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# In-situ biogas upgrading in thermophilic granular UASB reactor: key factors affecting the hydrogen mass transfer rate

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## Highlights

- Biogas upgrading to 82% $CH_4$ is feasible in a thermophilic granular UASB reactor.
- $H_2$ is introduced in a separate chamber having a volume of 25% the reactor.
- $H_2$ low gas-liquid mass transfer rate limits the availability of $H_2$ for methanogens.
- $H_2$ distribution can be improved using porous inert devices, like ceramic sponge.
- Gas recirculation and chamber configuration help to maximize $CO_2$ conversion to $CH_4$.


## Abstract

Biological biogas upgrading coupling $CO_2$ with external $H_2$ to form biomethane opens new avenues for sustainable biofuel production. For developing this technology efficient $H_2$ to liquid transfer is fundamental. This study proposes an innovative setup for in-situ biogas upgrading converting the $CO_2$ in the biogas into $CH_4$, via hydrogenotrophic methanogenesis. The setup consisted of a granular reactor connected to a separate chamber, where $H_2$ was injected. Different packing materials (rashig rings and alumina ceramic sponge) were tested to increase gas-liquid mass transfer. This aspect was optimized by liquid and gas recirculation and chamber configuration. It was shown that by distributing $H_2$ through a metallic diffuser followed by ceramic sponge in a separate chamber, having a volume of 25% of the reactor, and by applying a mild gas recirculation, $CO_2$ content in the biogas dropped from 42 to 10% and the final biogas was upgraded from 58 to 82% $CH_4$ content.



## Keywords

In-situ biogas upgrading; Hydrogen; Gas-liquid mass transfer rate; UASB; Granules; Anaerobic digestion

## 1. Introduction

Anaerobic Digestion (AD) of organic waste is a promising technology for sustainable energy production (Weiland, 2010). The potato-starch processing industry produces, as byproduct, up to 1 $m^3$ of potato juice per ton of potatoes (Abeling and Seyfried, 1993). Potato-starch wastewater contains high concentration of biodegradable compounds, such as starch and proteins, suitable for biogas production via AD (Barampouti et al., 2005). Biogas typically contains ~50-70% $CH_4$ and 30-50% $CO_2$. Biogas upgrading to $CH_4$ content higher than 90% increases its heating value and its potential applications as alternative to natural gas (Deng and Hägg, 2010).

Methods currently available for biogas upgrading are mainly based on physicochemical $CO_2$ removal. Nevertheless, these technologies require use of additional materials and chemicals considerably increasing the cost of the process and energy input. Alternatively, biogas can be upgraded by biologically coupling $H_2$, derived from water electrolysis, with $CO_2$ present in the biogas to convert them to $CH_4$. $H_2$ can be produced using the electricity generated by the surplus of energy from wind mills or photovoltaic facilities, which may result from variable weather conditions. This reaction is carried out by a group of microorganisms known as hydrogenotrophic methanogenic archaea that utilize $CO_2$, as carbon source, and $H_2$, as electron donor, to produce $CH_4$ via hydrogenotrophic methanogenesis (Muñoz et al., 2015). Previous studies demonstrated that the addition of $H_2$ to a conventional biogas reactor can lead to

20 to 40% increase in $CH_4$ production rate, as result of the conversion of the $CO_2$ present in the biogas to additional $CH_4$ (Luo and Angelidaki, 2013; Luo et al., 2012).

Although biological biogas upgrading offers economical and technical advantages compared to traditional methods (Nordberg et al., 2012), $H_2$ mediated biogas upgrading is still challenging. One of the main limitations is the low $H_2$ gas-liquid mass transfer rate (Bassani et al., 2015; Luo and Angelidaki, 2012; Luo et al., 2012).

$H_2$ gas-liquid mass transfer rate can be described by the following equation (1):

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

where $r_t$ (L/(L-day)) is the $H_2$ gas–liquid mass transfer rate, 22.4 (L/mol) is the gas volume to mole ratio (1 mol gas corresponds to 22.4 L at STP), $k_L a$ ($day^{-1}$) is the gas transfer coefficient, $H_{2gTh}$ (mol/L) represent the $H_2$ concentration in the gas phase while $H_{2l}$ (mol/L) the $H_2$ dissolved in the liquid phase. One way to increase $H_2$ gas–liquid mass transfer rate is by increasing $k_L a$. This coefficient is specific for given reactor configuration and operating conditions (Pauss et al., 1990). Therefore, $k_L a$ can be modulated by changing parameters such as mixing speed (Bhattacharyya and Singh, 2010; Luo and Angelidaki, 2012), gas recirculation (Guiot et al., 2011) and $H_2$ diffusion device (Luo and Angelidaki, 2013; Díaz et al., 2015).

Besides, high-rate anaerobic treatment using up-flow anaerobic sludge blanket (UASB) reactors is commonly applied in industrial wastewater treatment plants (Gomec, 2010; Sevilla-Espinosa et al., 2010). Moreover, typically a UASB process is expected to provide higher methane content in the biogas than a CSTR process (Nizami et al., 2012).

UASB reactors' technology is based on the presence of granular sludge comprised of microorganisms responsible for catalyzing the biological conversion of organic matter

to biogas. High recirculation flow rates and consequent high up-flow velocities have an in important role for the hydraulic mixing improving the wastewater to granules contact (Powar et al., 2013; Zheng et al., 2012). It has been previously reported that carbohydrate degraders and hydrogenotrophic methanogens are predominant in starch-grown granules, likely due to their role in the interspecies $H_2$ transfer with syntrophic bacteria (Lu et al., 2015). Moreover, previous studies on $H_2$ mediated biogas upgrading demonstrated that $H_2$ affected the microbial community composition enhancing the hydrogenotrophic methanogenic pathway and the syntrophic relationship between bacteria and hydrogenotrophic methanogens (Bassani et al., 2015).

