Innovative *ex-situ* biological biogas upgrading using immobilized biomethanation bioreactor (IBBR)

Katie Baransi-Karkaby, Mahdi Hassanin, Sharihan Muhsein, Nedal Massalha and Isam Sabbah

**ABSTRACT**

Biogas, which typically consists of about 50–70% of methane gas, is produced by anaerobic digestion of organic waste and wastewater. Biogas is considered an important energy resource with much potential; however, its application is low due to its low quality. In this regard, upgrading it to natural gas quality (above 90% methane) will broaden its application. In this research, a novel *ex-situ* immobilized biomethanation bioreactor (IBBR) was developed for biologically upgrading biogas by reducing CO2 to CH4 using hydrogen gas as an electron donor. The developed process is based on immobilized microorganisms within a polymeric matrix enabling the application of high recirculation to increase the hydrogen bioavailability. This generates an increase in the consumption rate of hydrogen and the production rate of methane. This process was successfully demonstrated at laboratory-scale system, where the developed process led to a production of 80–89% methane with consumption of more than 93% of the fed hydrogen. However, a lower methane content was achieved in the bench-scale system, likely as a result of lower hydrogen consumption (63–90%). To conclude, the IBBRs show promising results with a potential for simple and effective biogas upgrading.

**Key words** | biogas upgrading, biological upgrading, biomethanation, *ex-situ* biogas upgrading, immobilized bioreactor, polyfoam bioreactor

**HIGHLIGHTS**

- A novel *ex-situ* immobilized biomethanation bioreactor was developed.
- The process was demonstrated at laboratory- and bench-scale systems.
- CH4 content of 80-89% was achieved with consumption of over 90% of hydrogen.

**ABBREVIATIONS**

AD Anaerobic digestion  
IBBR Immobilized biomethanation bioreactor  
UASB Upflow anaerobic sludge blanket  
EGSB Expanded granular sludge bed  
HRT Hydraulic retention time

**INTRODUCTION**

Biogas is the product of a microbial process known as anaerobic digestion (AD). AD occurs in oxygen-free environments where organic waste is decomposed by complex metabolic pathways, eventually leading to the formation of biogas (*Angelidaki et al. 2018*). Biogas typically consists of 50–70% methane and 30–50% CO2, and often contains additional undesirable gases (i.e. traces of water vapor, H2S and H2, and other contaminants) (*Angelidaki et al. 2018*). Although biogas can be used for heating or to generate power, its large CO2 content reduces its heating value and limits its economic feasibility (*Comeau et al. 2002; Zhao et al. 2010; Muñoz et al. 2015*). In this regard, upgrading biogas to natural gas-quality (CH4% over 90%) will broaden its applications.
Most of the methods for biogas upgrading are based on physico-chemical techniques that remove CO₂ with minimal loss of CH₄ (Niesner et al. 2013). However, the high energy and chemical requirements of these techniques severely limit the degree to which the upgraded biogas can be considered a source of renewable energy (Muñoz et al. 2015). Alternatively, biogas can be upgraded biologically, by using chemoautotrophic or photosynthetic technologies. In chemoautotrophic biogas upgrading, hydrogenotrophic methanogens utilize H₂ (from an external source) as an electron donor to reduce CO₂ to CH₄ (Equation (1)) (Luo et al. 2012; Kougias et al. 2016; Rachbauer et al. 2016). This is done primarily through two operational modes: in-situ and ex-situ biogas upgrading.

\[ 4H_2 + CO_2 \rightarrow CH_4 + 2 H_2O \] (1)

During in-situ biogas upgrading, H₂ is injected directly into the liquid phase of the anaerobic digester in order to in-situ convert the produced CO₂ to methane by hydrogenotrophic methanogens (Strevert et al. 1995; Luo & Angelidaki 2012; Luo et al. 2012; Bassani et al. 2016). However, some technical challenges might occur during in-situ biogas upgrading, such as the increment of pH above 8.5 as a result of bicarbonate consumption, which can inhibit methanogenesis (Luo et al. 2012). Additionally, volatile fatty acids accumulation and process inhibition may occur as a result of increasing hydrogen partial pressure (concentration) inside the anaerobic reactor (Batstone et al. 2002; Liu & Whitman 2008; Agnessens et al. 2017). Alternately, biogas can be upgraded using the ex-situ mode, wherein the biogas is injected with the addition of H₂ into the liquid phase of a separate anaerobic reactor containing hydrogenotrophic methanogens (Kougias et al. 2016; Angelidaki et al. 2018). Ex-situ upgrading in a separate reactor unit ensures stability of the anaerobic digestion (avoiding the inhibition effect of H₂ on the volatile fatty acids consuming bacteria), leading to a more controlled upgrading process. However, the low solubility of hydrogen in water media and the low mass transfer of the gas-liquid phase of hydrogen limits the bioavailability of hydrogen, hindering both in-situ and ex-situ upgrading processes (Guiot et al. 2011; Kougias et al. 2016; Angelidaki et al. 2018).

