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Biological CO_2 fixation in up-flow reactors via exogenous H_2 addition

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Abstract

Gas fermentation for the production of building block molecules and biofuels is lately gaining attention as a means to eliminate the greenhouse gases emissions. Especially CO_2 capture and recycling are in focus. Thus, the biological coupling of CO_2 and H_2 is of high interest. Therefore, the focus of the present work was to evaluate the performances of two up-flow reactors for CO_2 and H_2 assimilation. Process monitoring showed that the gas-liquid H_2 transfer was highly affected by reactor design. A reactor filled with Raschig rings could lift up gases utilization leading to a CH_4 content of 81% at 6 h gas retention time and 8.8 $L/L_R.h$ gas recirculation rate. In contrast, limited biomethanation was achieved in the absence of Raschig rings highlighting the positive role of packing material to the performance of up-flow-reactors. Additionally, high-throughput 16S rRNA sequencing revealed that the microbial community was ultimately resided by *Methanothermobacter* methanogens.

Keywords

CO_2 fixation; packed column reactors; hydrogenotrophic methanogenesis; homoacetogenesis; anaerobic digestion

1. Introduction

In EU countries, the upgraded biogas is injected in the gas grid or used as transportation fuel depending on the national legislation (Browne et al., 2011). Nowadays, various commercial upgrading technologies exist, and several new concepts are under development. Conventional methods for biogas upgrade using high pressure

(e.g. pressure swing adsorption), water (e.g. water physical scrubbing) and/or chemicals (e.g. amine chemical scrubbing) are available (Baena-Moreno et al., 2020). Despite the high efficiency of these technologies, the increased costs initiated a further research for alternative options (Bassani et al., 2017; Sun et al., 2015). Recently, the biological process coupling of CO_2 and H_2 is a rapidly growing platform since it is less energy demanding and retains the efficiency of the physicochemical methods (Angelidaki et al., 2018). Moreover, biological upgrading is considered as second generation upgrading since CO_2 is not only deposited or released to the atmosphere but is coupled with H_2 by the action of hydrogenotrophic methanogens forming additional amounts of CH_4 (Bassani et al., 2015; Luo and Angelidaki, 2012; Vo et al., 2018). Therefore, biological biogas upgrading can be considered as CO_2 capturing and recycling technology. In addition, H_2 derived from inexpensive renewable electricity in periods with intense wind peak loads for wind mills and sunny days for photovoltaics ensures no energy wasting, while it maintains a stable electricity grid and improves sustainability (Alfaro et al., 2018; De Vrieze et al., 2019; Strübing et al., 2018). Hence, it can be considered as energy storage technology.

Biological biogas upgrading can be conducted either simultaneously with the anaerobic digestion (AD) process or in a separate consecutive step. In the first option, which is called "in-situ process" (Kougias et al., 2017), there is a risk of exceeding the acceptable pH range for AD (i.e. pH of 6.5-8.5) due to capturing the endogenously produced CO_2, which will lead to irreversible inhibition for the methanogenic communities (Bassani et al., 2016). In contrast, during "ex-situ processes" H_2 and CO_2 are externally provided in a separated chamber and therefore the pH is not affected (Kougias et al., 2017). However, in both processes, the increased H_2 partial pressure

might provoke volatile fatty acids (VFA) accumulation and subsequently, inhibition of the methanogenic archaea (Kougias et al., 2017).

Previous research works reported that the injection of H_2 into hydrogenotrophic up-flow reactors enhanced tremendously the CH_4 content from 23% to 96% during an ex-situ process (Bassani et al., 2017). Specifically, the up-flow reactors were filled with either alumina ceramic sponges or membranes and were associated with high biomethanation efficiency, despite the stepwise reductions of gas retention time (GRT) from 15 to 4 h. High CH_4 output at low GRT ensures high production capacity which is very important in order to ensure economic feasibility at scaling-up (Sieborg et al., 2020). However, the previously used materials can be easily clogged and also, are rather expensive. Thus, they are linked to rather inconvenient daily operation/maintenance and also, reduced potential investments in real-life.

