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**Biogas upgrading and biochemical production from gas fermentation:
impact of microbial community and gas composition**

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Abstract

The present study proposes a novel alternative method of the current biogas upgrading techniques by converting CO₂ (in the biogas) into valuable chemicals (e.g., volatile fatty acids) using H₂ as energy source and acetogenic mixed culture as biocatalyst. The influence of thermal treatment (90°C) on the inhibition of the methanogenic archaea and enriching the acetogenic bacteria in different inocula (mesophilic and thermophilic) was initially tested. The most efficient inoculum that achieved the highest performance through the fermentation process was further used to define the optimum H₂/CO₂ gas ratio that secures maximum production yield of chemicals and maximum biogas upgrading efficiency. In addition, 16S rRNA analysis of the microbial community was conducted at the end of the experimental period to target functional microbes. The maximum biogas content (77% (v/v)) and acetate yield (72%) were achieved for 2H₂:1CO₂ ratio (v/v), with *Moorella* sp. 4 as the most dominant thermophilic acetogenic bacterium.

Keywords: Biogas upgrading; Gas fermentation; Microbial community; Acetogens; Acetyl-CoA pathway

1 Introduction

The growing concern about climate change, fossil fuels depletion and environmental impacts of current energy sources has together stimulated the search for sustainable, renewable and environment-friendly energy sources. Biogas is attracting significant attention as a clean and CO₂-neutral energy carrier (Awe et al., 2017). Biogas is generally composed of carbon dioxide (25-60%) and methane (40-75%) with several minor compounds depending on the organic source (Rotunno et al., 2017). The relatively high concentration of CO₂ in raw biogas significantly reduces its calorific value. Biogas (e.g., 60% CH₄ and 40% CO₂, v/v) has a low heat value (LHV) of 17.7 MJ/kg, while a 100% CH₄ gas has a LHV of 50.2 MJ/kg (Abdeen et al., 2016). Moreover, CO₂ in biogas can increase carbon monoxide and hydrocarbon emissions during combustion and the corrosion in pipelines during transportation (Toledo-Cervantes et al., 2016). Therefore, the common application of biogas is restricted for heating and electricity production (Leonzio, 2016).

Biogas upgrading is one of the most efficient technologies that receives great interest in bioenergy industry (Kokkoli et al., 2018). It aims to concentrate the methane in biogas by removal or transformation of CO₂ and other impurities (Kougias et al., 2017). The upgraded biogas with more than 95% CH₄ content is known as biomethane and can be injected directly into the existing natural gas grid or used as vehicle fuel (Micale, 2015). Several commercial technologies are currently used for biogas upgrading which are mainly based on physicochemical CO₂ removal (Bassani et al., 2016). The high cost and the methane loss during separation of CO₂ from biogas make these technologies economically and environmentally unfavourable (Angelidaki et al., 2018).

Alternatively, biogas can be upgraded through the biological conversion of CO₂ (in biogas) to valuable liquid products such as alcohols and volatile fatty acids using H₂ as an

energy source (Angelidaki et al., 2018). Acetate, butyrate and the other produced fatty acids are important precursor for high value biofuels (Liew et al., 2016). Various microorganisms are capable of converting CO_2 and H_2 to liquid products (Schiel-Bengelsdorf and Dürre, 2012). Most of these microorganisms are acetogens, which efficiently ferment C1 compounds (including CO_2) using H_2 as an electron donor and produce valuable biofuels and chemicals (Fernández-naveira et al., 2017). However, various gas fermentation studies have been performed using pure culture, which faces several limitations. Low adaptation capacity to various substrates (e.g. toxic components) and high operation cost to sustain sterilization conditions make it less suitable for applying to large industrial systems (Fernández-naveira et al., 2017). On the other hand, mixed culture can be more suitable for applying to large industrial systems (Redl et al., 2017).

In practice, Hydrogen required for the fermentation process could be generated from large point sources such as soda manufacture, petroleum refinery, petrochemical plants and coal gasification (Luo and Angelidaki, 2012). The concept of renewable electricity utilization for H_2 production has attracted great attention in recent years (Angelidaki et al., 2018). This sustainable technology which also known as power to gas (P2G) technology relies on water electrolysis using excess electricity generated by renewable energy sources (RES) such as wind mills and solar panels. Utilisation of H_2 as a renewable energy carrier faces various challenges that are related to its very low volumetric energy density that requires infrastructure with high storage volume and high transportation cost (Kougias et al., 2017). In contrary, H_2 generated from P2G technology can be used as energy source to separate CO_2 from biogas mixture and convert it to valuable biochemical (e.g., volatile fatty acids) using acetogenic bacteria. This new method is very promising as it integrates wind or solar energy technology with biochemical and biogas upgrading process.