In this study an innovative setup consisting of a UASB granular reactor connected to a separate chamber, where the $H_2$ was injected, was designed to mediate efficient $H_2$ transfer to liquid phase for biological conversion of $H_2$ and $CO_2$ to $CH_4$. Key factors affecting the $H_2$ gas-liquid mass transfer rate were evaluated. More specifically, the effect of different operating conditions aiming in increasing $k_L a$ of $H_2$ to gas, and thereby increase the gas to liquid transfer, were studied to elucidate their role in improving $CO_2$ and $H_2$ conversion to $CH_4$. Parameters examined were liquid and gas recirculation and configuration of diffusion devices. Moreover, the addition of packing materials as a mean to minimize the gas bubble size and thus increase the gas dissolution in the liquid was tested. Finally, the effect of gas retention time was evaluated using single or serial chamber configurations with different working volumes.

## 2. Materials And Methods

### 2.1 Substrate characteristics and feedstock preparation

Potato-starch wastewater substrate was obtained from Karup Kartoffelmelfabrik potato-starch processing factory, Denmark. Because potato-starch processing involves an up-concentration step, the provided substrate was diluted 10 times with water and Basal Anaerobic (BA) medium, to adjust the volatile solids (VS) content to the required operation conditions. Successively, the substrate was stored at -20°C, in 5 L bottles and thawed at 4°C for 2-3 days, before usage. BA medium was prepared as described in Supplementary Information (SI). The diluted substrate had a pH of 6.05, chemical oxygen demand (COD) of 21.76±0.15 g/L, total solids (TS) and VS content of 26.14±0.17 and 18.73±0.12 g/L, respectively. The concentration of total volatile fatty acids (VFA) was 49.29±4.94 mg/L. Total Kjeldahl Nitrogen (TKN) and ammonium nitrogen $NH^{+4}$ ($NH_4$–N) were 1.24 ± 0.01 and 0.30 ± 0.01 g-N/L, respectively.

## 2.2 Setup and operation of the reactors

Each setup was composed of a UASB reactor with a working volume of 1.4 L, connected to a separate $H_2$-injection chamber with a working volume of 0.2 L. The feeding was introduced from the bottom of the UASB. The reactors were inoculated with 550 g of mesophilic granules, obtained from Colsen wastewater treatment plant treating potato starch wastewater (The Netherlands) and BA medium. The granules were adapted to thermophilic conditions for 25 days by feeding the reactors with diluted potato starch wastewater at hydraulic retention time (HRT) of 7 days and organic loading rate (OLR) of 2.79 gVS/L.day. A double net-separator was located in the upper part of each UASB to prevent the wash out of granules. One setup (R1) was used as upgrading reactor, while the other (R2) was utilized as control reactor operated throughout the experiment without $H_2$ injection. Both reactors were maintained at

126 thermophilic conditions (55 ± 1 °C) by circulating hot water through a water jacket
127 around the UASB reactors glass walls.
128 After the startup phase, the whole experiment was divided in 8 periods. During period I
129 the OLR was increased to 3.73 gVS/L day shortening the HRT to 5 days (Pre $H_2$ phase).
130 The recirculation flow rate was set to 4 L/h. From period II, $H_2$ was continuously
131 injected to R1 through a diffuser placed at the bottom of the $H_2$-injection chamber (In-
132 situ phase). Rashig rings (5 mm internal diameter) were inserted into the separate
133 chamber of both reactors to maximize the $H_2$ gas-liquid mass transfer rate in case of R1.
134 The volumetric $H_2$ flow rate was set to 4 times the $CO_2$ production rate (in the gas
135 phase) recorded before the $H_2$ addition, according to Luo and Angelidaki (2013b), i.e.
136 3.5 L/L.day, and then reduced to improve the $H_2$ consumption. In period III, the
137 recirculation flow rate of both reactors was increased to 7 L/h. Successively, in period
138 IV, rashig rings were replaced by an inert alumina ceramic sponge, while in periods V
139 and VI different gas recirculation flow were applied. In order to evaluate the effect of
140 the gas retention time, the $H_2$-injection chamber volume was doubled to 400 mL by
141 connecting two chambers in series (Period VII) or by assembling them as a single
142 chamber with extended length (Period VIII).
143 The percentage of $H_2$ utilized was calculated according to the following equation (2):

$$H_2 \text{utilization efficiency} = \frac{H_2 \text{ injected } \left(\frac{L}{L-day}\right) - H_2 \text{ in biogas } \left(\frac{L}{L-day}\right)}{H_2 \text{ injected } \left(\frac{L}{L-day}\right)} * 100$$

144 The percentage of $CH_4$ derived from the conversion of $CO_2$ and $H_2$ was calculated
145 according to the equation 3:

$$\text{CH}_4\text{from CO}_2\text{ and H}_2\text{conversion (\%)} =$$

$$\left(\frac{(\text{CH}_4\text{ production rate in R1 }\left(\frac{\text{L}}{\text{L.day}}\right)-\text{CH}_4\text{ production rate in R2 }\left(\frac{\text{L}}{\text{L.day}}\right))}{\text{CH}_4\text{ production rate in R2 }\left(\frac{\text{L}}{\text{L.day}}\right)+\text{CH}_4\text{ production rate equivalent to VFA in R2 }\left(\frac{\text{L}}{\text{L.day}}\right))}+\right.$$

$$\left.\frac{(\text{CH}_4\text{ production rate equivalent to VFA in R1 }\left(\frac{\text{L}}{\text{L.day}}\right)-\text{CH}_4\text{ production rate equivalent to VFA in R2 }\left(\frac{\text{L}}{\text{L.day}}\right))}{\text{CH}_4\text{ production rate in R2 }\left(\frac{\text{L}}{\text{L.day}}\right)+\text{CH}_4\text{ production rate equivalent to VFA in R2 }\left(\frac{\text{L}}{\text{L.day}}\right))}\right)*100$$

Where $CH_4$ production rate represents the volume of $CH_4$ produced per liter of reactor, per day, measured at the outflow of the reactor. While $CH_4$ production rate equivalent to VFA was calculated converting VFA concentrations, in the reactors, to $CH_4$ production equivalent according the following conversion reactions:

Acetate $CH_3COOH \rightarrow CH_4 + CO_2$

Propionate $CH_3CH_2COOH + 0.5\ H_2O \rightarrow 1.75\ CH_4 + 1.25\ CO_2$

Butyrate $CH_3CH_2CH_2COOH + H_2O \rightarrow 2.5\ CH_4 + 1.5\ CO_2$

Valerate $CH_3(CH_2)_3COOH + 1.5\ H_2O \rightarrow 3.25\ CH_4 + 1.75\ CO_2$

This was done to take into account the biomethanation inhibition caused by the injection of $H_2$ in the upgrading reactor and provide a more accurate estimation of the $CH_4$ produced from the conversion of $CO_2$ and $H_2$.