Among crucial parameters, the H_2 transfer from gas to liquid phase is the most important bottleneck to succeeding a high biomethane content (Dupnock and Deshusses, 2019). In addition, an increased contact area between liquid and gas flow and modulation of gas recirculation rate are key factors for high efficiency. Specifically, the contact area between the gas and liquid phases is markedly increased when filling the reactor with packing material (Bassani et al., 2017) and there is a variety of literature examining different carriers. For example, Lee *et al.* (2012) examined reticulated polyester urethane sponge as packing material which led to efficient biological uptake of gases at 3.8 h while the bioconversion was decreased at 2 h. Similarly, Burkhardt and Busch (2013) exploited the Bioflow 40 from Rauschert that was associated with high H_2 conversion at 4 h but lower performance was observed after that point. In addition, Rachbauer *et al.* (2016) filled a trickle-bed reactor with

polypropylene packing rings as carrier material leading to efficient bioconversion efficiencies up to 2 h GRT. Despite the high efficiency of trickle-bed reactors, the usage of submerged bed carriers in up-flow reactors could potentially be advantageous as no liquid recirculation will be needed avoiding extra operational costs. Furthermore, the introduction of gas recirculation seems to positively affect the gas conversion (Voelklein et al., 2019; Yun et al., 2017). Despite the positive effect of both parameters (packing and recirculation), the exact effect that each factor has on methane formation is still not clear.

Biological upgrading is mediated by dynamic microbial communities that can progressively shape during H_2 injection periods (Treu et al., 2018). Among archaea, hydrogenotrophic methanogens belonging to *Methanoculleus* or *Methanothermobacter* genera are commonly abundant during well-performing ex-situ processes (Bassani et al., 2015; Kougias et al., 2017). However, the increased H_2 partial pressure caused by the injection of exogenous H_2, can change the thermodynamic equilibrium promoting other metabolic pathways that act as sink of H_2 utilization. Hence, the establishment of archaeal members could be challenging.

The aim of the present work was to investigate the effect of packing material on CO_2 fixation in up-flow reactors fed with exogenous H_2. The purpose of using packed column reactors was to increase the gas-liquid mass transfer by achieving a more efficient dispersion of CO_2 and H_2. It was not attempted to optimize biomethanation, but it was aimed to monitor the end-products from the CO_2 hydrogenation and to compare the reactor configuration with a control system that was operating in the absence of packing material. Moreover, the microbial community changes upon exogenous CO_2 and H_2 addition were identified by performing high throughput 16S rRNA gene

amplicon sequencing.

2. Materials and methods

2.1. Inoculum and nutrient media

An active mixed hydrogenotrophic culture was collected from the effluent tank of well-performing biogas upgrading reactor and used as inoculum (Bassani et al., 2015). Fully degassed digestate was collected from the effluent tank of the Snertinge biogas plant (Denmark) and used for providing necessary nutrients for the microbial consortia in the upgrading reactor. The digestate was filtered through a 2 mm net to remove large particles and stored at a thermophilic incubator 55 ° C for more than two months in order to achieve complete decomposition of the degradable organic matter and to ensure the absence of biogas production. Before usage, orthophosphoric acid droplets were added to the digestate to decrease the pH from 9.6 to 8.0 and keep it within the optimal range for methanogenesis (Weiland, 2010). The chemical composition of the hydrogenotrophic inoculum and the degassed digestate are presented in Table 1.

2.2. Reactors configuration and operation

The experimental setup consisted of two cylindrical up-flow reactors with a working volume of 1.4 L. Enriched hydrogenotrophic inoculum was used to fill the control reactor (R_c) prior to start-up. Apart from inoculum, Raschig rings were added as packing material to the second reactor ($R_{r\text{-}r}$) in order to increase the gas-liquid contact and improve H_2 transfer to the liquid phase providing a surface area of 1.3 m^2. Both reactors were fed twice a day with 50 mL of degassed digestate as nutrient feedstock. The reactors were operated at thermophilic conditions (55 ± 1 °C) by recirculating hot