Biogas upgrading and biosynthesis of volatile fatty acids through mixed culture fermentation of CO₂ and H₂ were reported in our previous study (Omar et al., 2018). In that study, CO₂ and H₂ were successfully converted into volatile fatty acids (mainly acetate) using chemically treated mesophilic mixed culture. While, the methane content of the biogas was significantly increased. As this research area is still at its infancy, so more development with respect to microorganisms and conditions are necessary.

Therefore, the present study proposes a novel biogas upgrading method by fermenting CO₂ (in the biogas) into chemicals using different mixed culture acetogenic consortia as biocatalyst and externally added H₂ as energy source under different temperature. To achieve the main aim of the current study, a preliminary batch experiment was performed to select the most suitable microbial source of acetogens. Subsequently the most efficient inoculum through fermentation process was used to define the optimum H₂/CO₂ gas ratio that secures maximum biogas upgrading efficiency and maximum production yield of chemicals. The characterization of the microbial community was also conducted during the upgrading process.

2 Materials and methods

2.1 Inocula and medium

Mesophilic ($37 \pm 1^\circ\text{C}$) and thermophilic ($55 \pm 1^\circ\text{C}$) digested effluent derived from Danish full-scale biogas plants (Hashøj and Snertinge, respectively) were used as inocula in this study. Both plants are fed with pig and cow manure (70 to 90% w/w) and organic waste (10 to 30% w/w). The inocula were sieved after collection, through a 2 mm net to remove large particles and then were kept in incubators at the corresponding temperatures. Table 1 illustrates the basic characteristics of both inocula.

Basic anaerobic medium (BA-medium) was used for both inocula (Angelidaki et al., 1990) with the exception that all carbon sources (e.g., NaHCO_3 and glucose) were omitted to minimize the interference of carbon species during the fermentation.

2.2 Experimental setup

All the fermentative experiments were carried out in triplicate using 540 mL serum bottles with 100 mL working volume and 10% (v/v) inocula. The bottles with the BA-media were sealed with butyl rubber stopper and aluminium cap and then sterilized at 121°C and 15 psi for 20 min. After sterilization, vitamin solution was added using 0.2 μm sterilized filter. Before inoculation the serum bottles were reduced with sodium sulphide solution to a final concentration of 0.25 g/L. 2 N KOH and 2 N HCl solutions had been used to adjust the initial pH of the medium at 6.0 ± 0.1 . All the serum bottles were incubated horizontally in an IKA[®] KS 4000i control orbital shaker at $37 \pm 1^\circ\text{C}$ for mesophilic and $55 \pm 1^\circ\text{C}$ for thermophilic conditions and with 150 rpm constant speed.

2.2.1 Screening experiment: effect of heat treatment methods

Inhibition of methanogenic archaea in inocula was a crucial step in the present study. Therefore, batch experiments were performed to study the effect of thermal treatment of the different inocula on the microbial community and biochemical production. For the thermal treatment, the inocula were heat shocked at 90°C for 30 min, followed by cooling to room temperature (Nam et al., 2016). After treatment, the inocula were kept in the incubators at their corresponding temperature. The treated inocula were then fed with a synthetic gas composition ($\text{H}_2:\text{CO}_2:\text{CH}_4$, 2:1:1.5) to a final pressure of 1.5 bar.

2.2.2 Optimization experiment: effect of different gas ratios

Based on the results from the screening experiments, a selected inoculum was taken up for further study to investigate the influence of different gas ratios on biogas upgrading and

biochemical production process. The selected inoculum was fed with a mixture of biogas (40% CO₂: 60% CH₄) and pure (100%) H₂. The difference between the four gas ratios (GR_{1-4x}) was based on different ratios between H₂ and CO₂. The four feed gas composition was GR_(1x): (H₂/ CO₂/1.5 CH₄), GR_(2x): (2 H₂/ CO₂/1.5 CH₄), GR_(3x): (3 H₂/ CO₂/1.5 CH₄) and GR_(4x): (4 H₂/ CO₂/1.5 CH₄), respectively. For the controls, the mixed cultures were kept under 100% N₂ in the headspace without presence of other gases.