One way to overcome these limitations is the application of liquid-phase recirculation regimes to enhance the surface contact and time in the bioreactor. Usually, high recirculation rates (high velocity) can damage the structure and functionality of the anaerobic sludge, which contains the hydrogenotrophic methanogens (Muñoz et al. 2015), or even lead to biomass washout from the reactor in high rate anaerobic reactor systems (i.e. upflow anaerobic sludge blanket (UASB), expanded granular sludge bed (EGSB)). In this research, an innovative immobilized bio-methanation bioreactor (IBBR) was developed for ex-situ biogas upgrading. The developed IBBR is based on our recently developed immobilization technique of microorganisms from granular sludge within a polymeric-based matrix, polyfoam (Massalha et al. 2015a, 2015b; Sabbah et al. 2016). This immobilization technique can prevent washout of the active biomass while applying high recirculation rates. In this research, ex-situ biological biogas upgrading using IBBR was evaluated using different operating modes such as H₂ injection and liquid recirculation at different flow rates. The experiments were conducted at laboratory and bench scale.

MATERIALS AND METHODS

Preparation of composite polyfoam

Hydrophilic polyurethane pre-polymer, ‘Hypol FHP 2002™’, was purchased from Dow Chemical Company (‘Dow’). ‘Hypol FHP 2002™’ is a toluene diisocyanate (TDI) pre-polymer. All technical grade chemicals were purchased from Romical, a local supplier.

The preparation of the composite polyfoam containing the anaerobic sludge was according to Agrobics® patent (Sabbah et al. 2016). The polymerization process was performed in a 2 L plastic beaker, where the solution was vigorously mixed for 20 seconds using a mechanical stirrer and then poured into a rectangular glass tank. As the foaming reaction progressed, the hydrophilic polyurethane foam expanded to fill the mold. The foam was left for 40 minutes to reach its maximal strength before the mold was removed. The thin impermeable layer that formed on the outer surface of the foam was removed by an electric foam cutter (Bosch GSG 300). The foam was cut into nine cubes with an average volume of 10 cm³ for each cube, filling the laboratory-scale IBBR. The bench IBBR contained a ‘rope’ of polymer with a volume of 1,920 cm³. The foam specimens were washed by submerging them in deionized water for 15 minutes, squeezing and releasing them several times (Massalha et al. 2015a).

Reactor setup

Three IBBRs were used in this study. The two laboratory-scale IBBRs were glass-made anaerobic reactors with
440 mL active volume having an inner diameter of 3.8 cm and a height of 39 cm. Each reactor contained 9 cubes of anaerobic sludge-based hydrophilic polyurethane matrix corresponding to about 90 cm³. Estimation of the initial wet anaerobic biomass/sludge within the polyfoam was about 3.6 g (0.27 g of VS). The third reactor was a bench-scale IBBR made of polypropylene with 2.7 L active volume, having an inner diameter of 5.2 cm and a height of 128 cm; the reactor contained a ‘rope’ of polyfoam corresponding to about 1,920 cm³. Estimation of the initial wet anaerobic biomass/sludge within the polyfoam was about 76.8 g (5.76 g of VS).

The IBBRs were equipped with a cylindrical double jacket to keep a constant temperature of 37 °C by circulating heated water using a submerged pump. The reactors have two inlets, a liquid inlet of mineral media at the upper part of the reactors and a gas inlet through aquarium stone diffuser at the bottom of the reactors. The liquid outlet was located at the bottom of the reactors and the effluent was collected in a closed bottle (0.1 L for the laboratory-scale IBBR, and 1 L for the bench-scale IBBR). The mineral medium was continuously recirculated from the upper part of the reactors towards the bottom in a closed loop using a peristaltic pump. The gas was collected into multi-layer foil gas sampling bags (RESTEK®, USA) connected to the upper outlet of the reactor. A schematic diagram of the reactor configurations is given in Figure 1.