water. The glass walls were covered with insulating material to prevent heat loss and avoid light penetration. Reactors mixing was achieved by continuous gas recirculation. The reactors were provided with a gas mixture of 62% H_2, 15% CO_2 and 23% CH_4, (AGA A/S, Denmark), sparged through two stainless steel diffusers with pore size of 2.0 μm. The Q_{RC} was set to approximately 42 times the gas injection rate as previously described (Kougias et al., 2017). The experiment was divided into 5 distinguished periods, in which, either the gas injection rate (L/L_R/day) was increased in order to decrease the gas retention time (GRT) from 8 to 5 hours or the gas recirculation rate (Q_{RC}) was increased from 5.3 to 8.8 L/L_R/day in order to evaluate the effect of these two important operating conditions on CO_2 and H_2 utilization. The utilisation efficiency of H_2 and CO_2, η_{CO_2} and η_{H_2}, respectively, were determined in each period.

2.3. Analytical methods

Analyses of pH, TS, VS, TKN and NH_4-N were conducted according to Standard Methods for the examination of water and wastewater (APHA, 2005). The individual VFAs were quantified by gas chromatograph (GC Shimadzu) with flame ionisation detector (FID) based on Kougias *et al.* (2015). Biogas composition (CH_4, CO_2, H_2) was determined twice per week by gas chromatography equipped with thermal conductivity detectors (GC-TCD) as previously described (Bassani et al., 2017). H_2 gas transfer coefficient (day^{-1}) was calculated according to the following equation:

$$r_t = 22.4 k_L a (H_{2gTH} - H_{2l})$$

where, r_t (L/(L.day)) represents the H_2 gas to liquid mass transfer rate, 22.4 (L/mol) is the gas volume to mole ratio at STP, $k_L a$ (day-1) represents the gas transfer coefficient, H_{2gTH} (mol/L) represents the H_2 content in the gas phase while H_{2l} (mol/L) represents

168 the H_2 dissolved in the liquid phase.

169

170 **2.4. Microbial community composition**

171 Samples were obtained at the end of the experiment to ensure microbial community
172 stability. Genomic DNA was extracted from reactors' liquid samples, in triplicates, with
173 PowerSoil® DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA). The followed
174 procedure was previously described by Bassani *et al.* (2015). NanoDrop (ThermoFisher
175 Scientific, Waltham, MA) and QubitTM fluorimeter (Life Technologies, Carlsbad, CA)
176 were used for quality and quantity control of the extracted DNA. Universal primers
177 were used for 16S rRNA gene V4 hypervariable region amplification (Caporaso et al.,
178 2012). The 16S rRNA genes were sequenced using Illumina MiSeq sequencing
179 technology. The obtained reads were submitted to the NCBI sequence read archive
180 database (SRA) under the BioProject ID PRJNA607234. Detailed sample IDs are
181 presented in Supplementary Data. The 16S rRNA gene sequences were analyzed
182 according to Kougias *et al.* (2017) and subsequently, additional taxonomic verification
183 was conducted using BLASTN against NCBI 16S rRNA. Alpha diversity was
184 calculated from the number of operational taxonomic units (OTUs), while beta diversity
185 was calculated with the Bray-Curtis matrix and represented as Principal Coordinate
186 Analysis (PCoA). The relative abundance and fold change of most relevant OTUs are
187 presented in heat map drawn using Multiexperiment Viewer software (MeV) (Howe et
188 al., 2010).

189

190 **2.5. Statistical analysis**

191 Descriptive statistics were performed for all data, mean values and standard

192 deviations were calculated using Graphpad Prism 5 program (Graphpad Software, Inc.,
193 San Diego, CA). Anova Analysis with a Post Hoc test (Tukey HSD) was carried out to
194 compare and determine significant differences ($p < 0.05$) among the different
195 experimental periods. Concerning the AD microbiome, the significant abundance
196 differences for each microorganism were conducted using STAMP.
197