## 2.3 Analytical methods

The biogas production was recorded in daily basis. TS, VS, $NH_4$–N and TKN were measured according to the Standard Methods for Examination of Water and Wastewater (APHA, 2005). Liquid samples from the reactors were collected for pH and VFA analysis every second day. VFA and pH were measured according to Kougias et al., (2015) as described in SI. Detailed description of chromatographs utilized to measure biogas composition and $CH_4$ production (for batch assays) are given in SI. Detection limits for the measurement of $CH_4$, $CO_2$ and $H_2$ by GC were defined by the calibration curve (5−100%), while the detection limits for VFA were 5−1500 mg/L.

### 2.4 Specific methanogenic activity test

Specific methanogenic activity (SMA) assays were conducted during reactors' steady state operation. 1 g of granules and 9 mL of liquid sample obtained from the reactors were immediately transferred to 36 ml serum bottles under anaerobic conditions. The bottles were supplemented with acetate (20 mM) or $H_2/CO_2$ (80:20, 1 atm). Bottles with glucose (10 mM) or water as substrate were prepared as control and blank, respectively. All the tests were prepared in triplicates, flushed with $N_2$, sealed with rubber stoppers and aluminum caps and incubated at 55 °C and 155 rpm.

## 3. Results And Discussion

### 3.1 Process performances and biogas upgrade

Operational data from upgrading (R1) and control (R2) reactor under steady state conditions are reported in Table 1 and 2.

#### 3.1.1 Period I: the pre $H_2$ phase

In the pre $H_2$ phase (Period I), the two reactors showed similar performance in terms of biogas production rate (on average 2147 mL/L-reactor.day) and $CH_4$ yield (335 mL/gVS, corresponding to ~70% of the theoretical) (Table 1). This result is in accordance with previous studies on biogas production from starch biomasses (Frigon and Guiot, 2010). The average $CH_4$ content of the reactors was ~59% (Table 1 and Fig. 1), the pH was ~7.5 and the total VFA content >1 g/L (Table 1 and Fig. 2).

### 3.1.2 Period II: effect of rashig rings as $H_2$ distribution device on biogas upgrading performance

To increase the $k_L a$ and thereby enhance gas-liquid transfer, rashig rings were placed in the $H_2$-injection chamber to break $H_2$ bubbles and thus increase contact surface area between gas and liquid phases (Kramer and Bailey, 1991). Once steady state conditions were achieved, $H_2$ was continuously injected (3.5 L/L.day), through a metallic diffuser, in the $H_2$-injection chamber (In-situ phase). By comparing reactors' performance, in R1, 45% higher $CH_4$ production rate was observed (Table 1 and Fig. 3). Additionally, a pH increase to 7.9 was recorded in R1, as a result of the $CO_2$ removal (Table 1 and Fig. 2a). Nevertheless, because of the low $H_2$ gas-liquid mass transfer rate, only 51% of the $H_2$ injected was utilized leading to a high amount of unutilized $H_2$ in the output gas (45%) (Table 1 and Fig 1a). Additionally, a remarkable increase in VFA levels, reaching 3.4 g/L, was recorded in the upgrading reactor, while VFA concentration in the control reactor remained stable (Table 1 and Fig. 2b). This is likely due to the high $H_2$ partial pressure that affected negatively acidogenic VFA conversion resulting in their accumulation. Moreover, the continuous $H_2$ injection led to a progressive higher $H_2$ partial pressure, which shifted the metabolic pathway towards homoacetogenesis inhibiting methanogenesis (Cord-Ruwisch et al., 1997). This argument was supported by the predominance and accumulation of acetate over other VFA in R1 accounting for 55% of total VFA (Table 1). Moreover, this level was 4 % higher than the correspondent level in R2, which, together with higher total VFA concentrations, demonstrates the instability caused by the excessive $H_2$ flow rate provided in R1. Therefore, to provide a more accurate estimation of the increment of the $CH_4$ production rate due to $CO_2$ and $H_2$ conversion, the total VFA concentrations in the two systems

were converted in equivalent $CH_4$ production, as described in section 2.2. The difference in the VFA concentration between the two reactors was taken into account to estimate the inhibition of liquid substrate degradation occurring in the upgrading reactor and allow the reactors' performances to be comparable. Thus, the $CH_4$ derived from $CO_2$ and $H_2$ conversion was calculated (equation 3) based on the difference between the $CH_4$ production rates of the two systems after normalization of VFA.

To overcome the negative effect of the $H_2$ on the biomethanation process and improve the $H_2$ consumption, in the last part of this period the $H_2$ flow rate was reduced to 2.6 L/L.day reducing the unutilized $H_2$ to 34% of the output gas and increasing the $CH_4$ content to 47%.

### 3.1.3 Period III: effect of liquid recirculation on upgrading performance

Good mixing is known to be crucial to make substrates available for microorganisms (Bhattacharyya and Singh, 2010; Luo and Angelidaki, 2012). Moreover good mixing increases the $k_L a$ for gasses, which is function of the surface area per unit volume, thereby increasing gas-liquid contact (Kramer and Bailey, 1991). Therefore, to improve $H_2$-liquid contact, the liquid recirculation flow was increased from 4 to 7 L/h, while the $H_2$ flow rate was maintained to 2.6 L/L.day leading to a slight increase of the utilized $H_2$ (53%) (Table1). The unutilized $H_2$ and the $CH_4$ content in the output gas stabilized to 37% and 45%, respectively (Table 1 and Fig. 1a). Similarly, in this period in R1 36% higher $CH_4$ production rate was recorded, compared to R2 (Table 1 and Fig. 3). As these results did not markedly differ from the last part of period I (i.e. $H_2$ flow rate was reduced to 2.6 L/L.day), it can be concluded that the improved upgrading efficiency was mainly attributed to the lower $H_2$ flow rate applied, rather than to the higher liquid

recirculation flow. In fact, upon $H_2$ addition, the granular bed appeared less expanded, probably due to reduced dissolved $CO_2$ concentration in the liquid, due to the hydrogenotrophic consumption of $CO_2$ to $CH_4$ (Ohsumi et al., 1992; Song et al., 2005). Therefore, the positive effect of the higher liquid recirculation on biogas production and upgrading was not achieved.