The laboratory-scale experiments examined the produced methane by enriching the hydrogenotrophic methanogens in the IBBRs. The enrichment process was conducted by preparing different mixtures of either 1:4 or 1:5 of CO₂ and H₂. The gas mixture was prepared on a daily basis and stored in multi-layer foil gas sampling bags (RESTEK®, USA), and thereafter was supplied continuously into the bottom of the IBBRs using a peristaltic pump at a feed flow rate of 1.3–1.7 ml/min.

The bench-scale experiments also included an initial enrichment of the hydrogenotrophic methanogens in the IBBR by supplying a mixture of 1:4 of CO₂:H₂, at a feed flow rate of 12–20 ml/min. Thereafter, for testing biogas upgrading, synthetic biogas (59% of methane and 41% of CO₂) was injected through the diffusers continuously using a peristaltic pump at a biogas feed flow rate of 9 ml/min, while H₂ was fed from a gas cylinder at 4 folds of the CO₂ feed flow rate, which is equivalent to a flow rate of 14–14.7 ml/min.

The liquid recirculation flow rate was 13–25.5 ml/min for the laboratory-scale IBBR and 120–500 ml/min for the bench-scale IBBR. A plastic screen was installed above the immobilized composite polyfoam in order to prevent clogging of the outlet pipelines.

Analytical methods

The produced biogas in the anaerobic system was collected in 3–40 L multi-layer foil gas sampling bags (RESTEK®, USA). Collected biogas volume was measured daily and the methane and CO₂ contents were analyzed with special methane sensors (Guardian Plus, model 97460, Edinburgh Sensors and Guardian NG, Edinburgh Sensors, respectively). H₂ was analyzed using an (F-12D ATI) ATI Hydrogen Sensor by using a Gas Transmitter. The alkalinity was analyzed using the potentiometric titration method (Method 2320B). The total alkalinity was calculated using the amount of acid needed to titrate the sample from the starting pH to pH 4 (APHA 2005).

Inoculum and substrate

Anaerobic granular biomass was collected from a well-operated up-flow anaerobic sludge blanket (UASB) bioreactor used to treat the wastewater of a citrus-based soft drink factory (PRIGAT) at Kibbutz Givat Haim, Israel. After immobilization, the system was fed with media of minerals containing three stock solutions on a weekly basis (concentrations of the chemicals given below are in g L⁻¹ in distilled water):

A. NH₄Cl, 100; MgCl₂·6H₂O, 10; NaCl, 10; CaCl₂·2H₂O, 5;
B. K₂HPO₄·3H₂O, 15.26
C. Trace-metal and saline solution: FeCl₂·4H₂O, 2; H₃BO₃, 0.05; ZnCl₂, 0.05; CuCl₂·2H₂O, 0.038; MnCl₂·4H₂O,
0.05; (NH₄)₂Mo₇O₂₄·4H₂O, 0.05; AlCl₃, 0.05; CoCl₂·6H₂O, 0.05; NiCl₂·6H₂O, 0.092; ethylenediamine tetra acetate, 0.5; concentrated HCl, 1 ml; Na₂SeO₃·5H₂O, 0.1.

10 mL of stock solution (A), 2 mL of stock solution (B), 1 mL of stock solution (C) were added to a volume of 900 mL of distilled water. 2.6 g NaHCO₃ and 0.25 g Na₂S·9H₂O were also added.

