Enrichment of the hydrogenotrophic methanogens for, *in-situ* biogas up-gradation by recirculation of gases and supply of hydrogen in methanogenic reactor.

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Abstract

During *in situ* biogas up-gradation by supplying hydrogen from an external source and enrichment of hydrogenotrophic methanogens, high pressure of H_2 negatively affects hydrolytic and fermentative activities. To overcome this problem, the present study aimed to enrich the hydrogenotrophic methanogens by optimization of various parameters associated with gas recirculation along-with hydrogen supply from the external source. Due to recirculation of gases and supplied hydrogen, methane generation was two-fold higher in the optimal condition than in conventional anaerobic digestion, with the highest methane content of 99%. Additionally, the hydrogenotrophic methanogens were enriched, with a decrease in acetoclastic methanogens and an increase in *Bathyarchaeia* population, which utilizes H_2 and CO_2 to produce acetate and lactate as end products. The study concludes that recirculation increases methane production by converting H_2 and CO_2 into methane and enhances the degradation of organic matter left over undigested in the hydrolytic reactor.

Keywords

Biogas up-gradation; anaerobic digestion; *in situ* upgrading; Methane content; Hydrogenotrophic methanogens.

Introduction

The usage of petroleum derivatives is rising continuously with the expansion of the human population, and is linked with GHG emissions (Perea-Moreno et al., 2019), such as carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) (Shukla et al., 2019), thus warming up the planet. To minimize GHG emissions and fulfill the energy needs of modern society, fossil fuels must be replaced by renewable energy, which is both a viable solution to global warming and a cost-effective alternative source of energy (Sinitsyn & Sinitsyna, 2021). Over the last decade, global patterns of renewable energy consumption have shifted substantially. United States, Brazil, and Germany are the leading countries that generate biofuel (Dudley, 2018). Biogas, which is produced from a variety of wastes using an environmentally benign and low-cost technique known as anaerobic digestion, has the potential to replace natural gas (Antar et al., 2021). Various organic wastes have been used as feedstocks for biogas generation, including agricultural wastes, food waste, and organic fractions of domestic waste (Xu et al., 2017). This waste to energy conversion has gotten greater attention in recent decades as the greatest alternative to fossil fuels (Zhu et al., 2019). Biogas is mostly composed of 50-70% CH_4 and 30-50% CO_2, depending upon the feedstock type and process parameters, and also have a trace quantity of other gases (Kadam & Panwar, 2017). Biogas is often used for cooking and heating and power production, and if upgraded can be used as vehicle fuel (Rosa et al., 2016), which requires the removal of CO_2 and other residual gases (Sun et al., 2015). Up-gradation of biogas is mostly carried out through excision of CO_2 by physical or chemical techniques, such as water scrubbers, chemical scrubbers, pressure swing adsorption and membrane separation on a wide scale (Allegue et al., 2012; Lemmer et al., 2015). The drawbacks of these techniques are losing 1-8% CH_4 in the process, using costly chemicals and membranes, and consuming a considerable amount of water for scrubbing (Sarker et al., 2018).

Biological up-gradation has overcome these limitations; biological up-gradation can accomplish in-situ (within a methane digester) or ex-situ (in an externally connected reactor). The most notable advantage of bio-up-gradation is that CO_2 gets reused into CH4 instead of being removed, which reduces CO_2 burden in the environment and increases the amount of CH4 produced during anaerobic digestion process. During ex-situ biogas up-grading concept, external H_2 and the biogas produced in AD are fed to a separate anaerobic digester comprising hydrogenotrophic cultures and transformed to CH_4 (Kapoor et al., 2019). Using an additional reactor for biogas up-grading is a constraint of ex-situ up-grading approach, which raises the process's capital and operational costs (Voelklein et al., 2019). During in-situ biogas up-gradation, the organic substrate and additional H_2 are introduced to the same digester where biogas is generated where CO_2 and H_2 are transformed into CH_4 by hydrogenotrophic methanogens (Kapoor et al., 2019). While the hybrid mode of biogas up-gradation combines in-situ and ex-situ systems, a portion of the CO_2 is initially converted into CH_4 inside the same reactor by H_2 supply, and then it is further up-graded *ex-situ* (Angelidaki et al., 2018). The most advantageous of all these techniques is *in-situ* biogas up-gradation because it allows using the existing hydrogenotrophic methanogens while minimizing the need for additional infrastructure for post-gas processing and is a low cost gas-to-power technology (Aryal et al., 2018). Even the partial conversion of CO_2 to CH_4 can be cost effective due to low capital and operational cost of the process (Voelklein et al., 2019). Furthermore, because hydrogenotrophic methanogenesis is an exothermic process, which can lower the reactor's heating cost by 27–56% (Jensen et al., 2021). Aside from these potential advantages, there are some drawbacks of adding H_2 from an external source to a reactor, such as increase in pH due to lower CO_2 concentration (Luo & Angelidaki, 2013), VFAs accumulation due to high partial pressure of H_2 (Aryal et al., 2018), low efficiency due to low solubility of H_2 in water, and a change