### 3.1.4 Period IV: effect of alumina ceramic sponge as $H_2$ distribution device on upgrading performance

An alternative method to reduce $H_2$ bubbles size and thus increase gas-liquid contact is by increasing the surface area of the material over which the bubbles travelled and thereby breaking them to a smaller size. Based on that, the rashig rings in the $H_2$-injection chamber were replaced with alumina ceramic sponge. Alumina ceramic sponge introduced in the chamber had 16 $m^2$ (0.3 $m^2$/g) surface area which is significantly higher compared to the surface area in rashig rings (0.1 $m^2$, corresponding to 0.002 $m^2$/g). Interestingly, in this period, the $H_2$ utilization and the $CH_4$ production rate derived from $CO_2$ and $H_2$ conversion increased (Table 1 and Fig. 3). On average, 67% of the $H_2$ injected was utilized reducing the $H_2$ content in the output gas to 31% and increasing the $CH_4$ content to 52% (Table 1 and Fig. 1a). These results clearly show the influence of the $H_2$ distribution on the upgrading performances indicating the importance of porosity and pore size of the $H_2$ distribution device for an effective $H_2$ utilization by microorganisms.

In this period lower biogas and $CH_4$ production rates were observed in particular in R2 (Table 1 and Fig. 3). Previous studies have demonstrated that aluminum oxide does not cause any toxic effects on microorganisms' growth (Ingham et al., 2012).

Additionally, state indicators of the biomethanation process, such as VFA and pH, did not demonstrate any imbalance. More specifically, the VFA levels recorded in this period and particularly for R1 were at the lowest levels compared to the other periods (Table 1 and Fig. 2b). Therefore, we assume that ceramic sponge pores could have retained undigested biomass particles with consequent decrease of $CH_4$ production.

In the last part of this period, in order to reduce the unutilized $H_2$, the $H_2$ flow rate was further decreased to 2 L/L.day resulting in reduced $H_2$ and increased $CH_4$ content in the output gas to 20% and 57%, respectively.

### 3.1.5 Period V and VI: effect of gas recirculation on upgrading performance

As previously described, gas recirculation would have a positive effect on $k_L a$ coefficient, increasing $H_2$ gas-liquid mass transfer rate (Equation 1) (Guiot et al., 2011).

Therefore, in period V, 4 mL/min gas recirculation (then increased to 6 mL/min, in period VI) were applied to R1 improving the $H_2$ dissolution and thus significantly increasing the $CO_2$ conversion. In fact, in these periods on average 87% of the $H_2$ injected was utilized leading to 37% higher $CH_4$ production rate (Table 2 and Fig. 3). Nevertheless, an increase in the pH value to 8.2 was recorded as a result of the $CO_2$ removal (Table 2 and Fig. 2a). The $CH_4$ content in the biogas markedly increased to 66% and the unutilized $H_2$ decreased to 14% (Table 2 and Fig. 1a). To further decrease the unutilized $H_2$, at the end of the period the $H_2$ flow rate was reduced to 1.8 L/L.day (corresponding to ~2.5 times the $CO_2$ production rate recorded in R2). Nevertheless, no substantial difference in biogas composition and upgrading performances was recorded. In previous studies, $H_2$ distribution in the reactor's liquid phase was optimized by the application of gas recirculation flow rates ~4-folds higher than the input gas flow rate

289 (Díaz et al., 2015). Unfortunately, in this experiment, beside the positive effect on
290 upgrading performances, the application of such a high gas recirculation flow rate led to
291 an excessive pressure through the diffuser and to turbulent movements causing granules
292 disintegration. The subsequent reduction of reactor's active biomass can explain the
293 lower $CH_4$ production rate and VFA levels higher than 5 g/L observed in R1 from
294 period V (Table 2, Fig. 2b and Fig. 3).
295
296 **3.1.6 Period VII and VIII: Effect of gas retention time using $H_2$-injection chamber**
297 **configuration on upgrading process performance**
298 To increase the contact area between $H_2$ bubbles and liquid, and therefore increase $H_2$
299 transfer coefficient (Equation 1), the ceramic sponge surface area was doubled. This
300 was done by doubling $H_2$-injection chamber volume, either by connecting two chambers
301 in series (Period VII), or by assembling them in a single longer chamber (Period VIII).
302 The connection of two chambers in series did not lead to a substantial improvement of
303 upgrading performances, indicating that chamber's volume itself has not a direct
304 correlation with $H_2$ distribution. Nevertheless, by assembling two chambers in a single
305 longer one, a higher $H_2$ percentage was utilized (94%) resulting in only 8% $H_2$
306 unutilized (Table 2 and Fig. 1a). Therefore, $CO_2$ and $CH_4$ contents in the output biogas
307 dropped to 10% and increased to 81% (with a maximum of 82%) respectively (Table 2
308 and Fig. 1a). However, in this period the pH raised to 8.4 as a consequence of the high
309 $CO_2$ conversion (Table2 and Fig. 2a). The results clearly demonstrate the importance of
310 a proper reactor configuration design that increases the gas retention time leading to
311 more efficient $H_2$ distribution and $CO_2$ conversion to $CH_4$.