2.3 Analytical methods

A digital PHM210 pH meter connected to the Gel pH electrode (pHC3105–8; Radiometer analytical) was used to measure pH of samples. Standard Methods were applied for total ammonia (NH₄⁺-N), total Kjeldahl nitrogen (TKN), total solid (TS) and volatile solid (VS) determination (APHA, 2005). The gas chromatograph (Shimadzu GC-2010, Kyoto, Japan) was used to determine the volatile fatty acid samples that prepared as previously described (Symsaris et al., 2015). The CH₄ and CO₂ content in headspace gas samples were measured with a gas-chromatograph (Thermoscientific GC-8A, Japan), while the H₂ gas was determined with a gas chromatograph (Shimadzu GC-11A, Tokyo-Japan). The characteristics of both GC were previously described (Omar et al., 2018). Triplicate samples were analysis for the all processes.

2.4 Microbial analysis

RNA PowerSoil® DNA Elution Accessory Kits (MO BIO Laboratories, Carlsbad, CA) were used for the extraction and purification of genomic DNA from thermophilic inocula (untreated and thermal treated) and liquid samples from the optimisation process. Animal fibers residues were initially eliminated by filtering the samples through a 100 µm nylon cell strainer filter. Afterward, samples were purified using (Phe: Chl: IAA) solutions. Evaluation

of the quantity and the quality of extracted DNA was performed using Qbit fluorimeter (Life Technologies, Carlsbad, CA) and NanoDrop (ThermoFisher Scientific, Waltham, MA).

Through the current study, BA01 and BA02 were referred to untreated and treated inoculum at 90°C, respectively. While, BA03, BA04, BA05, BA06 and BA07 were referred to treated inoculum (at 90°C) exposed to different gas ratio $GR_{(1x)}$, $GR_{(2x)}$, $GR_{(3x)}$, $GR_{(4x)}$ and control samples (without fermentation gas), respectively. Samples were then sequenced in the Ramaciotti Centre for Genomics (UNSW, Sydney) where MiSeq platform (Illumina) and universal primers 515F/806R were used. CLC Workbench software (V.8.0.2) with microbial genomics module plug in (QIAGEN) was used for Bioinformatics' analyses conduction on raw data (Kougias et al., 2017). Raw reads were deposited in Sequence Read Archive (SRA) database (<http://www.ncbi.nlm.nih.gov/sra>) under the project number PRJNA388850 (E-supplementary data for this work can be found in e-version of this paper online).

2.5 Statistical analyses and calculation

The obtained data were analysed statistically by ANOVA with F test. Comparison of treatment means was performed using MSTAT-C statistical computer package (Michigan State University, East Lansing, MI, USA) with Duncan's multiple range Test. The figure was performed by the OriginLab program (OriginLab Corporation, Northampton, Massachusetts). The calculations of mass balance through the current study were depended on the Chemical Oxygen Demand (COD) balance.

3 Results and Discussion

3.1 Effect of different treatment methods on upgrading and chemical production processes

The effect of heating treatment of the different inocula on the fermentation process was primarily evaluated in terms of hydrogen consumption, pH and VFA production. Under the thermophilic condition, there was a lag phase of five days, after which H₂ utilisation increased rapidly till reached to the maximum consumption of 86% after 12 days of incubation (Fig. 1a). In contrast, the mesophilic inoculum has shown a poor adaptation to the gas mixture. The different behaviour of the both inoculum could be attributed to the different diversity in microbial population and cell density after heating pre-treatment (Wang and Wan, 2008). Wang and Yin (2017) investigated the influence of temperature on the microbial survival and reported various significant effects of the high temperature on the bacteria such as deterioration of microbial protein, disruption of the chemical bonds of the cell wall and membrane and solubilization of the cell components. Another reason for the low adaptation of the mesophilic inoculum to fermentation condition could be the absence of spore-forming bacteria that can resist the high temperature.