**Calculations**

Consumption percentage of H₂ was calculated as the difference between the hydrogen feed flow rate and the hydrogen flow rate out of the reactor (in the gas sampling bag), divided by the hydrogen feed flow rate, using the following equation:

\[
\% \text{ H}_2 \text{ consumption} = \frac{\text{H}_2 \text{ injected}[\text{ml/min}] - \text{H}_2 \text{ in biogas}[\text{ml/min}]}{\text{H}_2 \text{ injected}[\text{ml/min}]} \times 100
\]

The CH₄ production rate was calculated as the difference between the outlet and the inlet methane flow rates in the reactor as seen in Equation (3):

\[
\text{CH}_4 \text{ production rate}[\text{ml/h}] = \frac{\text{CH}_4 \text{ (out)}[\text{ml}] - \text{CH}_4 \text{ (in)}[\text{ml}]}{\text{time [h]}}
\]

Similarly, the H₂ consumption rate was calculated as the difference between the hydrogen inlet and outlet flow rates (in the gas sampling bag), using Equation (4):

\[
\text{H}_2 \text{ consumption rate}[\text{ml/h}] = \frac{\text{H}_2 \text{ out}[\text{ml}] - \text{H}_2 \text{ in}[\text{ml}]}{\text{time [h]}}
\]

Hydrogen consumption could be increased by increasing the overall volumetric mass transfer coefficient kLa. One way to increase kLa is by increasing the mixing inside the reactor (Luo et al. 2012). In this study, kLa of hydrogen at the different operational conditions within the bench IBBR was estimated.

kLa of hydrogen was calculated according to Equation (5) (Pauss et al. 1990):

\[
(k_L a)_{H_2} = \frac{\text{Q}_g}{V_L} \frac{K_H R T}{[\text{gas}]_L} \left(\frac{[\text{gas}]_L}{[\text{gas}]_L^o} - 1\right)
\]

where D is the gas diffusivity coefficient [cm²/s⁻¹] of each gas, taken from Pauss et al. (1990) at 353 K (Pauss et al. 1990). The diffusion coefficients were corrected to 310 K by assuming diffusion coefficients' dependence on temperature as T³/² according to Hudson et al. 2007. kLa of methane was calculated based on Equation (6):

\[
k_L a = \frac{Q_g}{V_L} \frac{K_H R T}{[\text{gas}]_L} \left(\frac{[\text{gas}]_L}{[\text{gas}]_L^o} - 1\right)
\]

where Qg is the gas flow rate [L/h]; V_L is the volume of the liquid phase [L]; K_H is Henry's law constant [mol L⁻¹ Pa⁻¹]; R is the ideal gas constant [8,314 Pa mol⁻¹ K⁻¹]; T is the temperature [K]; [gas]_L is the concentration of dissolved gaseous species in the reactor (normally equal to the concentration in the effluent) [mol/L]; [gas]_L^o is the concentration of dissolved gaseous species in the reactor at thermodynamic equilibrium [moles/L] (equal to P_g × K_H) (Pauss et al. 1990).

In order to estimate kLa, Q_g was assumed to include the circulated flow rate (volume). In addition, for V_L it was assumed that the void volume of the polyurethane foam equals 90% (Massalha et al. 2015a). Moreover, K_H values at 310 K were taken from compilation of Henry's law constants (Sander 2015). Finally, the concentration of dissolved methane was estimated to be 19 mg/L at 293 K based on laboratory measurements from anaerobic digestors fed with the same mineral medium. The measurements were according to Souza et al. 2011 and the correction for 310 K was done according to the maximal solubility of methane at 310 K (Engineering ToolBox 2008).

**RESULTS AND DISCUSSION**

**Conversion of H₂ and CO₂ to CH₄ by hydrogenotrophic methanogens in laboratory-scale IBBR**

The objective of this study was to develop an innovative process of up flow immobilized biomethanation bioreactor (IBBR) for ex-situ reduction of CO₂ by H₂ as an electron donor to CH₄. The immobilized anaerobic sludge in the polyfoam matrix contains anaerobic microorganisms including hydrogenotrophic methanogens that can be enriched (Bassani et al. 2011) by feeding a gas mixture of CO₂ and H₂ at a stoichiometric ratio of 1:4.

The first experiment examined the produced methane from the laboratory-scale IBBR by injecting CO₂ and H₂ at 1322
a ratio of 1:4 at a flow rate of 1.3 ml/min (see reactor set up section in materials and methods). A methane content of about 89% was achieved after 28 days of operation (see Figure 2). The recirculation ratio of this experiment was 10 folds of the gas feed flow. This ratio is within an optimal range identified through previous unpublished results.