87 in microbial population (Zhu et al., 2020). To address these issues, multiple studies have used
88 varied flow rates of H_2, ranging from 1.5-7.2 ml/min which increased the CH_4 content from 62 to
89 70%, with limited efficiency and a drop in CH_4 content with a higher flow rate, as well as an
90 increase in VFAs concentration (Zhu et al., 2020). The main constraints during *in-situ* up-gradation
91 are low process efficiency and significant VFAs accumulation.

92 Recirculation of biogas is a potential approach to deal with the aforementioned concerns, which
93 extends the contact duration between gases and hydrogenotrophic methanogens during *in-situ* up-
94 grading approach for enhanced bio-methanation. Because biogas contains a considerable amount
95 of CO_2; recirculating it into the AD system will cause CO_2 and H_2 to dissolve in the liquid phase,
96 increasing CH_4 concentration of the biogas. Besides, recirculation also boosts mixing of the reactor
97 increasing homogeneity of the microbes, nutrients, substrate, and alkalinity, as well as the release
98 of trapped bubbles in the reactor, thus playing a significant role in biogas production (Wang et al.,
99 2017). Many different mixing techniques have been previously documented, including the most
100 widely utilized mechanical, hydraulic, and pneumatic mixing. However, the kind, intensity,
101 frequency, and speed of the mixing have an impact on biogas generation (Lindmark et al., 2014).
102 Gas recycling was previously used for the enrichment of hydrogenotrophic methanogens and
103 biogas up-grading in a separate reactor during *in-situ* biogas up-gradation (Yun et al., 2017).
104 Furthermore, distinct types of bacteria and archaea carry out anaerobic digestion, which might
105 alter with time and conditions (Yenigün & Demirel, 2013). The microbial groups' activities have
106 a directly affect the process and can result in digester failure (Fernández et al., 1999). Different
107 parameters, such as H_2 concentration, temperature, pH, and VFAs, affect the microflora in
108 anaerobic reactors, which must be monitored (Cho et al., 2017). With gas recirculation and H_2
109 supply, a rise in hydrogenotrophic methanogens as well as increase in CH_4 concentration was

observed (Yun et al., 2017). Wahid & Horn, 2021 found that gas recirculation with H_2 supply increased CH_4 content in continuous stirred tank reactors (CSTRs), but to our knowledge, no study has been done to employ gas recirculation for *in-situ* biogas up-gradation and enhancement of biogas production due to the mixing impact of recirculation.

According to the literature, CH_4 content of biogas can be raised by supplying H_2 from an external source, but *in-situ* up-gradation can have a detrimental impact on biogas production. To address this issue, the current research looked into the impact of *in-situ* H_2 supply and gases recirculation into methanogenic reactor on biogas output and its composition. The process failure/reduced CH_4 production caused by high partial pressure of H_2 can be overcome by supplying H_2 in a continuous mode and hence the study aims to find the optimum flow rate for H_2 supply, and optimum flow rate and duration for gases recirculation.

2. Material and Methods

2.1. Substrates collection and characterization

Cattle manure collected from a local dairy farm and greengrocery waste collected from the local fruit and vegetable market in Islamabad, Pakistan were used as the substrates for biogas production. The total solids and volatile solids of cattle manure and greengrocery waste were determined using the National Renewable Energy Laboratory's standard procedures for total and volatile solids determination (Sluiter et al., 2008). The cattle manure was co-digested with greengrocery waste mixed in 1:1 based on VS to optimize the C/N ratio for high biogas yield.