VFA profile is another indicator of the ability of thermophilic inoculum to achieve the main goal of the present study. Acetate was the main metabolite produced by the thermophilic inoculum with maximum net production of 504.2 mg/L (Fig. 1b), while the mesophilic inoculum produced significantly lower concentration (44 mg/L) ($p < 0.05$). The maximum acetate productivity under the thermophilic condition was 86 fold of that produced in the control, which confirmed that acetate was mainly produced from CO₂ and H₂ fermentation. On the contrary, non-significant difference between control and the tests under the mesophilic condition was observed ($p < 0.05$). The maximum acetate concentration

achieved through the current study was lower than that previously reported (Wang et al., 2017). The enhancement of the liquid-gas mass transfer which leads to a high utilization of H_2 was the main reason for the relatively higher acetate production in previous study. In addition, using a highly effective inhibitor to methanogens (e.g., BES) in that study was another important reason contributing to the enhancement of acetate production. While, the heating treatment of the thermophilic inoculum through the current study did not completely block the activity of methanogens which significantly affected the productivity of acetate due to the ability of methanogens to use H_2 - CO_2 directly or generated acetate indirectly to produce methane (Kimmel et al., 1991).

The significant difference in the behaviour of the both inocula reflected also on the pH fluctuation throughout the test (Fig. 1c). Under the thermophilic condition, pH increased after four days from 6 to 7 and then started to decrease due to the accumulation of acetate until reached 5 at the end of experiment, while non-significant difference ($p > 0.05$) of pH was observed in the mesophilic condition and also in the control reactor. Thus, the thermophilic mixed culture after heating pre-treatment was selected for the following experiments.

3.2 Effect of H_2/CO_2 ratios on the thermophilic fermentation process

3.2.1 Biochemical production and pH profile

Through the thermophilic fermentation, acetate was the most dominant product at all tested gas ratios. The acetate production started after four days of inoculation by the thermophilic inoculum and then rapidly increased till the end of fermentation, which was consistent with the rapid consumption of hydrogen gas illustrated in Fig. 2. There was a significant difference between the samples and the control in the production of acetate, as the maximum acetate produced at $GR_{(2x)}$ was 78 fold of that produced in the control bottles. The highest acetate yield of 72% was achieved at $GR_{(2x)}$ (Table 2), which was also consistent with the consumption of H_2 shown in Fig. 2. The COD balance is illustrated in Table 2. The

presence of methanogens in the thermophilic inoculum was the main reason for the lower acetate production in the present study comparing to other fermentation process in previous studies (Savage and Drake, 1986; Sakai et al., 2005; Kim et al., 2016 ; Wang et al., 2017). (E-supplementary data for this work can be found in e-version of this paper online). The uninhibited methanogens after heating pre-treatment could compete with acetogens for H_2 and CO_2 and produce CH_4 (Wang et al., 2017). The rapid increase in the consumption of the gaseous substrate and accumulation of acetate had a significant influence on the pH values. pH increased from 6 to 7 with the all gas ratios till the fourth day, after which the values started to decrease due to the accumulation of acetate (Table 2). The lowest pH was observed at $GR_{(2x)}$ which could be due to the highest acetate concentration over other gas ratios. $GR_{(1x)}$ had a higher final pH, which could be due to the less available electron donor (i.e., H_2) for acetate production. In the control, pH increased through the first four days from 6.1 to 7.65 and remained stable till the end of the experiment.

3.2.2 Biogas upgrading

The maximum biomethane concentration of 77% (v/v) was achieved at $GR_{(2x)}$ after 12 days of incubation (Fig. 3). $GR_{(3x)}$ and $GR_{(4x)}$ achieved maximum biomethane concentration of 56% and 39% which was significantly ($p < 0.05$) 26.7% and 49.5% lower than $GR_{(2x)}$ ($p < 0.05$). This was associated with the high consumption of both CO_2 and H_2 at $GR_{(2x)}$ with 8.95% CO_2 and 13.98% H_2 left (Fig. 3). Moreover, this gas ratio achieved the maximum yield of acetate (Table 2). However, the difference in maximum concentration of biomethane between $GR_{(1x)}$ and $GR_{(2x)}$ was non-significant ($p > 0.05$), but the relative concentration of lefted CO_2 at $GR_{(2x)}$ was 61% lower than lefted at $GR_{(1x)}$. The biogas upgrading efficiency in the current study was lower than that achieved by the other biological biogas upgrading techniques. Methane concentration above 96% with ($<4\%$ and $<0.1\%$ residual CO_2 and H_2 , respectively) was achieved through biogas upgrading process using hydrogenotrophic

community in a trickle-bed reactor (Rachbauer et al., 2016). In another microbial upgrading technique performed by microalgae *Chlorella* sp., CH₄ concentration in upgraded biogas reach to 93.7% with 1.57% CO₂ left (Yan and Zheng, 2014). Compared to the gas quality standards regarding to biogas utilization, the upgraded biogas from the current study cannot be used a vehicle fuel or injected into natural gas grids, due to its lower methane content and higher content of H₂ and CO₂ (Sun et al., 2015b). The incomplete inhibition of methanogenic archaea by thermal treatment was the main reason of lower biogas upgrading and acetate production compared to the other studies. However, the upgraded biogas from the current study can be used in a new class of power-generation technologies (fuel cells) which converting chemical energy of fuels directly and with relatively high efficiency in to electricity at reduced harmful emissions (Budzianowski, 2016).

