storage systems. The study has also established a reactor system which has the potential of accelerating biotechnology developments and deployment of other novel C1 gas routes to low carbon products.

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1. Introduction

Hydrogenotrophic methanogenesis has recently gained significant attention due to its potential as a CO₂ utilization process [1–3]. The low temperature and pressure operating conditions required as well as the inexpensiveness of the microbial catalyst

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make the biological synthesis of CH₄ from CO₂ an attractive alternative to conventional biogas upgrading methods [4], by increasing both quantity and quality of the end product. Furthermore, the conversion of excess renewable electricity to methane (Power to Methane, PtM) has the potential to resolve many of the current inadequacies of existing power back up technologies, such as storage capacity, cost and geographical limitations [5] and support renewable energy developments in terms of minimizing power curtailment and facilitating wider deployment of renewable energy generation in regions with limited electricity grid availability. Additionally, in comparison to H₂, green CH₄ can be readily added to the gas grid [1] since it does not alter the characteristics of the existing fuel.

Methanogens have been typically cultivated in homogenous aqueous solutions due to the ease of control over the entire population. Because of this, continuously stirred tank reactors (CSTRs) have over the years become the standard in anaerobic processes such as the digestion of organic waste as, if designed and operated appropriately, they provide even distribution of temperature and soluble and insoluble constituents as well as high levels of consistency in sampling. The anaerobic digestion of organic feedstock relies considerably on the acetoclastic methanogenic route [6–8], which is the terminal stage in a series of solid/liquid substrate conversions. However, in the case of ex-situ hydrogenotrophic methanogenesis the very presence of the aqueous solution hinders gas transferability. Hydrogen is a gas with a particularly low solubility coefficient (1.35×10^{-5} v/v in water, 35 °C), meaning that its rate of diffusion into the liquid media is considered to be the main limiting factor for the process [9].

A generally accepted model describing the rate of diffusion of a single gas in a liquid is given by Eq. (1):

$$dq/dt = k_L \alpha (C_g - C_l) \quad (1)$$

where (k_L) is the linear mass transfer coefficient, (α) is the specific surface area of contact between the gas and the liquid and ($C_g - C_l$) is the concentration difference between the gas phase and the liquid phase.

The $k_L \alpha$ factor depends on a large number of parameters, some of which cannot be altered (e.g. the molecular weight of the gas), or are difficult to control, especially in biological reactors, (e.g. the rheological behaviour of the liquid). Theoretical models that predict diffusivity gradients in stirred tank bioreactors [10–12] rely on simplified versions of the actual systems (single gas - single liquid matrix). Hydrogenotrophic methanogenesis however, creates a much more complex multi-component system (gas - liquid - solid matrices) with continuous changes in all three phases due to the biological reactions involved. The process designer is therefore typically left with just three controllable options; pressure, gas hold-up and gas-liquid surface area of contact.

Higher pressure generally results in better gas diffusion since it increases the concentration gradient between the gas phase and the liquid phase. Furthermore, there is evidence that several hydrogenotrophic species are not only resistant to high pressure conditions (>100 atm) but also exhibit improved growth and methanogenesis rates [13,14].

Gas hold-up and interfacial area of contact are interlinked and in liquid flooded reactor configurations are maximized with the reduction of bubble size, which is typically achieved through intensive mixing. CSTRs have been shown to be efficient at high angular velocities (>1200 rpm) [15,16]. However, intensive mixing also has a profound negative effect on the energy balance of the system. Power dissipation is directly linked to the gas-liquid mass transfer in CSTRs. With increasing power dissipation the bubble diameter decreases which, in turn, increases the interfacial surface area [11,17]. Bubbles break apart because the surface tension forces are overcome by a

higher power density. Therefore CSTR systems depend on high impeller rotational speeds in order to increase gas diffusivity.

Due to the turbulent regime in stirred gas-liquid contactors the power drawn by the impeller is typically given by Eq. (2):

$$P = P_o \rho N^3 D^5 \quad (2)$$

where (P_o) is the power number and depends on the structural characteristics of the mixing system like the geometry of the impeller and the vessel, (ρ) is the density of the fluid, (N) is the angular velocity of the impeller and (D) is the diameter of the impeller. From Eq. (2) it is evident that power consumption increases exponentially with increasing agitation rates [18].

The microbial cultures that are used as catalysts in biomethanation may also be influenced by the shear forces created by the impeller. Although there is no reference of inhibition due to shear in pure culture biomethanation systems, cell damage cannot be excluded. In mixed culture systems there is also the added possibility of reduced cell to cell interaction. For example, syntrophic relationships between different groups have been found to be hindered by shear in CSTRs treating animal manure [19]. Experiments with lab-scale CSTRs treating sewage sludge also indicate that there is a mixing speed threshold above which biogas production declines significantly [20].

The major inefficiency in the energy balance of any biomethanation reactor that is flooded with an aqueous solution occurs because most of the working volume is occupied by water which acts as a barrier between the microbes and their gaseous feed. If the feeding gas could be in contact with the culture without the need for intense mixing, then energy consumption would not necessarily be linked to the gas-liquid mass transfer factor, therefore delivering a far more energy efficient process. The ability of mixed bacterial and methanogenic cultures to form colonies attached to various materials could be utilised to achieve such a system. The microbes would still need to be wetted and in contact with essential nutrients in solution but this could be achieved by reducing the volume of the solution that surrounds the biomass to a minimum level compared with flooded systems.

Eq. (1) is a derivative of Fick's first law of diffusion which states that the molar flux of a species (i) is proportional to its concentration gradient across the interface between the gas phase and the liquid phase (two film model) as shown by Eq. (3):

$$j_i = -D_i \nabla_{ci} \quad (3)$$

where D_i is the diffusion coefficient and C_i is the concentration of the species

For transfer along a vector z ,

$$\nabla_{ci} = \frac{d_{ci}}{dz} \quad (4)$$

which means that the rate of transfer is inversely proportional to the separation distance of the two media. A thin liquid layer means that diffused gases will get converted closer to the interface, thus increasing the concentration gradient and therefore diffusivity.