The second experiment was conducted with a similar IBBR as the previous one, but at a ratio of 1:5 (CO₂:H₂) of the injected gas mixture in order to test the effect of H₂ excess to the system on the biogas upgrading process. Similar to the results shown in Figure 2, about 30 days were needed to enrich the hydrogenotrophic methanogens and achieve a steady state (results not shown). Figure 3 shows the average composition of the gas at the outlet of the laboratory-scale IBBRs at a steady state. This figure shows the effect of the different flow rates and recirculation ratios (the upper legend of the figure) on the upgrading performance. The goal of this experiment was to test the effect of increasing the flow rate and reducing the hydraulic retention time (HRT) on the system performance.

As can be seen in Figure 3, a methane content of 88% was obtained in the first operational run, where the feed flow contained CO₂ and H₂ at a ratio of 1:4, while the unconsumed CO₂ and H₂ were 7% and 4% respectively (see first set of columns in Figure 3). In the second operational run, a lower flow rate (1.3 ml/min) was applied and the feed flow contained CO₂ and H₂ at a ratio of 1:5. This corresponds to an HRT of 5.6 h compared to 4.9 h for the flow rate of 1.5 ml/min. In this run, the methane content was lower than that obtained in the first operational run. Nonetheless, during the third operational run, when a similar flow rate of 1.5 ml/min was applied and a recirculation ratio of 10, the methane obtained was around 80%. Additionally, in the last operational run, when the flow rate and the recirculation ratio were increased to 1.7 ml/min (HRT = 4.3 h) and 15 respectively, a methane content of 75% was observed.

These results show that the ratio of H₂: CO₂ of 4:1 (stoichiometric) is crucial for better upgrading performance in order to achieve a high methane content, as well as high hydrogen consumption. These results are in line with findings reported by Rachbauer et al. (2016). In their study, the H₂/CO₂ ratio of the inlet gas was gradually reduced from 6.7 to 3.7 and the optimum ratio for maximum methane content was found to be between 3.67 and 4.15 (Rachbauer et al. 2016).

Figure 2 | CH₄, CO₂ and H₂ content in the laboratory-scale IBBR over time. Operational conditions: flow rate of 1.3 ml/min of CO₂ and H₂ at a ratio of 1:4, recirculation ratio of 10.

Figure 3 | CH₄, CO₂ and H₂ content in the laboratory-scale IBBR at different operational conditions. The recirculation ratios and CO₂:H₂ ratios are shown in the upper legend of the figure. Diagonal striped columns correspond to a CO₂:H₂ ratio of 1:4, solid fill columns correspond to a CO₂:H₂ ratio of 1:5.
Figure 4 shows the average consumption of hydrogen in the laboratory-scale IBBRs under different operational conditions calculated according to Equation (2). This figure demonstrates that consumption of hydrogen was above 90% in all operational runs. The applied high recirculation ratio of 10–15 folds of the flow rate gave rise to this high consumption of hydrogen. This recirculation ratio corresponds to a velocity of 0.69–1.35 m/h in the laboratory-scale IBBR (3.4–14 m/h in the bench-scale IBBR), which is within the typical range of velocities in conventional high rate systems (0.5–1.2 m/h for UASB (Van Lier et al. 2010)). The applied velocity in the developed IBBR at bench scale was significantly higher than the typical velocity.

It is important to mention that this high consumption of hydrogen was achieved without using any sophisticated systems (i.e. high porous diffusers or special membranes) compared to the in-situ (Luo & Angelidaki 2015a; Bassani et al. 2016) and ex-situ biogas upgrading systems (Bassani et al. 2017).

Conversion of H₂ and CO₂ to CH₄ by hydrogenotrophic methanogens in the bench-scale IBBR

The next set of experiments was conducted in a bench-scale system. A mixture of CO₂ and H₂ was injected into the IBBR at a 1:4 ratio respectively (the optimal ratio). After a stable operation of 200 days, a mixture of synthetic biogas (59% of methane and 41% of CO₂) and hydrogen was supplied at the optimal ratio (CO₂:H₂ of 1:4) for testing biogas upgrading (the last two sets of columns in Figure 5).

Figure 5 shows the composition of gas in the outlet of the IBBR at different feed flows and recirculation ratios (the upper legend of the figure). It can be seen from this figure that during the different operating conditions, the methane content was between 40% and 60%. Moreover, the unconsumed CO₂ and H₂ in the bench system were higher than the laboratory-scale system. This low performance can be attributed to a different reactor configuration, likely due to limited distribution or optimal gas-liquid interface.