3.3 Microbial community composition through the optimization processes

Alpha diversity based on the number of operational taxonomic units (OTUs) showed that the sequencing depth was adequate to cover the microbial species richness (E-supplementary data for this work can be found in e-version of this paper online). The thermal treatment of the inoculum and different gas ratios significantly affected the complexity of the microbial community. The lowest microbial complexity was observed in the control (BA07), while the highest was observed with preheated sample at 90°C (BA02). All the microbial communities exposed to thermal treatment and different gas ratios and the abundance of the identified OTUs were represented by phylogenetic tree (Fig. 4) and a heat map (Fig. 5). The significant effect of the thermal treatment and different gas ratios were also clarified by principal coordinate analyses (PCoA) (E-supplementary data for this work can be found in e-version of this paper online).

3.3.1 Effect of thermal treatment on microbial community

The thermal treatment of the inoculum has significantly affected the microbial community. The most dominant bacterium in the untreated inoculum (from the Biogas Plant), BA01, was *Bacteroidales* sp. 16 (27.12%) following by *MBA08* sp. 1 (6.74%) and *Syntrophaceticus schinkii* 51 (6.72%). *MBA08* cluster was previously reported in thermophilic garbage digester (Cheon et al., 2007), thermophilic manure based biogas reactors processing straw (Sun et al., 2013) and thermophilic ex-situ biogas upgrading reactor (Kougias et al., 2017). After thermal treatment, the relative abundance of *MBA08* sp. 1 was 4.75 fold compared to the untreated inoculum, while the abundance of *Bacteroidales* sp.16 in untreated inoculum was 1.71 fold compared to the treated one, which reflected the significant effect of treatment on the different microbes. Similar trends had been also reported in previous studies (Sun et al., 2013; Sun et al., 2015a; Ziganshin et al., 2013). Increasing temperature degrees through these studies led to an increase in the relative abundance of Clostridia affiliates and a decrease in the relative abundance of Bacteroidetes. In addition, the relative abundance of *Anaerobacillus macyae* 40 (Phylum Firmicutes) was 8.16 fold compared to that in the untreated inoculum. *A. macyae* has the ability to form resistant endospores, allowing this bacterium to survive for a prolonged period of time in adverse circumstances (Wang et al., 2015).

3.3.2 Effect of different gas ratios on the microbial composition

Through the second batch experiment, the gas composition had a significant influence on the microbial community compared to control samples (BA07). Based on 16S rRNA sequence analysis, *Clostridium caenicola* 75 was the most dominant bacterium in the control and was 100% sequence similarity to *Clostridium caenicola*. This obligatory anaerobic, thermophilic and chemo-organotrophic bacterium was isolated from anaerobic sludge of a cellulose-degrading methanogenic bioreactor (Shiratori et al., 2009). *Clostridia* sp.10 and

MBA08 sp. 1 were the second most abundant bacteria with a relative abundance of 13.02% and 13.65%, respectively. By performing a BLASTn search against the NCBI database (16S rRNA sequence database), these bacteria were 91% and 90%, respectively similar to “*Hydrogenispora ethanolica*” which was newly identified as a carbohydrate-fermenting bacterium (Liu et al., 2014). Moreover, exposing the samples to different gas ratio led to a significant difference in the composition of the microbial community. *Moorella* sp. 4 was the most dominant bacterium in the samples with gas ratio GR_(2x) (AB04) and GR_(3x) (AB05) with a relative abundance of 23.38% and 28.92%, respectively. This dominant bacterium was 99% sequence similarity to both *Moorella thermoacetica* and *Moorella thermoautotrophica*. These thermophilic model acetogenic bacteria have been studied extensively for their ability to reductively synthesize acetate from CO₂ via the acetyl-CoA pathway (Hu et al., 2013; Redl and Nielsen, 2016; Seifritz et al., 1999). While the relative abundance of this acetogenic bacterium was lower in samples with other gas ratio GR_(1x) (AB03) and GR_(4x) (AB06) and was completely absent in the control samples (AB07). The relative abundance of *MBA08* sp.1 (Phylum Firmicutes) was high in all gas ratio and control, except the sample with gas ratio GR_(3x) (AB05) which achieved the lowest relative abundance (2.25%) of this bacterium. BLASTn search against the NCBI database (16S rRNA sequence database) revealed 90% similarity to the newly identified carbohydrate-fermenting bacterium “*Hydrogenispora ethanolica*” (Liu et al., 2014). As the sequence identity score was less than the genera classification threshold (>94.5%), the taxonomy of this OTU remains uncertain, suggesting this microorganism as a new species (Yarza et al., 2014). However the high abundance of this microbe revealed important function through the fermentation process.