Novel gas delivery systems that rely on the formation of biofilm after inoculation with mixed methanogenic cultures have been used as candidates to increase biomass while reducing the energy consumption of biomethanation. Fixed bed [21,22], trickling bed [23,24] and hollow fibre membrane reactors [25] have been studied in the past with high gas conversion efficiencies (>98% of CH₄ in the effluent gas). However, the volume of gas per working volume of reactor (v/v) conversion throughputs of these systems have been reported to be at significantly lower levels ($\approx 1-6$ v/v/d) to those of intense mixing systems ($\approx 18-28$ v/v/d) that used similar mixed methanogenic inocula [26,27]. In the case of flooded fixed film reactors (fixed bed and hollow fibre reactors), the disparity in the conversion rates can be explained by the fact that the

attached microbes were submerged in a liquid media and the presence of attachment media prevented thorough gas-liquid mixing leading to low gas dilution rates and localized conversion. In the case of the trickling bed reactors the low conversion rates could be linked to the geometry of the reactor vessels which did not allow for enough contact time between the biofilm and the gas phase.

In the last decade, the upsurge in the number of studies on ex-situ biomethanation is a result of an increased interest in PtG technologies for the valorisation of CO₂ and as an aid to a more sustainable deployment of intermittent renewable energy sources [28,29]. However, there is presently a gap in literature regarding the parasitic energy losses that accompany the process, especially in systems that rely on agitation in order to increase gas-to-liquid mass transfer. In a process that already suffers from the energy losses that accompany the conversion of electricity to hydrogen, steps need to be taken in order for any further energy conversion losses to be minimized. It is envisaged that for such minimization to occur, biomethanation efficiency needs to be de-coupled from agitation through a more efficient way of biofilm utilization.

In biofilm reactors where the availability of the gas phase and the liquid phase are increased and reduced respectively, it is suggested that the geometry of the reactor should achieve two things: (i) reduce the thickness of the liquid layer around the biofilm to the bare essential minimum, and (ii) maximize the gas residence time by increasing the distance the gas molecules need to travel before they lose contact with the biofilm. In the present study, the above hypothesis was tested with the use of a mixed anaerobic culture. A novel reactor consisting of a single tube filled with microbial attachment media was constructed and evaluated for its ability to convert H₂ and CO₂ input gases to CH₄ with lower energy inputs than for CSTRs or other liquid media flooded reactor designs.

2. Materials and methods

2.1. Inoculum

The inoculum used was anaerobically digested sewage sludge collected from Cog Moors Wastewater treatment Plant in Cardiff, South Wales. Prior to use the sludge was filtered through a 125 µm stainless steel sieve and then left to settle for 48 h at room temperature. The reactor was then filled with approximately 0.75 L of the supernatant at a concentration of 4.6 g/L TS, 3.7 g/L VS.

2.2. Nutrient solution

A nutrient solution was created from the same batch of digested sewage sludge as the inoculum, centrifuged and filtered down to 0.45 µm and was kept at 4 °C. The nutritional profile of the solution including 17 elements essential for a wide range of methanogens is given in Table 1.

2.3. Feeding gas

The feeding gas comprised H₂ and CO₂ delivered by pressurised gas cylinders (>99.9% purity, Air Liquide, UK) and mixed at a

stoichiometric ratio of 4/1 (H₂/CO₂). These were pre-mixed in a glass vessel before being injected into the reactor by controlling the individual gas flows with peristaltic pumps (Watson-Marlow Ltd, UK). A peristaltic pump was also used to control the feeding rate of the mixed gas.

2.4. Analytical methods

Gas composition was analysed in real time by infra-red sensors (Premier Series 0–100% Vol CO₂/CH₄ Voltage output 0.4–2.0 V, Dynament Ltd). Gas composition was also periodically analysed with a gas chromatograph (Varian Inc., CP-4900) equipped with two columns, one for CO₂ (Porapack Q, Varian – 10 m × 0.15 mm) and one for CH₄, H₂, N₂ and O₂ (Molsieve 5A Plot, Varian – 10 m × 0.32 mm). The carrier gas used was Ar.

Volumetric gas flow rates were measured by bespoke manufactured tip meters. Elemental analysis was carried out by ICP-MS (Inductively coupled plasma mass spectrometry).

Volatile Fatty Acids (VFAs) were determined according to [30] using a head space autosampler gas chromatograph (Perkin Elmer, AutosystemXL) equipped with a flame ionization detector and a Supelco Ltd. column (30 m × 0.32 mm). The carrier gas was N₂.

The pH of the microbial media was measured in real time with the use of a pH electrode HI-1001 (Hannah Instruments, UK). Total solids (TS) and Volatile solids (VS) were measured according to [31]. For the quantification of the TS and VS of the biofilm, the tube was cut into 10 cm pieces and its contents were scrubbed into a known amount of de-ionised water within less than 1 h. Data for gas flow and gas composition were logged every 5 min using LabVIEW™ software (National Instruments™, UK).