312 Moreover, from the comparison of reactors $CH_4$ production rate, it was shown that, in
313 the upgrading reactor, on average the $CH_4$ produced from the conversion of $CO_2$
314 represented ~37% of the total recorded $CH_4$ production rate (Table 1 and 2 and Fig. 3).
315 Finally, it should be mentioned that the lower $CH_4$ production and higher VFA levels
316 of control reactor observed in period VII were due to the disassembly of the separate
317 chamber in order to be mounted in the upgrading reactor (Table 2 and Fig. 2b and 3).
318 The $CH_4$ productivity and the VFA concentration of the control reactor were recovered
319 in period VIII.
320

321 **3.2 Specific methanogenic activity test**

322 $H_2$ addition is known to promote the hydrogenotrophic methanogenic pathway (Bassani
323 et al., 2015; Luo and Angelidaki, 2013a, 2013b). Therefore, in this experiment, SMA
324 tests were performed to validate the effect of the $H_2$ addition on methanogenesis
325 pathways. Granules and liquid samples were taken from the reactors at steady state of
326 periods IV (introduction of ceramic sponge as $H_2$ distribution device) and V
327 (application of gas recirculation). It was shown that the preferable methanogenic
328 pathway in both reactors (i.e. R1 and R2) was hydrogenotrophic (Table 3). This result
329 was expected because hydrogenotrophic methanogens are known to be predominant in
330 starch-grown granules (Lu et al., 2015).
331 In period IV, $CH_4$ production rate achieved by batches fed with $H_2/CO_2$ did not show
332 markedly difference between the two reactors. Conversely, in period V, higher
333 hydrogenotrophic activity was observed in R1 compared to the control reactor, likely
334 due to the gas recirculation enhancing the effect of $H_2$ addition on microbial community
335 composition and thus stimulating hydrogenotrophic methanogenic pathway.

Both tests showed low aceticlastic activity which can be explained by the high acetate levels detected in the reactors before the tests which further increased in period V (~3.3 g/L in R1 and ~1.5 g/L in R2; Table 2). Moreover, by comparing the concentration of unutilized acetate at the end of SMA tests and in the UASB reactors, it was shown that acetate levels markedly decreased in all batches (from 3 to 2.5 g/L in the upgrading system and from 1.4 to 1.3 g/L in the control treatment), apart from batches fed with acetate, where acetate levels increased to 3.3 and 1.8 g/L in R1 and R2, respectively. These results indicate that high acetate levels in the inoculum obtained from the reactor probably inhibited the process not allowing the further degradation of the supplemental amount of acetate that was added in the batch bottles (Gorris et al., 1989).

Finally, it was found that the specific microbial activity for the degradation of glucose was lower in period V compared to period IV. This could be possibly due to the negative effect of gas recirculation on the granules as previously discussed in the continuous reactor operation (Tables 1, 2 and 3).

## 4. Conclusions

The current research demonstrated the feasibility of in-situ biogas upgrading using an external chamber with 25% of the conventional biogas reactor volume. Key factors affecting the $H_2$ gas-liquid mass transfer rate were tested to improve the efficiency of the overall process. It was shown that the use of porous devices benefit the $H_2$ uptake as the active contact area is increasing and the gas retention time is extended. Moreover, the gas recirculation flow rate and the chamber design are fundamental elements that must be considered to maximize the gas retention time and thus the $H_2$ dissolution to the liquid media.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at

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458 **Table captions:**

459 **Table 1:** Upgrading (R1) and control (R2) reactor performances under steady state
460 conditions (Periods I-IV).

461 **Table 2:** Upgrading (R1) and control (R2) reactor performances under steady state
462 conditions (Periods V-VIII).

463 **Table 3:** Specific methanogenic activity (SMA) results, expressed as $CH_4$ production
464 rate (mL/L.day), under steady state conditions.

**Figure captions:**

**Fig. 1:** Biogas composition ($CH_4$ (●), $CO_2$ (○) and $H_2$ (■) %) of (a) upgrading and (b) control reactor.

**Fig. 2:** pH (a) and total VFA (b) of upgrading **(**♦) and control (○**)** reactor.

**Fig. 3:** $CH_4$ production rate of upgrading (♦) and control (○**)** reactor.

**Table 1**

| Phase | Pre $H_2$ | | In-situ | | | | | |
|---|---|---|---|---|---|---|---|---|
| Period | I | | II | | III | | IV | |
| $H_2$ distribution device | - | | rashig rings | | rashig rings | | ceramic sponge | |
| Reactor | R1 | R2 | R1 | R2 | R1 | R2 | R1 | R2 |
| Liquid recirculation flow (L/h) | 4 | 4 | 4 | 4 | 7 | 7 | 7 | 7 |
| Gas recirculation flow (mL/min) | NA* | / | NA* | / | NA* | / | NA* | / |
| Biogas production rate (mL/L.day) | 2167±180 | 2127±180 | 2093±232 | 2229±129 | 2072±102 | 2015±75 | 1953±97 | 1787±57 |
| Biogas composition (%): | | | | | | | | |
| $CH_4$ | 58.2±3.4 | 60.3±3.0 | 40.4±4.3 | 60.6±1.8 | 44.9±2.3 | 60.9±1.0 | 52.0±1.9 | 62.5±0.3 |
| $CO_2$ | 41.8±3.4 | 39.7±3.0 | 14.9±3.2 | 39.4±1.8 | 18.5±3.2 | 39.1±1.0 | 17.0±0.7 | 37.5±0.3 |
| $H_2$ | NA* | / | 44.6±6.7 | / | 36.6±1.9 | / | 31.0±1.9 | / |
| $CH_4$ production rate (mL/L.day) | 1255±54 | 1277±61 | 1528±147 | 1350±74 | 1497±73 | 1227±53 | 1471±72 | 1117±39 |
| $CO_2$ in output gas (mL/L.day) | 912±148 | 850±134 | 565±115 | 878±73 | 618±55 | 789±33 | 482±34 | 670±19 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| $H_2$ flow rate (mL/L.day) | NA* | / | 3477±594 | / | 2636±89 | / | 2629±93 | / |
| $H_2$ consumption rate (mL/L.day) | NA* | / | 1769±330 | / | 1412±212 | / | 1756±121 | / |
| pH | 7.46±0.03 | 7.49±0.06 | 7.92±0.11 | 7.59±0.09 | 7.90±0.06 | 7.60±0.05 | 7.93±0.12 | 7.56±0.09 |
| Total VFA (g/L) | 1.69±0.37 | 1.21±0.25 | 3.40±0.31 | 1.41±0.28 | 3.60±0.23 | 2.26±0.11 | 2.81±0.46 | 2.37±0.32 |
| Acetate content in VFA (%) | 41.3±4.3 | 49.0±3.9 | 55.3±4.0 | 51.5±3.8 | 51.8±2.3 | 47.3±3.7 | 49.7±3.8 | 47.2±4.2 |