Thermoanaerobacterium sp.11 was another interesting bacterium that observed in all gas ratios with a lowest relative abundance (0.09%) in (AB03) and was completely absent in the control samples (AB07). This interesting bacterium was 100% similar to

Thermoanaerobacterium xylanolyticum/ *Thermoanaerobacterium thermosaccharolyticum*/ *Thermoanaerobacterium caldifontis* compared to the NCBI database. *Thermoanaerobacterium* (Phyla Firmicutes) are obligate anaerobic fermentative microorganisms and also reported as hydrogen-producing bacteria under thermophilic conditions (Ratti et al., 2015). Thermophilic anaerobic bacteria like *Thermoanaerobacterium* and *Moorella* spp. are usually habitated in numerous hot environments such as: geothermal hot springs and hydrothermal vents or sometimes warm environments like compost and manure (André et al., 2013). *Thermoanaerobacterium* and *Moorella* spp. were observed in assays for H₂ production by thermophilic anaerobic microflora (Ueno et al., 2006). The presence of fermentation gases also stimulated the activity of methanogenic archaea “*Methanosarcina thermophila* 1” which was observed in all gas ratios with maximum relative abundance of 1.74% for BA05, while BA04 has shown the lowest relative abundance of *M. thermoacetica* (0.8%) which explains the highest acetate yield achieved by this gas ratio (Table 2). *M. thermophila* 1 can grow on acetate, methanol, or methylated amines and can also grow slowly on H₂:CO₂, which explain the methane production through the fermentation process and the lower acetate productivity compared to other studies (Zinder et al., 1985).

3.4. Future directions for improving the biogas upgrading and biochemical production process

The present study provided a new method for biogas upgrading and VFA production through the mixed culture fermentation of CO₂ and H₂. However, further improvement should be applied to enhance the efficiency of both processes. These improvements including:

- Treatment method: Using other pre-treatment methods (e.g., chemical treatment) that are more efficient for methanogenesis inhibition and enriching acetogenic bacteria.
- Biocatalyst type: Providing the process with effluents from other anaerobic reactor using acetogenic bacteria for the production of VFAs and alcohols. By using these enriched bacteria, pre-treatment step and its related problems (e.g., cost) can be omitted.
- Gas liquid mass transfer: Increasing the availability of H_2 to the acetogenic bacteria is another significant factor that can enhance the efficiency of the whole process. Replacing batch system with reactor provided with gas dispersion systems (e.g. hollow fibre membranes) can guarantee high mass transfer rates of H_2 gas and thus increases the productivity of the process.

Applying these suggested improvements on the present novel method can open new avenues in the bioenergy field.

4. Conclusions

Through the current study, a novel bioprocess for chemicals production and biogas upgrading was optimised. This new bioprocess was based on the fermentation of CO_2 and H_2 to valuable chemicals (e.g., acetate) by acetogenic mixed culture. The effects of thermal treatment of two different inocula on the microbial community and biochemical production was initially investigated under thermophilic and mesophilic condition. Thermophilic inoculum has shown higher ability to inhibit methanogenesis and produced higher acetate compared to mesophilic inoculum. Thermophilic fermentation by the gas ratio $2H_2:1CO_2$ achieved the maximum biogas content and acetate yield compared to the other gas ratios.

"E-supplementary data for this work can be found in e-version of this paper online".

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Figure Captions

Fig.1 a) H_2 consumption, b) acetate production and c) pH fluctuation in the mesophilic and thermophilic inocula experiments.

Fig. 2 Hydrogen consumption profile at the different gas ratios.