2.2. Reactor design and operation

The effect of biogas recirculation and H_2 supply on the production and up-gradation of biogas was studied in a two-stage reactor. Two glass reactors, i.e. hydrolytic reactor (R1) and methanogenic reactor (R2), were interconnected, with a total volume of 2.5 liters while the working volume was kept 2 liters. Hydrogen supply and recirculation of gases into the methanogenic reactor was accomplished via a conduit connecting from the gas bag to the reactor's bottom through a gas sparger to improve gas contact with the reactor's slurry. Gas recirculation was accomplished using a peristaltic pump. The reactors were operated at 37 ±1˚C in an incubator. Based on the highest quantity of CO_2 produced daily, the amount of H_2 supplied daily to the methanogenic reactor was determined stoichiometrically [H_2: CO_2 (80:20)] (Yun et al., 2017).

The reactor was fed with organic loading rate of 3.5 gVSL-1day-1 with hydraulic retention time of 10 days to determine the effect of biogas recirculation and supply of H_2 on CH_4 production, *in-situ* biogas up-gradation, and diversity of methanogenic microorganisms in methanogenic reactor during two-stage anaerobic digestion. Cattle manure and greengrocery waste was mixed in 1:1 to provide an optimal C/N ratio for anaerobic digestion (Table S1). The experiment was divided into four phases, the first of which was a Control phase (C) in which no biogas was recirculated and no H_2 from an external source was given. Recirculation of gases (RC) in the methanogenic reactor was carried out after 30 days during phase 2, and the effect of gas recirculation at various flow rates (32, 64, 96, and 128 mL/min) was also investigated. During Phase 3, varied flow rates of hydrogen (HS) were fed into the reactor. In phase 4 (HS+RC), H_2 was added to the gas collecting system and recycled at varying flow rates of 32, 64, 96, and 128 mL/min. During Phase 4, the effect of recirculation time was investigated by recirculating the gas at 32 ml/min (the optimum flow rate discovered during Phase 3) for different time intervals (3, 6, 9, and 12 hours). Daily biogas production and pH were recorded, and CH_4 content was measured during steady state. The

reactor was run for three retention times (30 days) to evaluate the effect of variables, and samples for microbiological examination were taken during the steady state.

2.4 Metagenomic analysis

For the metagenomic analysis, 4-5 samples during the steady state were taken from each phase. DNA was extracted using a DNA extraction kit (DNeasy PowerLyzer PowerSoil Kit, QIAGEN, Germany) and kept at -20 °C according to the manufacturer's instructions. The V3-V4 regions of bacterial and archaeal 16S ribosomal RNA (rRNA) genes were amplified using 515F/926R primer, and the 16S metagenomic sequencing library was constructed using Illumina instructions (Illumina, USA). The 16S amplicon was then sent to GENEWIZ France Ltd, for Illumina sequencing. We plotted the top 25 most abundant archaeal species using the Qiime2 pipeline and the DADA2 algorithm following the same procedure as given at author's recent publication (Trego et al., 2021).

2.5. Analytical methods

The amount of gas produced was measured with the help of a 60 ml syringe on daily basis. The CH_4 concentration of biogas was determined by passing one liter of biogas through a 1M NAOH solution and calculating the volume reduction as CO_2. Titration with 1 M H_2SO_4 solution confirmed the amount of CO_2 fixed in the scrubbing solution, according to a procedure validated by (Goertzen et al., 2010).

The methanogenic reactor's VFA concentration and alkalinity were measured at 5-day intervals using Standard Methods for the Examination of Water and Wastewater, 23rd Edition (Baird, 2017). CHNS analysis was performed using a CNHS analyzer (vario EL cube) at the College of Chemistry and Chemical Engineering Lanzhou University, China.

175 **2.6. Statistical analysis**

176 Prism-5 software was used for graphical representation and statistical analysis of the data. CH_4
177 yield and CH_4 content in all four phases were presented as mean value and standard deviation.
178 One-way ANOVA was applied on the recorded data to evaluate the significant difference ($p <$
179 0.05) during each phase in comparison to control.