198 ## 3. Results and Discussion

199 ### 3.1. Overview of reactors performance

200 Coupling CO_2 and H_2 was examined under five separately distinguished operating
201 conditions. Experimental data from operation of the control reactor (R_c) and the reactor
202 filled with packing material (R_{r-r}) are summarized in Table 2. With respect to CO_2
203 fixation, H_2 utilization and CH_4 production; the performance of both reactors was
204 increasing from the beginning of the experiment since the end of the second
205 experimental period. Subsequently, the efficiency of R_c steadily decreased, reaching the
206 lowest performance at the end of the experiment, when the gas injection rate was set at a
207 GRT of 5 h. Indeed, the microbiome did not manage to sufficiently metabolize the extra
208 CO_2 and thus, the overall efficiency of the reactor decreased. On the contrary, R_{r-r}
209 retained a relatively more stable performance during the whole experiment. The added
210 packing material increased the surface area that was served for both biofilm formation
211 (Maegaard et al., 2019) and also, as a means for bubbles disruption of the injected gas
212 (Burkhardt and Busch, 2013). The comparison between the two reactors under all
213 experimental phases is further discussed in the following sections.
214 Regarding the pH, relatively unchanged values (8.53 ± 0.06) were measured during
215 the first two experimental periods. In contrast, different levels of VFAs and especially,

acetate were detected in two reactors in the same interval (Fig. 1). The detected accumulation indicated that the injected CO_2 and H_2 were not fully utilized by hydrogenotrophic methanogens but by homoacetogens, producing acetate instead of methane (Bassani et al., 2017). Consequently, the pH dropped from the end of period III until the end of period IV. In parallel, a second trend of acetate accumulation was observed during period IV (Fig. 1). In accordance, η_{CO_2} values were increased and η_{H_2} remained at high levels. Hence, we assumed presence of homoacetogenic microbes for CO_2 and H_2 fixation (Ragsdale and Pierce, 2008). This hypothesis was validated by the microbial analysis as shown in the following sections. Acetogenic CO_2/H_2 fermentation is lately gaining increased attention. Acetate can be produced as sole molecule containing trace by-products which makes the economic feasibility of its biological production route promising (De Vrieze et al., 2019). Hence, acetate production can be an alternative to biomethanation, offering flexibility to the gas fermentation system.

3.2. The role of packing materials

During the first experimental period, the performance of both reactors revealed a similar behaviour and an immediate start of CO_2 and H_2 utilisation for CH_4 production. At the end of period I, the control reactor had slightly higher CH_4 content (R_c: 62%) compared to the reactor filled with packing material ($R_{r\text{-}r}$: 60%). In the beginning of the experiment, the volume of the hydrogenotrophic inoculum was higher in R_c than $R_{r\text{-}r}$ which was partially filled with packing materials. Hence, the enriched microbial community could have positively affected the biomethanation due to the already high abundance of specialized microbes harbouring the key metabolic pathways to conduct the specific biological conversion (Herrero and Stuckey, 2015). In contrast, the

240 microbiome in R_{r-r} might have needed an adaptation period to create biofilm around the
241 Raschig rings where the microorganisms are immobilised and boost the biomethanation
242 at higher levels. On this topic, it has been previously observed that a tremendously more
243 prolonged adaptation period (>150 days) was necessary also under thermophilic
244 conditions to build-up adequate and stable amount of anaerobic biomass that could
245 efficiently bioconvert CO_2 and H_2 (Pokorna et al., 2019).

246 Subsequently, the performance of R_{r-r} was significantly improved ($p < 0.05$) during
247 the second period, indicating that sufficient biofilm was developed and consisted of
248 specialized microbes at increased abundance to enhance the biological absorption of
249 gases (Kim and Deshusses, 2003). Specifically, during period II, H_2 utilization was
250 significantly enhanced ($p < 0.05$) from 73.9% to 90.6% (Table 2) and the CH_4
251 concentration was 81%. Indeed, methanogenic archaea are growing rather slowly and
252 hence, a lag phase was needed in order to be amplified around the carrier material in
253 sufficient colonies for H_2 utilization (Siegert et al., 2014).