*NA: not applicable to this period

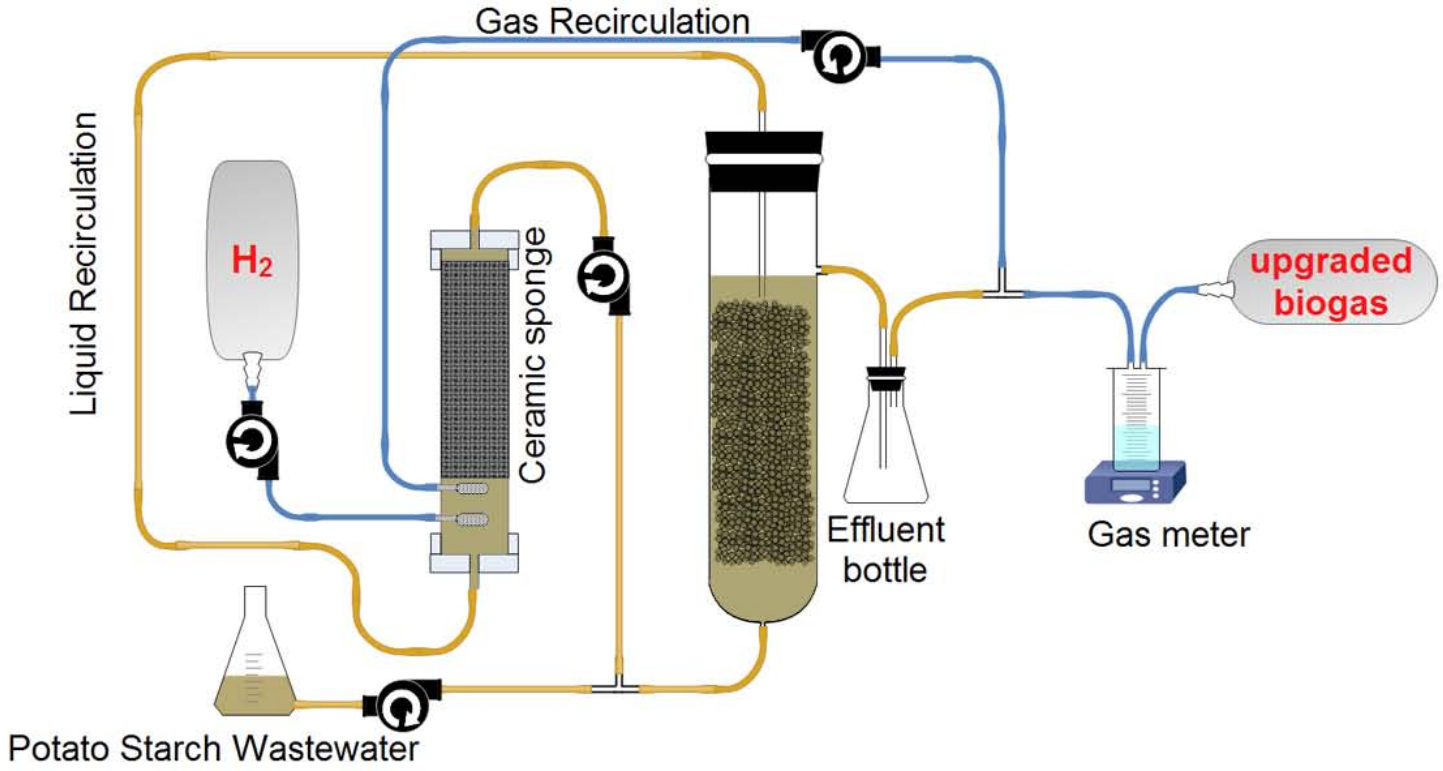
**Table 2**

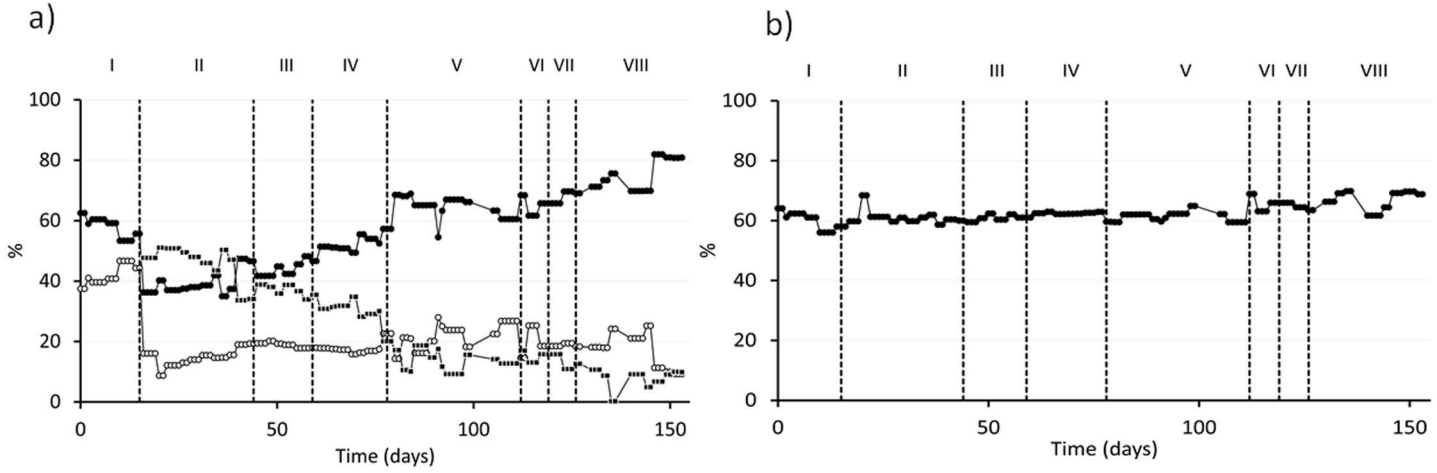
| Phase | In-situ | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Period | V | | VI | | VII | | VIII | |
| $H_2$ distribution device | ceramic sponge | | ceramic sponge | | serial chambers | | single chamber with extended length | |
| Reactor | R1 | R2 | R1 | R2 | R1 | R2 | R1 | R2 |
| Liquid recirculation flow (L/h) | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 |
| Gas recirculation flow (mL/min) | 4 | / | 6 | / | 6 | / | 6 | / |
| Biogas production rate (mL/L.day) | 1786±68 | 1900±85 | 1521±98 | 2018±275 | 1337±72 | 1175±138 | 1261±157 | 1558±188 |
| Biogas composition (%): | | | | | | | | |
| $CH_4$ | 66.4±1.9 | 61.1±1.2 | 66.0±2.5 | 65.0±2.4 | 67.6±2.0 | 65.0±1.0 | 81.3±0.6 | 66.7±2.8 |
| $CO_2$ | 20.5±4.0 | 38.9±1.2 | 18.35±3.9 | 35.0±2,4 | 18.8±0.5 | 35.0±1.0 | 10.2±1.0 | 33.2±2.8 |