Another way of analysing the results is by looking at the percentage of normalized methane, calculated as the percentage of methane from the biogas (%CH₄ + %CO₂) by subtracting the hydrogen excess, as a function of hydraulic retention time HRT (see Figure 6). HRT refers to the volume of the reactor divided by the volumetric flow rate of gas. In Figure 6, the HRT for biogas injections refers only to the HRT of CO₂ and H₂ without methane. From this figure, it can be seen that the obtained normalized methane was between 70% and 85% during the different operating runs. Moreover, when synthetic biogas with hydrogen was injected into the IBBR, biogas upgrading was apparent, with an increase in methane content from 59% to 80% (the recirculation ratio was between 15 and 20).

A 6% increase in normalized methane content was observed when the recirculation ratio was increased (same...
HRT). This result was in full agreement with the more recently reported work of Yanuka-Golub et al. (2019), suggesting that recirculation between the anaerobic digester and microbial electrolysis cell chambers enhances hydrogen solubility, leading to a higher bioavailability for the hydrogenotrophic community (Yanuka-Golub et al. 2019).

Additionally, a previous study demonstrated that an increase in the mixing speed of a continuous stirred tank reactor (CSTR)'s stirring devices led to higher biomethanation, attributing this to the better distribution of H₂ as a liquid substrate, thus allowing its utilization by the hydrogenotrophs (Luo & Angelidaki 2012).

On the other hand, when reducing the HRT at the same recirculation ratio, a reduction in methane content was observed. These findings are in agreement with the data of Rachbauer et al. (2016), in which the increase of H₂ loading (i.e. decrease in retention time) within a trickle-bed reactor containing immobilized hydrogenotrophic enrichment culture caused a decline in methane concentration (Rachbauer et al. 2016). Lee et al. (2012) also reported that biological conversion of H₂:CO₂ mixture to methane was complete at a ratio of 4:1 using an up-flow anaerobic fixed bed reactor at gas retention times of above 3.8 h, but decreased to 71% when the retention time was reduced to 2 h (Lee et al. 2012). This is also true when comparing the results of the bench-scale IBBR to the laboratory-scale IBBR, where the HRT was higher, which seems to increase the distribution and solubility of hydrogen, resulting in a better performance of the laboratory-scale IBBRs.

Figure 7 presents the portion of H₂ consumption (calculated according to Equation (2)) during the different operational conditions. From this figure, it can be seen

![Figure 6](image6.png)

**Figure 6** | Normalized CH₄ in the outlet of the bench-scale IBBR at different CO₂ and H₂ hydraulic retention time (HRT). The recirculation ratios are shown in the upper legend of the figure. Solid fill columns correspond to a CO₂:H₂ ratio of 1:4. Diagonal striped columns correspond to biogas upgrading at a CO₂ (in biogas):H₂ ratio of 1:4.

![Figure 7](image7.png)

**Figure 7** | The average H₂ consumption in the bench-scale IBBR at different CO₂ and H₂ flow rates. The recirculation ratios are shown in the upper legend of the figure. Solid fill columns correspond to a CO₂:H₂ ratio of 1:4. Diagonal striped columns correspond to biogas upgrading at a CO₂ (in biogas):H₂ ratio of 1:4.
that the hydrogen consumption during hydrogenotrophic methanogens enrichment (before biogas upgrading) ranged from 80 to 95%. However, when a biogas and hydrogen mixture was injected into the IBBR, the consumption of H2 dropped to 64%. The difference between injecting a mixture of H2 and CO2 in comparison to injecting biogas is that the latter contains high amounts of methane (>50%), affecting the partial pressure of the other gases (less concentration). Reduced partial pressure influences the gas-liquid mass transfer and reduces the solubility of the gases (Seifert et al. 2013), leading to a lower bioavailability.