2.5. Microbial profiling

DNA was extracted with the use of a PowerSoil DNA Isolation kit (Mo Bio Laboratories Inc.) according to the manufacturer's protocol. The concentration of purified DNA was measured based on absorbance at 260 nm with a NanoDrop 1000 spectrophotometer (Thermo Scientific, UK). Total bacteria were quantified according to the method described in [32] with the use of bacterial rDNA standards. The numbers of hydrogenotrophic and acetoclastic methanogens were estimated by using the method defined in [33]. Pure cultures supplied by the Leibniz Institute (DSMZ) were used for the extraction of the DNA used for positive controls. The species *Halorubrum saccharovorum* (DSM 1137) was used as control for total bacteria. With regards to the methanogenic populations, five different order and family levels were investigated by using the following species: *Methanosaeta concilii* (DSM 6752), *Methanosarcina barkeri* (DSM 800), *Methanobacterium bryantii* (DSM 863), *Methanomicrobium mobile* (DSM 1539), *Methanococcus voltae* (DSM 1537). Real-time PCR was executed on a Roche Light-Cycler nano system with the use of TaqMan primers/probe sets (Life Technologies, Thermo Scientific, U.K.) targeting 16S rRNA gene sequences. Calibration curves were produced by using known amounts of oligonucleotides that contained complementary

Table 1
The concentration of 17 important elements in the nutrient solution identified by ICP-MS.

Element	mg/L	Element	mg/L	Element	mg/L
Na	175.000	Fe	0.280	Ni	<0.013
K	149.000	Zn	0.016	Cu	<0.003
Ca	30.300	Co	0.013	Mn	<0.010
Mg	19.100	B	0.098	V	<0.003
P	14.000	Al	<0.110	Mo	0.000
S	14.000	Se	<0.019		

sequences to the primers and probes. All samples were analysed in triplicate.

2.6. Reactor configuration of first experimental stage

The reactor comprised a 7 m length of a single walled, clear, flexible PVC tube with a 13 mm internal diameter that was attached to a frame and positioned in such a way so as to form an arrangement of a series of vertical loops. The tube was filled with polyethylene wheels (Kaldnes k1) that served as the biofilm attachment media. The whole reactor system was placed inside an incubator for stable mesophilic conditions (37 °C).

The total working volume inside the tube (free space) was approximately 0.75 L. The gaseous feed (mixture of H₂ and CO₂) was continuous and passed through the tube once, whereas the liquid media was recirculated at a very slow rate (10 ml/min) to ensure that all the microbes were wetted and had equal access to nutrients. At one end the tube was connected to a container which helped with gas/liquid separation and also served as a sampling point. The arrangement of the tube into loops created two phases in the way the gas was delivered to the microbes, thus forming a series of up-flow and trickling bed sub-reactors that alternated with each other as depicted in Fig. 1. Six full loops were created out of the 7 m tube length.

After each liquid sampling occasion the container attached to the main reactor (gas/liquid separator) was topped up to its initial volume with filtered Cog Moors nutrient solution in order for the same chemical and nutritional characteristics to be maintained throughout the experiment.

2.7. Reactor configuration of second experimental stage

After the reactor had reached a steady state in terms of gas conversion it was detached from its frame and was positioned horizontally. As a result the reactor was emptied of most of its liquid content leaving only the attached biofilm in place. As such, the new arrangement did not include the previous sub-compartments and allowed for free and uniform gas flow through

the tube. The biofilm was kept wet by short intervals of liquid media addition (10 ml/min). The small internal diameter of the tube allowed the adhesion of the liquid media (mostly water) to the inner surfaces of the reactor (tube walls and biofilm) so that the liquid media was carried through the attachment media by the gas in short discs (see graphical abstract).

3. Results and discussion

3.1. First stage of operation – Vertical arrangement

In the first weeks it became clear that the two phases (i.e. up-flow/down-flow) exhibited totally different physical/biological characteristics. In the up-flow (flooded) compartments the feeding gas was observed to be rising rapidly as fairly large bubbles (cm range) whereas in the trickling compartments it remained in contact with the whole volume of microbes for longer periods. This was evident by the difference in biofilm formation between the two compartments as shown in Fig. 2a and b.

After 5 weeks, and as the conversion rates rose steadily, the up-flow parts of the reactor also started to show signs of biofilm formation. This was observed to occur more densely in the last compartments as the diffused gas concentration was gradually increasing in each loop (Fig. 2c). During the first stage the reactor reached acceptable conversion efficiencies (>98% CH₄), and exhibited the ability to work at a sequence of increasing gas throughputs.

Fig. 3 shows the performance of the reactor during the first stage of the experiment, which lasted for a period of 52 days. In the first 32 days the culture showed the ability to cope with gradual increases in gas feeding rates up to the level of approximately 100 v/v/d of influent gas. This is indicative of a methanogenic culture going through the enrichment phase. The next increase (day 32) to 135 v/v/d resulted in the reactor reaching a plateau at approximately 70% CH₄ in the outflow indicating a steady state at which the reactor was unable to deal with higher gas flows. During this last period (up to day 52) the mean CH₄ production rate was 25 v/v/d.

The large troughs and peaks in Fig. 3 indicate periods of inactivity due to equipment maintenance, disturbances in the gas flow