| $H_2$ | 13.0±4.3 | / | 15.7±1.4 | / | 13.5±2.4 | / | 8.5±1.5 | / |
| $CH_4$ production rate (mL/L.day) | 1365±52 | 1161±55 | 1188±55 | 1308±149 | 1046±57 | 763±92 | 1145±134 | 1039±121 |

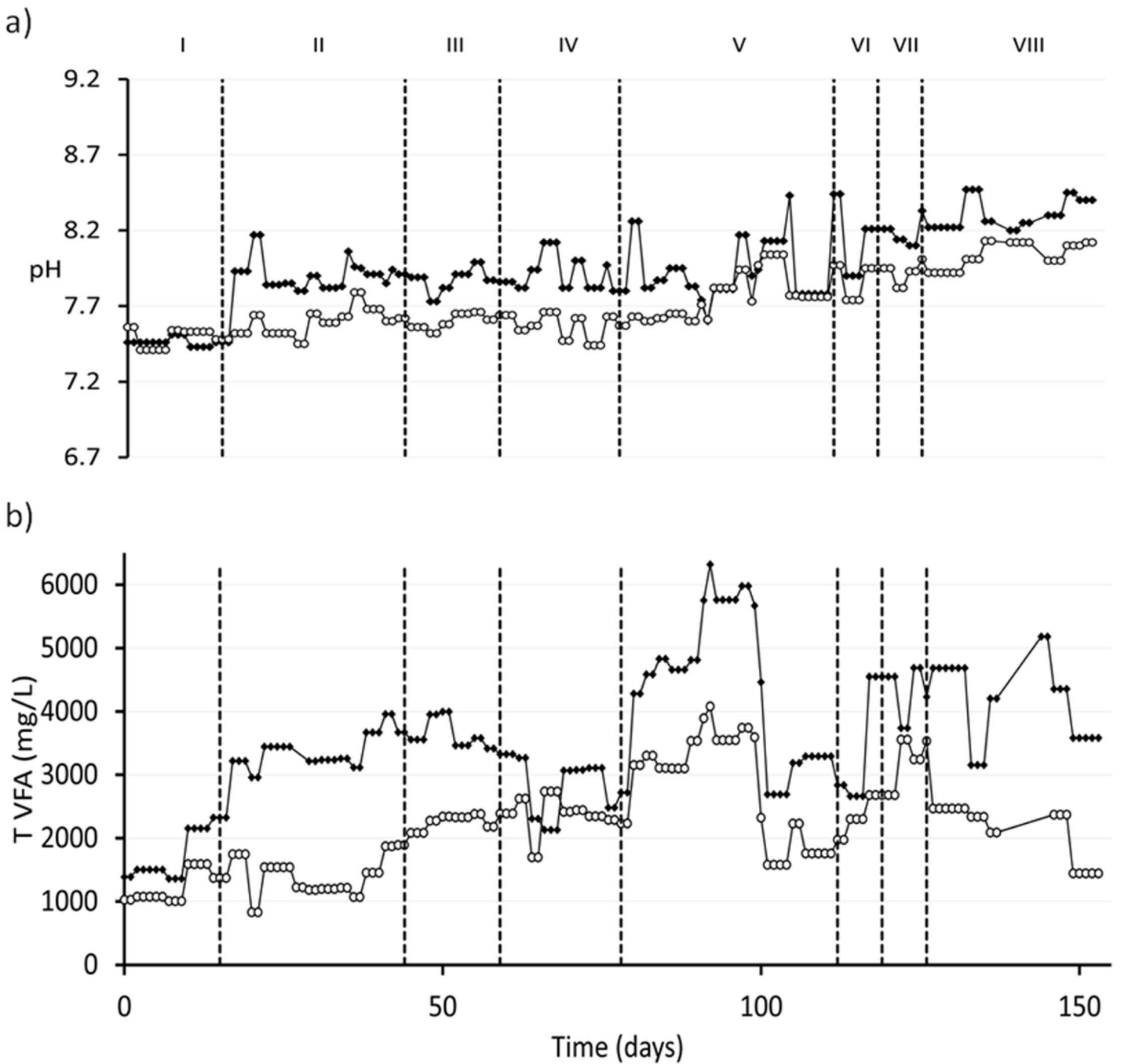
| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| $CO_2$ in output gas (mL/L.day) | 421±65 | 740±47 | 333±82 | 710±134 | 291±16 | 412±48 | 121±21 | 615±83 |
| $H_2$ flow rate (mL/L.day) | 2144±312 | / | 1834±30 | / | 1768±55 | / | 1828±14 | / |
| $H_2$ consumption rate (mL/L.day) | 1873±234 | / | 1551±44 | / | 1536±80 | / | 1717±23 | / |
| pH | 7.83±0.10 | 7.64±0.07 | 8.24±0.20 | 7.85±0.12 | 8.18±0.08 | 7.92±0.07 | 8.38±0.07 | 7.99±0.09 |
| Total VFA (g/L) | 5.11±0.06 | 3.24±0.48 | 3.66±0.97 | 2.37±0.27 | 4.34±0.40 | 3.21±0.39 | 3.87±0.40 | 2.36±0.15 |
| Acetate content in VFA (%) | 64.6±3.4 | 46.0±4.7 | 39.9±2.6 | 39.4±4.3 | 37.0±2.2 | 36.5±2.9 | 30.3±1.4 | 34.5±6.5 |