254 In accordance, the improved performance of the R_{r-r} containing Raschig rings was
255 revealed during the following experimental periods (III-V). Higher CO_2 fixation
256 efficiency was achieved in R_{r-r} (61.2-68.1%) in comparison with R_c (27.2-55.3%).
257 Moreover, the H_2 transfer coefficient was always higher in R_{r-r} compared to R_c (Table
258 2). In fact, it was previously shown that packing units can boost the performance of
259 biological conversion processes, providing extra surface area for microbial activity
260 (Singh and Prerna, 2009) and inducing the gas-liquid transfer (Billet and Schultes,
261 1993). The superiority of the R_{r-r} with the Raschig rings was more clearly detected at
262 the final experimental period in which the most intense experimental conditions were
263 applied (i.e. lowest GRT, highest Q_{RC}). Although the CH_4 concentration of R_{r-r} was

decreased at the end of period V, the packing materials managed to retain the CH_4 productivity at higher levels compared to the previous experimental periods. In contrast, the biomethanation process performance in R_c was dramatically dropped, supporting the hypothesis that the presence of packing materials with increased surface is prerequisite in order to have efficient gas-liquid mass transfer (Bassani et al., 2017) and create an immobilized biofilm to efficiently carry out the hydrogenotrophic methanogenesis (Sieborg et al., 2020). In fact, the η_{CO_2} and η_{H_2} had the lowest values during the whole experiment 30.5% and 64.9%, respectively, in the up-flow R_c without packing materials.

3.3. Effect of gas retention time and recirculation rate

Reactors monitoring showed that GRT reduction did not influence positively the biomethanation efficiencies. While the performance of $R_{r\text{-}r}$ remained at the same levels in the first periods (i.e. period II to III) with subsequently reduced GRTs, the efficiency of the control reactor R_c clearly deteriorated at low GRT. In contrast, the biomethanation process of both reactors failed in the final period with the lowest GRTs applied (i.e. period IV to V).

The GRT was altered by changing the input gas flow rate (i.e. feeding rate). However, increasing the gas feeding rate could negatively affect a previously well-performing upgrading community (Rachbauer et al., 2016). Thus, the decreased efficiency can be ascribed to the non-optimal operational conditions (e.g. low gas liquid mass transfer for the microbiome. Alteration of GRT was previously examined for ex-situ CO_2 and H_2 fixation (Burkhardt and Busch, 2013). In accordance, it was shown that at GRT lower than 6 h, the biomethanation efficiency was negatively affected as

advised by the lower H_2 degradation rates. Despite the limited biomethanation, the CO_2 convection rate of $R_{r\text{-}r}$ increased during the last GRT decrease. In agreement, Lee *et al.* (2012) found that high conversion CO_2 rate can be achieved with a GRT up to approximately 4 h. In parallel, in the same period there was a peak in acetate production, highlighting CO_2 and H_2 partial utilization for homoacetogenesis instead of methanogenesis.

Regarding the impact of the gas recirculation rate (Q_{RC}), distinct impacts were observed for both reactors during the first increase (i.e. period I to II). The methane production rate was increased by 52% and 35% in R_c and $R_{r\text{-}r}$, respectively. The positive effect of Q_{RC} was also detected in a recent biogas upgrading study (Alfaro et al., 2019). Interestingly, in the cited study a positive effect was revealed upon changing the Q_{RC} (i.e. from 5.3 to 7.0 $L/L_R.h$) to similar levels compared to the present work (i.e. from 4.2 to 8.3 $L/L_R.h$). The second Q_{RC} increment (period III to IV) contributed to η_{CO_2} increase, while retaining the η_{H_2} at high levels for both R_c and $R_{r\text{-}r}$. Indeed, the gas absorption rate is enhanced through increased recirculation (Jacob-Lopes et al., 2009) and accordingly, CO_2 fixation was finally induced. Regarding $R_{r\text{-}r}$, the increased recirculation rate did not manage to boost the biomethanation process. In agreement, Dupnock and Deshusses (2019) concluded that increasing Q_{RC} has not a clearly visible impact in reactors filled with packing materials. In contrast, intense Q_{RC} is a prerequisite to achieving high CH_4 purity in well-mixed reactors, by enhancing H_2 holdup and increasing the gas-liquid interfacial area.