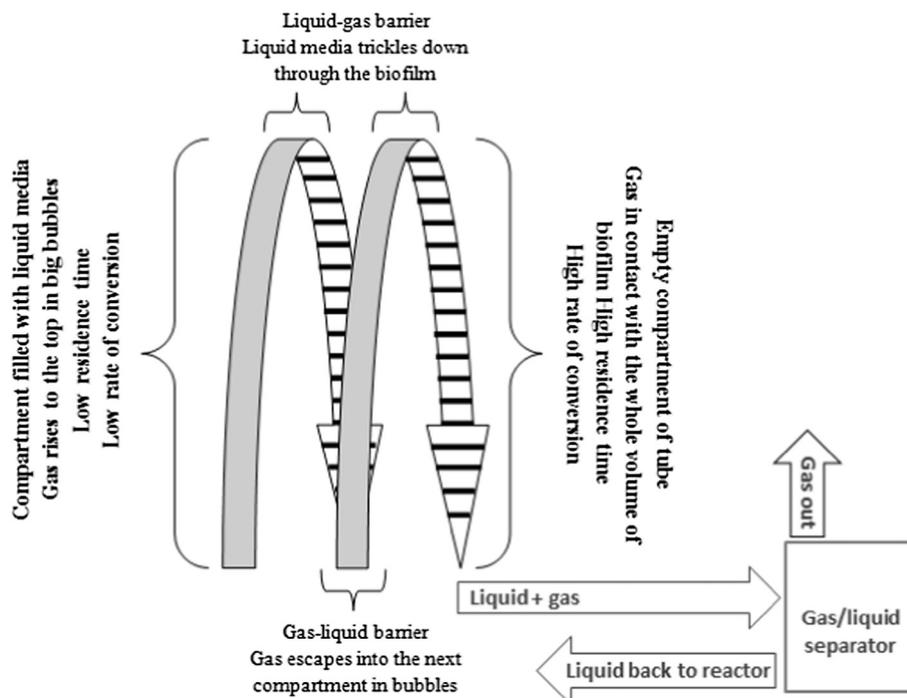


Fig. 1. A section of two vertical loops. The up-flow and trickling bed sub-reactors are alternating each other.

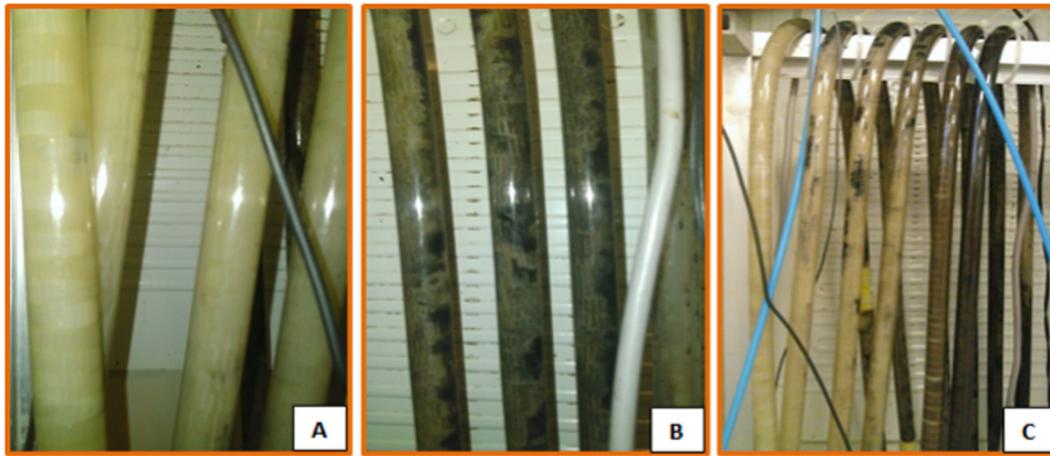


Fig. 2. Difference in biofilm formation between the upflow and the trickling bed compartments; after 3 weeks the up-flow compartments were still clear (a), while biofilm had already formed in the trickling bed compartments (b); after 5 weeks the up-flow compartments had also started to show some biofilm formation (c).

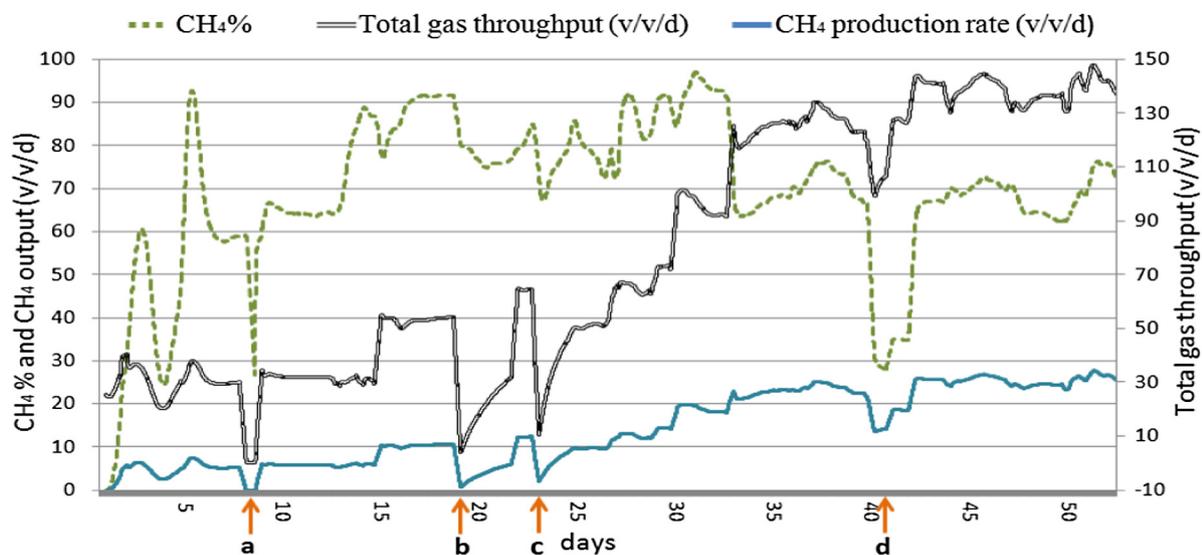


Fig. 3. First stage operation in vertical arrangement, CO_2 to CH_4 conversion efficiency relative to total gas inflow and CH_4 outflow. To aid clarity, data points have been averaged to 8 h periods.

due to sampling, and periods of adaptation to increased gas throughputs. More specifically, marked disturbances **a**, **b** and **c** were related to technical issues whereas disturbance **d** was due to a major change in H_2/CO_2 feeding gas ratio as explained later in the text.