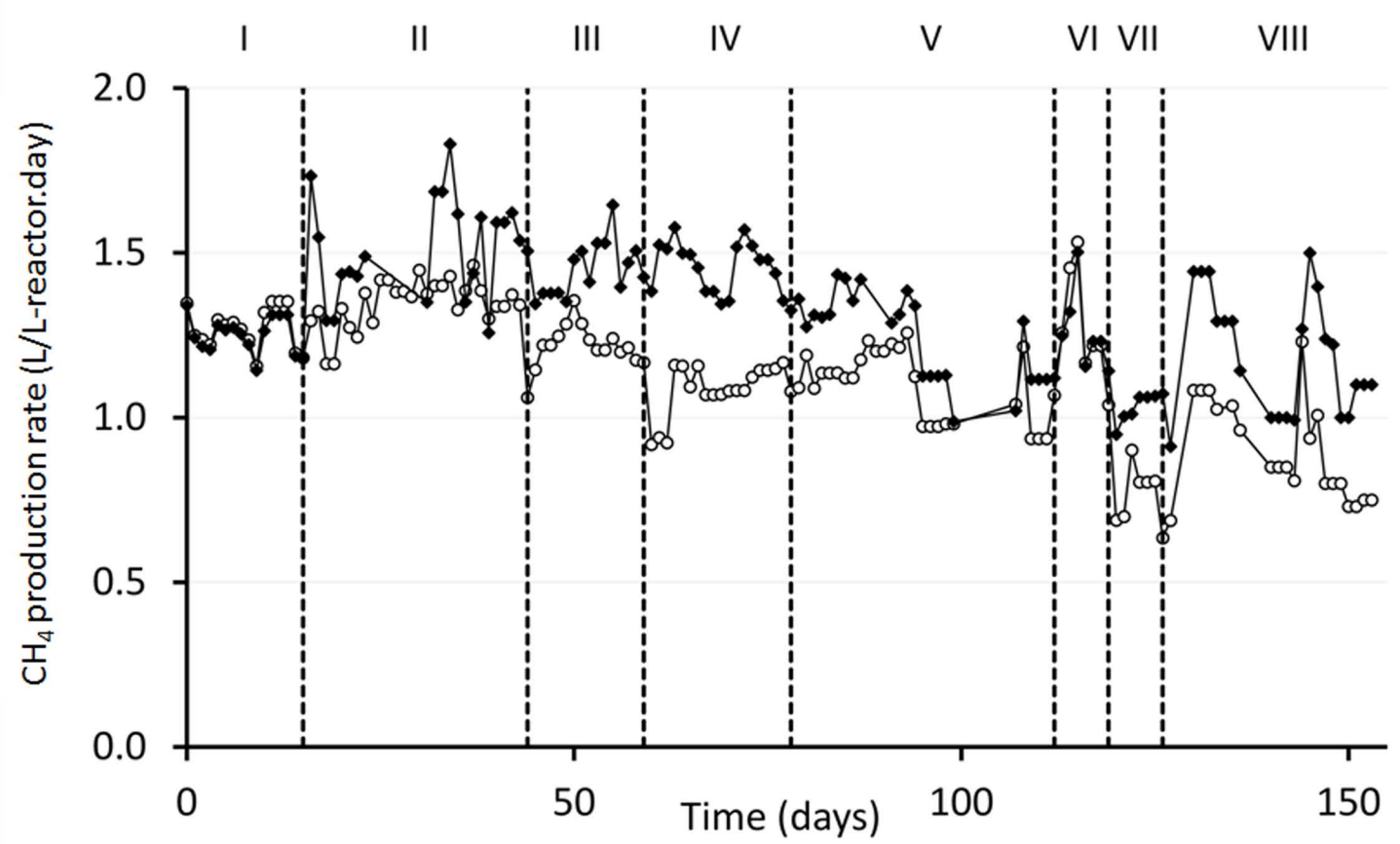
**Table 3**

| Period | IV | | V | |
|---|---|---|---|---|
| Reactor | R1 | R2 | R1 | R2 |
| Blank | 36±2 | 11±2 | 6±1 | 7±1 |
| Glucose | 589±67 | 219±6 | 73±22 | 23±12 |
| Acetate | 159±4 | 4±1 | 4±1 | 3±2 |
| $H_2/CO_2$ | 1270±20 | 1296±29 | 986±212 | 520±65 |


Gas Recirculation
Liquid Recirculation
$H_2$
Ceramic sponge
upgraded biogas
Effluent bottle
Gas meter
Potato Starch Wastewater


a)
I
II
III
IV
V
VI
VII
VIII
100
80
60
40
20
0
%
0
50
100
150
Time (days)
b)
I
II
III
IV
V
VI
VII
VIII
100
80
60
40
20
0
%
0
50
100
150
Time (days)


a)
I
II
III
IV
V
VI
VII
VIII
9.2
8.7
8.2
7.7
7.2
6.7
pH
b)
6000
5000
4000
3000
2000
1000
0
T VFA (mg/L)
0
50
100
150
Time (days)


I
II
III
IV
V
VI
VII
VIII
2.0
1.5
1.0
0.5
0.0
CH4 production rate (L/L-reactor.day)
0
50
100
150
Time (days)