Fig. 3 The relative concentration of the three gases through the fermentation process under different gas ratios: A) $GR_{(1x)}$ ($H_2:CO_2$), B) $GR_{(2x)}$ ($2H_2:CO_2$), C) $GR_{(3x)}$ ($3H_2:CO_2$), and D) $GR_{(4x)}$ ($4H_2:CO_2$), respectively.

Fig. 4 Phylogenetic tree of the complete microbial community of the thermophilic inoculum before and after thermal treatment and after exposing to different gas ratio.

Fig. 5 Heat map representing the OTUs with the higher abundance in the samples. The gradient scale above the heat map illustrates the correspondence between the colours and relative abundance.

Tables

Table 1 Characteristics of the mesophilic and thermophilic inocula used in this study.

Table 2 COD balance and fermentation performance of the pre-treated thermophilic inoculum at various gas ratios.

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Fig.1

Fig. 2

Fig. 3

Fig. 4

ACC

Table 1

Parameter	Mesophilic- inoculum value $\pm$ SD^a	Thermophilic- inoculum value $\pm$ SD^a
pH	8.13	8.6
TS (g/kg)	3.97 $\pm$ 0.98	23.8 $\pm$ 3.0
VS (g/kg)	27.82 $\pm$ 0.01	16.7 $\pm$ 0.10
Total VFA (g/L)	0.75 $\pm$ 0.028	0.43 $\pm$ 5.5
Ammonia (g NH₄⁺-N/kg)	3.56 $\pm$ 0.09	1.65 $\pm$ 0.02
Total Kjeldahl nitrogen (g N/kg)	5.15 $\pm$ 0.50	4 $\pm$ 0.06
^a Standard deviation		

Concentration								
	mg-COD _i /L *				mg-COD _f /L *			
	GR	G	GR	GR	G	G	G	G
	(1x)	R _(2x)	(3x)	(4x)	R _(1x)	R _(2x)	R _(3x)	R _(4x)
Ethanol	0.7	0.	0.8	0.6	1.	5.	3.	1.
	6	72	0	5	69	32	24	66
Propanol	12.	12	12.	10.	2.	3.	1.	2.
	74	.76	58	20	18	59	70	21
Butanol	1.1	2.	1.3	1.3	-	0.	-	-
	5	18	0	9		81		
1-Hexanol	0.8	0.	0.5	0.7	0.	0.	0.	-
	3	98	7	9	00	19	15	
Acetate	17.	16	17.	18.	19	56	39	42
	74	.31	72	27	1.50	1.34	1.37	3.27
Butyrate	2.6	2.	2.5	2.5	3.	3.	2.	3.
	4	66	4	5	51	43	00	40
Valerate	-	-	-	-	0.	0.	-	-
					31	21		
Propionate	6.2	6.	6.1	6.3	5.	9.	4.	4.
	9	35	9	1	62	90	20	98
Iso-Butyrate	3.1	3.	3.2	3.1	1.	3.	1.	1.
	4	00	2	2	65	81	63	85
1-Hexanoate	0.6	0.	0.6	0.6	0.	0.	0.	0.
	6	55	7	3	23	26	33	00
Iso-Valerate	6.8	7.	6.8	7.0	5.	8.	4.	6.
	7	04	5	0	68	00	32	53
Iso-Amylalcohol	0.1	-	0.3	-	-	-	-	-
	4		7					

H ₂	370	76	128	167	24	10	42	82
	.10	0.21	4.87	5.01	.08	8.84	3.82	4.65
CH ₄	518	48	483	499	56	54	52	48
	.80	0.38	.95	.87	4.53	5.16	7.95	9.27
Overall COD balance (%)					85	96	74	78
					.04	.73	.70	.97
Maximum acetate conc. (mg/l)					17	52	36	39
					8	1	3	3
Final Acetate yield (%)					47	72	29	24
Final pH					5.	4.	5.	5.
					92	92	10	17

Table 2

*COD_f : final COD, COD_i : initial COD

Highlights

- Anaerobic mixed culture for efficient biogas upgrading and bioproduction.
- Biological conversion of CO₂ and H₂ into acetate through acidogenesis.
- Biogas upgrading up to 77% CH₄ was achieved under thermophilic conditions.
- Acetate yield of 72% was observed for 2H₂:1CO₂ gas ratio.
- Thermophilic acetogen, *Moorella* sp. 4, was responsible of acetate production.