Variations in conversion efficiency were also present due to minor instabilities in the mixing system for the production of the feeding gas. The molar ratio of the H_2/CO_2 mix entering the reactor is directly responsible for the efficiency of the process due to the stoichiometry of the conversion (4 mol of H_2 with 1 mol of $\text{CO}_2 \rightarrow 1$ mol CH_4). Therefore, minor discrepancies in the composition of the feeding gas result in major variations in the % CH_4 output value of the end product. This is evident in Fig. 4 which shows the effect of the value of the H_2/CO_2 fraction on the % CH_4 output. It can be seen that the slopes close to the peak of the CH_4 function are so steep that they allow only for minute deviations away from the ideal molar ratio of 4:1 $\text{H}_2:\text{CO}_2$. In order to reach >98% conversion efficiency a gas analysis and control system must be able to achieve less than 1% resolution and repeatability. This was difficult to attain with the lab scale equipment used in this study but would be possible on a scaled up version.

On day 39 the CO_2 percentage in the feeding gas was intentionally lowered to 16% for a period of 3 days. The main reason was to investigate the effect of changes in the CO_2/H_2 ratio on the dynamics of the population since CO_2 was the main pH regulator. The first visible outcome (Fig. 3) was the expected drop in conversion efficiency to below 30% due to excess, unused H_2 ; however this was also followed by the depletion of the majority of the VFAs present in the liquid media (Fig. 5), in particular propionic acid which by day 39 was still rising despite a previous reduction of the acetic acid. Depletion of acetic acid could have been helped thermodynamically by an increase in pH followed by a metabolic shift from the family of *Methanosarcinaceae*. Members of this family can utilise a variety of substrates (acetate, CO_2/H_2 , methanol) but have a generally higher affinity towards autotrophy at 37 °C [34]. However, in the absence of CO_2/H_2 they have been observed to turn to acetate utilization [7,35]. Propionate oxidation occurs through syntrophic association with methanogenesis from acetate [36], and propionate could have therefore been depleted due to acetate degradation followed by an increase in pH to just above 8. This could have also been supported by a competitive response of the culture to the high levels of acetate by an accelerated growth of

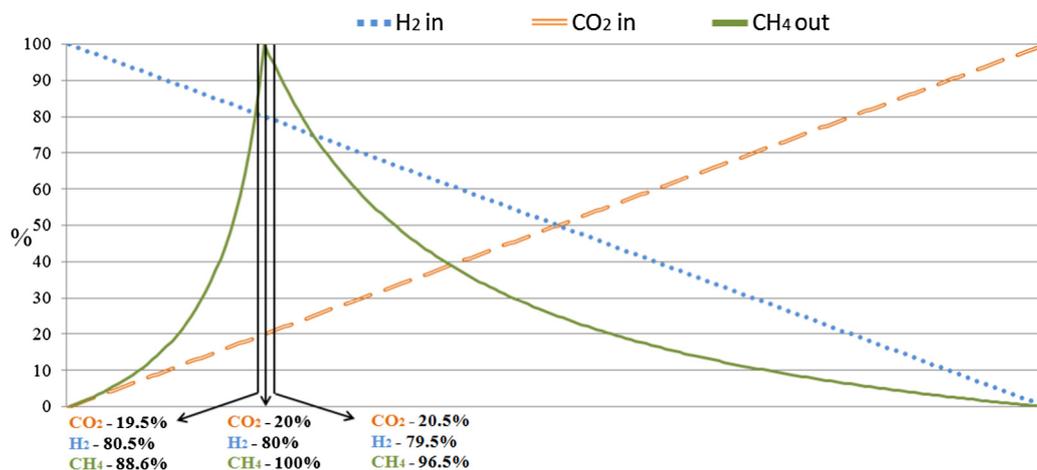


Fig. 4. The CH_4 function related to the molar H_2/CO_2 ratio of the feeding gas mix.

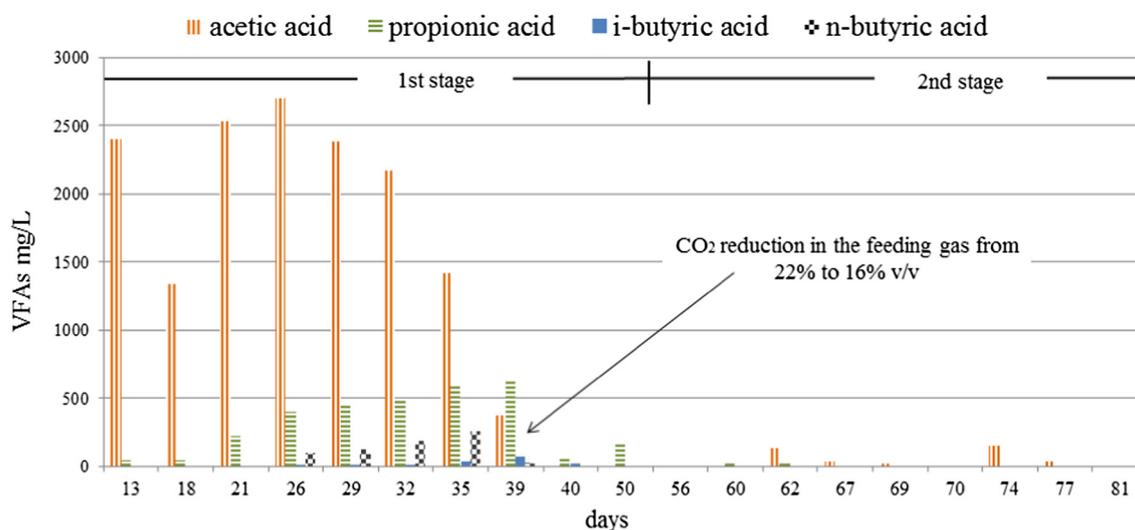


Fig. 5. Concentration of VFAs in the liquid media throughout the experiment.

members of the *Methanosaetaceae* family, which are obligatory acetotrophs. As shown by the end of the experiment, acetotrophic methanogens occupied more than half of the methanogenic population in the biofilm (Fig. 8).