The gas-liquid mass transfer rate of H2 is known to be very low, severely limiting H2 availability for methanogens (Luo & Angelidaki 2012; Luo et al. 2012; Bassani et al. 2015, 2017). The H2 gas-liquid mass transfer rate is proportionally correlated to the mass transfer coefficient, kLa, which in turn represents the rate of transfer in either direction (gas to liquid or liquid to gas) for the whole reactor (Pauss et al. 1990). kLa is dependent on specific operational parameters (Angelidaki et al. 2018) such as reactor configuration (Bassani et al. 2016; Kougias et al. 2016), gas recirculation flow rate (Guiot et al. 2011; Kougias et al. 2016), the gas diffusion device (Luo & Angelidaki 2013a; Díaz et al. 2015; Bassani et al. 2017), and the stirring intensity (Luo & Angelidaki 2013a, 2012). Previous studies on biogas upgrading using H2 reported kLa ranging from 1 to 106 days 1, depending on the operating conditions (Luo et al. 2012; Luo & Angelidaki 2013b; Díaz et al. 2015; Bassani et al. 2017).

In this study, kLa of hydrogen was calculated in order to estimate the direct impact of the different operational conditions on the upgrading performances. The values of the kLa at the different operational conditions can be seen in Table 1.

As can be seen in Table 1, the variations in kLa are within the reported range (Luo et al. 2012; Luo & Angelidaki 2013b; Díaz et al. 2015; Bassani et al. 2017).

Moreover, the changes in kLa are in correlation with the hydrogen consumption seen in Figure 7, where higher kLa values resulted in higher hydrogen consumption. It can also be noted that increasing the recirculation ratio at the same feed flow rate increased kLa. Furthermore, when injecting biogas for upgrading, kLa dropped. These results demonstrate the positive effect of recirculation on enhancing H2 bioavailability and also indicate that the combination of operational conditions, in this case the feed flow and recirculation ratio, have a significant effect on the kLa and the H2 gas-liquid mass transfer rate.

As a result of H2 consumption, methane is produced. The increase in the methane production rate is considered a highly important indicator of the efficiency in biomethanation processes. Figure 8 presents the change in the methane production rate and hydrogen consumption rate (calculated according to Equations (3) and (4) respectively), as a result of increasing the H2 and CO2 flow rates and the recirculation ratio.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Bench-scale IBBR operational conditions and kLa under steady state</th>
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<tbody>
<tr>
<td>Qgas [ml/min]</td>
<td>Recirculation ratio</td>
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<tr>
<td>12</td>
<td>10</td>
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<tr>
<td>12</td>
<td>20</td>
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<td>20</td>
<td>20</td>
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<tr>
<td>18 (25.2 biogas and H2)</td>
<td>15</td>
</tr>
<tr>
<td>18 (25.2 biogas and H2)</td>
<td>20</td>
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**Figure 8** The CH4 production rate and H2 consumption rate in the bench-scale IBBR at different CO2 and H2 flow rates. The recirculation ratios are shown in the upper legend of the figure. Solid fill columns correspond to a CO2:H2 ratio of 1:4. Diagonal striped columns correspond to biogas upgrading at a CO2 (in biogas):H2 ratio of 1:4.
According to Figure 8, increasing the flow rate resulted in an increase in the methane production rate. This result is in correlation with the relatively stable consumption percentage of hydrogen when the flow rate increased (reducing the HRT), as is shown in Figure 7. However, when injecting biogas and H₂, the methane production rate dropped as a result of the smaller partial pressure of hydrogen within the mixture of gases, leading to a lower consumption rate (see Figure 7). Still, the produced methane rate when biogas was injected into the system was similar to the first operational run.

The range of the methane production rate during the different operational runs was between 120 and 220 ml/h, which corresponds to 1.1–2 L CH₄/L_reactor-day. These values are within the range of reported and known volumetric CH₄ productivities from experimental studies on the chemoautotrophic CO₂ conversion to CH₄ (Muñoz et al. 2015).

**CONCLUSION**

The current research demonstrated successful ex-situ biogas upgrading using novel immobilized biomethanation bioreactors (IBBR) at laboratory and bench-scale systems. The system is based on immobilized anaerobic biomass within a polymeric matrix that enables the application of high recirculation ratios to increase the solubility and the bioavailability of H₂ without washing out the microorganisms. The hydrogenotrophic methanogens were easily enriched in the IBBRs by feeding a mixture of CO₂ and H₂. The developed process leads to a production of hydrogen when the HRT, as shown in Figure 7. However, when injecting biogas and H₂, the methane production rate dropped as a result of the smaller partial pressure of hydrogen within the mixture of gases, leading to a lower consumption rate (see Figure 7). Still, the produced methane rate when biogas was injected into the system was similar to the first operational run.

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