3.4. Microbial diversity and dynamicity

Alpha diversity based on the number of OTUs and the rarefaction curves showed

312 that the sequencing depth was adequate to deliver information about the microbial
313 species richness (Supplementary Data). PCoA analysis was also performed to evaluate
314 composition and variability of microorganisms (Supplementary Data).
315 In both reactors, the most abundant microorganisms belonged to the
316 *Pseudomonadaceae* family. *Pseudomonas caeni* 1 had a relative abundance of 11.29%
317 and 18.61% in R_c and $R_{r\text{-}r}$, respectively. Apart from *Pseudomonas caeni* 1, additional
318 members within *Pseudomonas* genus were detected (i.e. *Pseudomonas* sp. 4, 10, 11 and
319 30). This is in accordance with Kougias *et al.* (2017) who found that *Pseudomonas*
320 genera were remarkably enriched in the liquid phase of different ex-situ upgrading
321 reactor systems. Likewise, members of the genus *Pseudomonas* were detected during
322 biologically mediated CO_2 reduction containing 656 $mg_{\text{-acetate}}$ /L (Porté et al., 2019).
323 Moreover, the ability of *Pseudomonas* spp. to tolerate much higher concentrations (up
324 to 10 $g_{\text{-acetate}}$/L) was lately shown (Yang et al., 2019). Thus, the proliferation of these
325 acetate utilizers is in agreement with the high concentrations of the carboxylic acid
326 observed in R_c (743 $mg_{\text{-acetate}}$/L) and $R_{r\text{-}r}$ (539 $mg_{\text{-acetate}}$/L). In contrast,
327 *Porphyromonadaceae* sp. 5 was 1.9-fold higher in R_c compared to $R_{r\text{-}r}$ and on top of
328 this, diverse species of *Porphyromonadaceae* family (*Porphyromonadaceae* sp. 7, 21,
329 28, 37, 38 and 46) were detected in both reactor systems. The high abundance of
330 members within either *Pseudomonadaceae* or *Porphyromonadaceae* family in acetate-
331 rich media could be associated to a potential presence of homoacetogenic and
332 syntrophic bacteria within these families. Specifically, the function of *Pseudomonas*
333 genera to accelerate electron transfer was suggested as the reason for their co-habitation
334 with archaea during efficient methanogenesis (Yuan et al., 2020). On the other hand,
335 representatives of *Porphyromonadaceae* family dominated the bacterial community

during efficient hydrogenotrophic methanogenesis driven by *Methanobacteriaceae* archaea (Granada et al., 2018).

On this topic, the only highly abundant methanogen in both reactors was the *Methanothermobacter* sp. 12 which is known to create syntrophies with acetate oxidizers (Treu et al., 2018). Also, it should be noted that *Methanothermobacter* sp. 12 was 1.5-fold higher in $R_{r\text{-}r}$ which is explained by the higher upgrading efficiency of the reactor filled with Raschig rings. Hence, the colonization of acetate-utilizing bacteria could be favoured with the proliferation of *Methanothermobacter* species. The preferential utilisation of acetate via syntrophic oxidation pathway, instead of direct acetate methanation, is further supported by the absence of known aceticlastic methanogens, such as members of the genus *Methanothrix* (Karakashev et al., 2006) (Supplementary Data). Furthermore, the existence of syntrophic acetate oxidising (SAO) bacteria and hydrogenotrophic archaea during ex-situ methanation is known (Corbellini et al., 2018). As expected, potential SAO were also detected in the present work. According to the results of the BLASTn search, *Peptococcaceae* sp. 25 was closely related to *Desulfonispora thiosulfatigenes* with a sequence similarity of 93%. The identified OTU was 6.3-fold higher in R_c in which higher acetate concentrations were detected. Similarly, *Sulfurospirillum* sp. 29 was detected in both systems and was more abundant in the control operation. Despite the fact that H_2S was not monitored in the present study, it is known that the presence of sulphate reducing bacteria can deteriorate process performance and is reducing sulfate to H_2S. In addition, the metabolic role of *Sulfurospirillum* genera is uncertain; however, it was lately indicated that their growth could rely on acetate oxidation by SAO (Westerholm et al., 2018). Finally, *Thermoanaerobacteraceae* sp. 18 (90% similarity with *Moorella thermoacetica*

and *Moorella thermoautotrophica*) was 2.8-fold higher in R_c. Within this family well-known homo- acetogenic species producing acetate from H_2 and CO_2 are available (Angelidaki et al., 2018). Despite the fact that potential SAO candidates were available in both systems, higher relative abundances were detected in control operation in which higher concentrations of acetate were measured.