From Fig. 5 it appears that the decrease of acetate levels had already started after the 26th day of operation. The oxidation of acetate could have brought subsequent relief to acetogenic bacteria responsible for the oxidation of propionic and butyric acids since the levels of acetate in the media had decreased. However, even though there was a reduction of acetate from 2300 mg/l to 400 mg/l, propionic acid was still increasing at that point. Only when CO_2 % input was reduced from the 22 to 16% v/v was there a reduction of propionic acid from 700 mg/l to 100 mg/l. CO_2 reduction coupled with a low content of acetic acid may have jointly allowed a fast propionic acid conversion within 24 h. Finally, although little information exists on propionogenesis either from acetate or even directly from CO_2 , such pathways are possible and should be further investigated [37,38].

3.2. Second stage of operation – Horizontal arrangement

On day 55 the reactor was set up for the second phase where it was positioned horizontally and the whole length of the tube was

provided with an equal opportunity for biofilm formation. Due to the reactor re-adjustment a portion of the liquid media had to be removed and re-introduced into the reactor. The procedure was not performed anaerobically, thus leading to an initial drop in performance due to the introduction of oxygen. However, the culture recovered steadily as shown in Fig. 6 and after a period of 12 days it reached a conversion efficiency of 90% at a total gas throughput rate of approximately 150 v/v/d. At that point conversion capacity was evaluated by increasing the gas feeding rate to approximately 230 v/v/d, which resulted in the reduction of conversion efficiency to 50%. At this rate methane productivity rose to 40 v/v/d, which marked either the physiological limit of the hydrogenotrophic culture or the gas mass transfer limit of the system.

The reactor was left to run for 7 more days and then the gassing was stopped for 12 h before termination of the experiment. During the 12 h without gas feeding, liquid recirculation continued and the temperature was kept at 37 °C.

After termination of the second stage the contents of the reactor were emptied in order for the volume of liquid inside the reactor as well as the weight of the formed biofilm to be measured. Since the reactor was initially inoculated with filtered sludge supernatant, any remaining soluble organics were consumed during the operational period. Consequently it was reasonable to accept that the VS

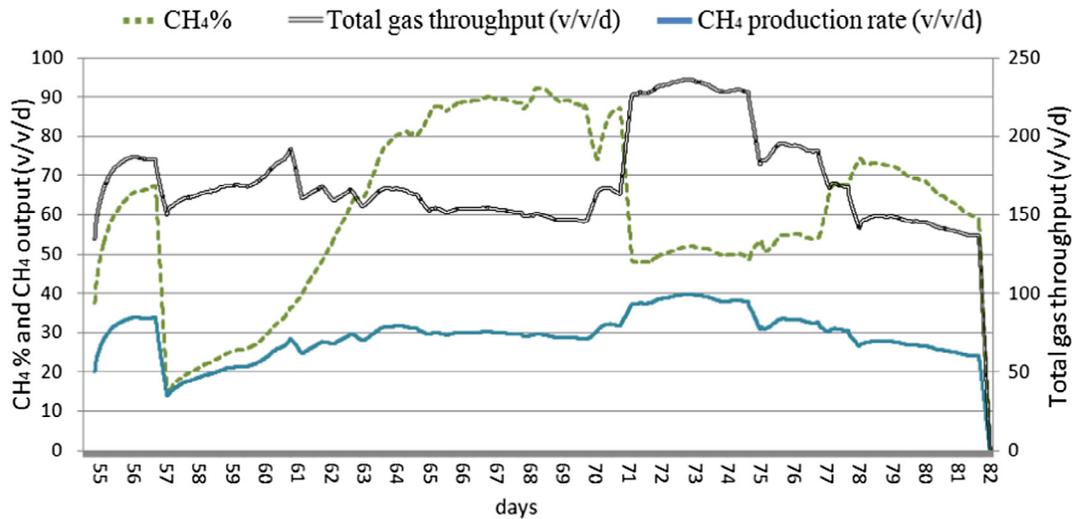


Fig. 6. Second stage; operation in horizontal arrangement; CO₂ to CH₄ conversion efficiency relative to total gas inflow and CH₄ outflow. To aid clarity, data points have been averaged to 8 h periods. Recovery after oxygen contamination can be seen starting day 57.

content of the reactor at the end of the experiment was predominantly cell mass.

Fig. 7 shows the TS and VS of the biofilm and in the liquid media and indicates that only 11% of the biomass was detached and recirculated in the liquid media around the reactor. According to previous experiments (data not shown) when H₂/CO₂ gas was allowed to bubble freely at similar rates from the bottom to the top of a 1 m vertical tube filled with the same enriched inoculum, conversion to CH₄ was close to zero. Consequently due to the existing conditions of the present reactor it can be reasonably assumed that this 11% of cells did not significantly contribute to the conversion rates achieved during the experiment.

3.3. Microbial profiling of the culture

Cell numbers per ml of sample can be seen in Fig. 8a for both attached and suspended bacteria, hydrogenotrophic and acetotrophic methanogens. The dominance of bacteria is profound with methanogenic cells accounting for 10% of the total biomass in the biofilm and 5% in the liquid media. Fig. 8b displays the results per g of VS so that differences in the density of the samples can be taken into account. The gene copy numbers of bacteria per g VS in the biofilm was 9.71×10^8 whereas the gene copy numbers of methanogens per g VS was 1.07×10^8 .

Characterization of microbial mixed cultures have so far been limited for ex-situ biomethanation systems with the exception of [39] referring to the abundance and diversity of the archaeal species. In this study, the dominance of bacteria was found to be in

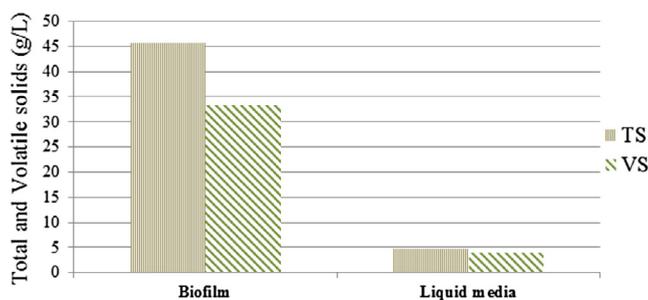


Fig. 7. Total and volatile solids of the biofilm and the liquid media measured after 82 days of operation.

accordance with previous work by [40] and appeared to be similarly a characteristic of a self-reproducing mixed culture. The high gene copy numbers of the bacterial population were most likely supporting the redistribution of nutrients through the hydrolysis and digestion of dead biomass as has been observed in closed nutrient systems [40]. However, the biofilm formation of the present reactor system was able to support a denser bacterial culture (3.23×10^{10} gene copies) than the complete liquid flooded reactor system (6.8×10^9 gene copies) used by those researchers.

With regards to hydrogenotrophic methanogens, the order of *Methanobacteriales* dominated. Their numbers, both attached (1.67×10^9) and in suspension (3.21×10^8), were more than two orders of magnitude higher than the rest of the hydrogenotrophic population which suggests that they were responsible for at least 99% of the methane produced autotrophically. The numbers of the obligate acetotrophs of the *Methanosaetaceae* family were equally high in the biofilm (1.9×10^9), which explains the almost complete absence of acetate in the media post day 40 (Fig. 8b). It is possible that the biofilm nature of this reactor and the higher throughput of the input gas promoted a higher density of acetoclastic methanogens.

In contrast to pure hydrogenotrophic methanogenic cultures in the delivery of biomethanation, mixed enriched cultures may entail the risk of accumulation of by-products (most notably organic acids) which can act as inhibitors to methanogenic populations. However, this was not the case in the present study after day 39.

Despite the high numbers of *Methanosaetaceae* archaea the amount of CH₄ produced from organic matter through the acetate route appears to have contributed less compared to the volumes of CH₄ produced autotrophically. This is evident from Fig. 6 in which it can be seen that during the period of 12 h of operation on day 82 without any H₂/CO₂ input methanogenesis rates fell close to zero. It can therefore be argued that acetotrophic methanogenesis related to the digestion of dead biomass was not a significant contributor to CH₄ evolution rates.

An alternative autotrophic pathway, that of the assimilation of H₂ and CO₂ through the homoacetogenesis route, should however be investigated. Under normal conditions and at mesophilic temperatures methanogenic hydrogen oxidation is more energetically favourable than homoacetogenic hydrogen oxidation [41] and the domination of homoacetogens has been typically observed in systems where the conditions were unfavourable for methanogens [42–45]. Nevertheless, the role of homoacetogenic bacteria has

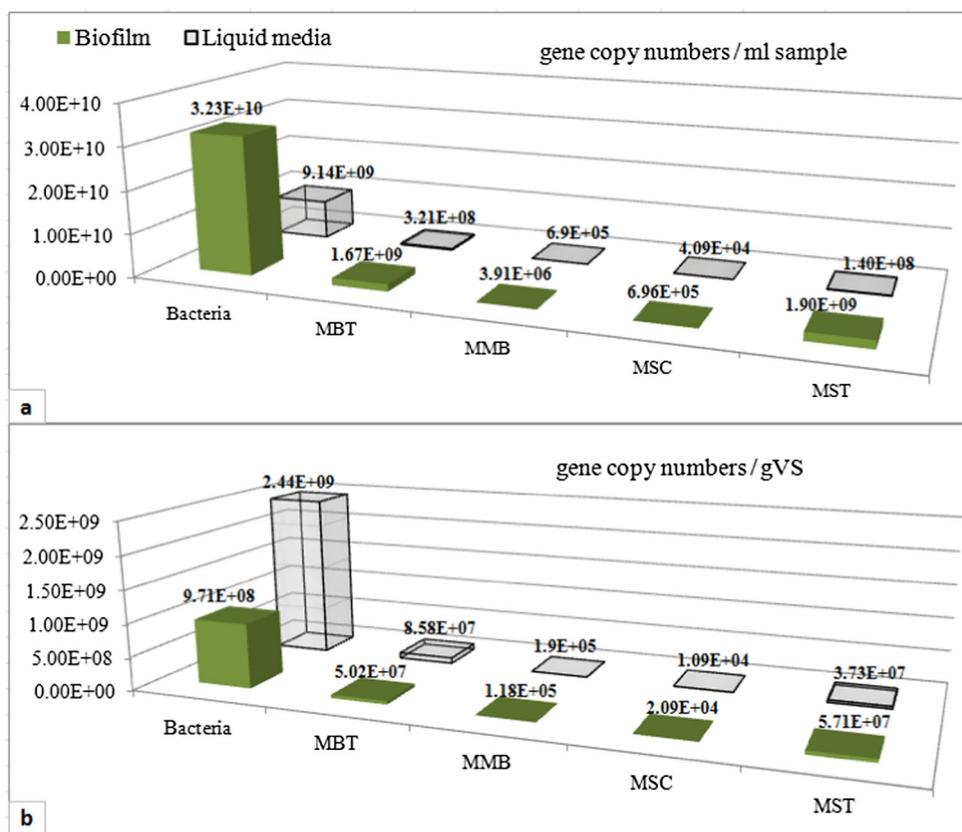


Fig. 8. Gene copy numbers per ml of sample (A) and per gram of VS (B) for bacteria and methanogenic cells in the biofilm and the liquid media after 82 days of operation (Acetoclastic methanogens cover the family of *Methanosaetaceae* (MST); hydrogenotrophic methanogens cover the orders of *Methanobacteriales* (MBT) and *Methanomicrobiales* (MMB) and the family of *Methanosarcinaceae* (MSC).

not been evaluated in high rate hydrogenotrophic methanogenic systems and therefore cannot be excluded. The homoacetogenesis and hydrogenotrophic methanogenesis reactions are represented by Eqs. (5) and (6) and yield 1 mol of acetic acid and 1 mol of CH_4 respectively per 4 mol of H_2 . The conversion of acetic acid to CH_4 by acetoclastic methanogens is represented by Eq. (7).