4. Conclusions

The current research showed that in order to achieve an improved biological biogas upgrading by coupling CO_2 with H_2, the addition of packing material is crucial for up-flow reactors. Challenges were achieved decreasing the gas retention time. Intense gas recirculation rate can partially favour the performance of ex-situ biogas upgrading. The obtained results can be further interpreted as engineering data to use alternative reactors design that can efficiently work at shorter retention times. Results from the microbial analysis showed that *Methanothermobacter* species dominated completely the archaeal population.

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Tables

Table 1. Characteristics of degassed digestate and hydrogenotrophic inoculum

	Digestate	**Inoculum**
pH	8.00±0.10	8.28±0.04
Total solids (TS), g/L	23.1±1.2	25.5±1.3
Volatile solids (VS), g/L	12.0±0.6	17.8±0.9
Total Kjeldahl Nitrogen (TKN), g/L	4.9±0.2	2.01±0.03
NH_4-N, g/L	3.6±0.2	1.67±0.01
Total volatile fatty acids (TVFA), mg/L	136.6± 6.8	290.1±13.6
Acetate, mg/L	64.9±3.2	239.2±11.2
Propionate, mg/L	39.9±2.0	26.8±0.4
Iso-butyrate, mg/L	17.9±0.9	7.9±1.4
Butyrate, mg/L	2.0±0.1	1.5±0.2
Iso-valerate, mg/L	11.3±0.6	14.7±1.2
Valerate, mg/L	0.3±0.0	0.1±0.0
n-hexanoate, mg/L	0.3±0.0	0.1±0.0

519 **Table 2.** Reactors' upgrading performance under different experimental periods

Period	GRT, h	Q_{RC}, L/L_R.h	Reactor	*P*CH_4, LCH_4/L_R.d	η_{CO_2}, %	η_{H_2}, %	k_La, 1/day
I	8	5.3	R_c	0.29±0.02	48.3±5.3	80.4±2.6	$8.95*10^2$
			R_{r-r}	0.32±0.03	54.8±1.4	73.9±0.8	$9.22*10^2$
II	8	7.0	R_c	0.44±0.02	63.0±4.1	94.7±0.7	$2.89*10^3$
			R_{r-r}	0.42±0.04	55.2±3.5	90.6±.1.3	$2.24*10^3$
III	6	7.0	R_c	0.48±0.01	55.3±13.5	81.8±2.4	$1.29*10^3$
			R_{r-r}	0.52±0.03	61.2±10.3	87.3±2.6	$2.40*10^3$
IV	6	8.8	R_c	0.43±0.01	62.9±0.5	74.5±0.5	$9.59*10^2$
			R_{r-r}	0.54±0.07	64.1±4.6	86.1±7.0	$2.11*10^3$
V	5	8.8	R_c	0.38±0.17	30.5±6.6	64.9±2.9	$1.59*10^3$
			R_{r-r}	0.67±0.17	72.5±8.1	74.7±3.8	$2.57*10^3$

GRT: gas retention time, Q_{RC}: gas recirculation rate, η_{CO_2}: CO_2 utilisation efficiency, η_{H_2}: H_2 utilisation efficiency, k_La: H_2 gas transfer coefficient

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Figures captions

Fig. 1. VFA concentration and pH in R_C (a) and $R_{r\text{-}r}$ (b)

Fig. 2. Biogas composition at the outlet of R_C (a) and $R_{r\text{-}r}$ (b)

Fig. 3. Heat maps of relative abundance (left side of the panel) and fold change in microbial composition (right side of the panel). In the relative abundance heat map, the colour scale varies from low abundance (blue) to high abundance (red); in the heat map of fold changes, an increase trend is reported in red, while a decrease is expressed in green. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)