In a hydrogenotrophic methanogenic system where the molar ratio of H_2/CO_2 is 4/1 any significant diversion of H_2 through a competing homoacetogenic route would require a H_2/CO_2 ratio of 2/1, which would therefore be noticeable. It is possible that these two conversions ran in parallel, which could explain the accumulation of acetic acid in the first month of operation (Fig. 5). However, with the further development of acetotrophic methanogens in the mixed microbial culture of the reactor, homoacetogenesis could have proceeded at the same rate as acetotrophic methanogenesis and therefore the ratio of input gases would revert back to 4/1. The role of the homoacetogenic bacteria within mixed culture biomethanation should therefore be further evaluated (e.g. with the use of inhibitors that specifically target methanogenic populations, through DNA sequencing and qPCR for quantification of microbial cultures, and isotope based experiments). These further studies would likely support improved operational regimes of mixed culture inoculation, enrichment and operation phases, starting with a more adequate H_2/CO_2 control management which may even lead to selection of pathways.

4. Theoretical process power requirements

With regards to energy input requirements, the microbial factor renders a direct comparison between different biomethanation reactor designs impractical. This is because the conditions applied to the culture (method and intensity of agitation, the presence or not, as well as the type of microbial attachment media) have a direct impact on the culture itself and thus its metabolic activity. Therefore, the biological, physical and chemical parameters responsible for this energy conversion process are closely inter-linked and inseparable. Nevertheless, by taking into account the conditions applied in previous ex-situ biomethanation studies it is possible to get an indication of the difference in the energy input requirements between typical CSTRs and the biofilm reactor of the present study.

CSTRs used in biomethanation experiments have typically comprised 1–5 L working volume baffled vessels, and the use of 2–3 Rushton type impellers for radial mixing of the liquid media at speeds of 600–1500 rpm [9,15,16,26,46,47]. The methane evolution rates achieved in those studies were highly disparate not only because of the different physicochemical parameters used (agitation rates, morphology of the reactor, temperature, pH, nutrient availability) but also due to a range of different biological factors (different microbial strains and densities). However, based on data from previous experiments with the same mixed inoculum (data not shown), similar gas conversion rates and efficiencies to the ones of the present study have been achieved with a 2 L and a 5 L working volume CSTR at an agitation rate of 1200 rpm.

By using Eq. (2) and by substituting the microbial liquid media with water (for simplification purposes) it can be calculated that a 2 L working volume CSTR equipped with 2 Rushton type impellers

that rotate at 1200 rpm would have a power consumption of the order of 28 kWh/m³_{reactor}/day. This means that for a CH₄ production rate of 40 v/v/d, the energy spent by the system would be approximately 7% of the energy contained in the end product (≈ 400 kWh/m³_{reactor}/day, LHV of CH₄). However, due to the strong dependence of the power consumption on the dimensions of the reactor and the impeller, a 5 L working volume CSTR would have a power consumption of 360 kWh/m³_{reactor}/day. This would increase the parasitic energy losses to 90%. The challenges arising from the scaling up of a CSTR methanation system are therefore evident. The present tubular reactor system negates the requirement for this energy expenditure by completely removing the agitation factor, while any pressure drop due to the recirculation of the liquid media at a rate of 10 ml/min is calculated to be insignificant. The scaling up of such a system could be accomplished by elongation of the tube up to a point that the pressure drop is still negligible and/or by running multiple systems in parallel.

In the study performed by [16] an argument is made regarding the importance of reactor design for the intensification of the *in situ* biomethanation process and it is proposed that a CH₄ production rate of 1000 mmol/litre of reactor/hour (≈ 538 v/v/d at NTP) is a realistic target value for an industrial process. As shown in the same study, such volumetric productivity is achievable with a pure submerged culture but at the expense of high impeller rotational speeds (1500 rpm) which are linked to high energy inputs. An increase in gas feeding rates and pressure has also been shown to positively contribute to CH₄ production rates.

The qPCR results of the present study show that the CH₄ production rate of 40 v/v/d has been realized by approximately 10% of the total biomass (methanogenic cells). This indicates that an increase in the ratio of methanogens/bacteria can significantly raise the CH₄ evolution rates close to those achieved by high intensity mixing systems, but without raising the energy requirements of the process. The possibility of such methanogenic enrichment needs to be verified by further research, however, since the process depends on the minimization of the liquid media layer surrounding the microbes, further intensification is deemed possible by reactor design modifications.

5. Conclusions

The de-coupling of energy input from gas-to-liquid mass transfer was demonstrated in a prototype biofilm plug flow biomethanation reactor. The study showed that it is possible to obtain high biomethanation conversion rates and efficiencies by changing the way a mixed microbial culture is utilized, with the specific aim of reducing the liquid volume in the reactor while increasing the gas residence time. The novelty of the present design (in horizontal mode) relies on the adhesive properties of water which allowed the minimization of the liquid media volume used for nutrient replenishment of the biofilm as opposed to trickling bed reactor designs. With minimal energy input for liquid media transfer, methane evolution rates of 40 v/v/d were achieved, the highest to date for mixed culture biomethanation systems. However, the stoichiometry of the H₂/CO₂ gaseous input will require careful control not just to help maintain an appropriate pH but also to deliver optimum CH₄ densities in the effluent gas. The biofilm plug flow design allows the application of simple gas flux models without complications from bubble kinetics and further process intensification is still possible. The results and modelling parameters of this study can aid the development of high rate, low parasitic energy biological methanation technologies for biogas upgrading and renewable power conversion and storage systems. Further work on the dynamics of fluid flow through modelling software is expected to yield improved reactor design for biomethane

generation. The study has established a system which also has the potential of accelerating biotechnology developments and deployment of other novel C1 gases conversion routes to low carbon products.

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