180 **3. Results and discussion**

181 The recirculation of gases along with hydrogen supply was the best approach for *in-situ* biogas up-
182 gradation. During *in-situ* biogas up-gradation, the flow rate of recirculation of gases, flow rate of
183 hydrogen supply, and introduction of hydrogen into the reactor at intervals showed significant
184 effect on the CH_4 yield and CH_4 content. The highest increase in the CH_4 yield and CH_4 content
185 was observed when H_2 was supplied (interval: After 12 hours of feeding, the hydrogen was
186 supplied for during of 12 hours with 1 hour interval) at flow rate 32 mL/min in the methanogenic
187 reactor and gases were recirculated for 12 hours at flow rate of 32 ml/minutes

188 **3.1. Feasibility of the *in-situ* up-gradation of biogas by hydrogen supply, and recirculation of**
189 **gases**

190 To check the feasibility of in-situ biogas up-gradation, the recirculation of biogas was carried out
191 for 12 hours during phase 2. Hydrogen was provided at a flow rate of 32 ml/min in phase 3, and
192 recirculation of gases along with H_2 supply was carried out at a flow rate of 32 ml/min in phase 4.
193 In each phase, the CH_4 yield and content of biogas were measured (Fig. 1). Due to the mixing
194 effect of recirculation, the CH_4 yield increased by 41% in phase 2 (RC) when biogas was
195 recirculated in comparison to the control (without mixing).The increase in CH_4 production owing
196 to the mixing effect is supported by Wang et al. 2017 who found that mixing at 10 rpm enhanced

197 CH_4 output by 77%. Mixing improves substrate liquefaction, substrate movement, and nutrient
198 transfer, resulting in increased CH_4 generation (Singh et al., 2020). However, because of the
199 digester's constant and high-speed mixing, CH_4 output can be reduced (Kim et al., 2017). Methane
200 production increased by 36% during phase 3 (HS) as compared to the control. Increased CH_4
201 production owing to external H_2 supply (Daz et al., 2020) is due to hydrogenotrophic methanogens
202 converting CO_2 and H_2 to CH_4 (Rittmann et al., 2015). The CH_4 yield was enhanced by 76% in
203 phase 4 (RC+HS). The increase is attributable to two factors: first, mixing increases substrate
204 liquefaction in methanogenic reactors, resulting in increased CH_4 production from the substrate
205 (Singh et al., 2020); and second, hydrogenotrophic methanogens convert CO_2 and H_2 to methane
206 (Rittmann et al., 2015). Following that, the flow rate for H_2 supply and recirculation of gases was
207 optimized for maximum up-gradation during each phase.

208 **3.2. Effect of flow rate of hydrogen supply, and recirculation of gases on methane yield and**
209 **quality of output gas**

210 Increasing the flow rate of H_2 supply and recirculation of gases decreased the CH_4 production (Fig.
211 2A). Increase in flow rate increases the partial pressure of H_2 and negatively affects the microbial
212 process resulting in reduction of CH_4 production (Giovannini et al., 2016). To determine the
213 optimum flow rate for hydrogen supply (phase 3) and hydrogen supply along with recirculation
214 (phase 4) both hydrogen supply and recirculation were carried out at different flow rates. The
215 highest methane yield was achieved in recirculation along with hydrogen supply which is 528
216 $NmLg^{-1}VS_{added}$ while supplying only hydrogen yield 408 $NmLg^{-1}VS_{added}$ of methane at a flow rate
217 of 32 ml/min (Fig. 2A). While the methane yield and content of biogas declined frequently as the
218 flow rate was increased. Similar trend was noted in methane content of biogas with highest
219 methane content 93% during recirculation along with hydrogen supply and 90% in only hydrogen

supply. Rachbauer et al., 2016 support the decrease in methane yield with increased flow rate due to high partial pressure of hydrogen (Luo et al., 2012) and high speed mixing of the slurry in the digester (Kim et al., 2017). Because of the high partial pressure of hydrogen, acetoclastic methanogens are inhibited, resulting in low methane output and VFAs buildup (Stronach et al., 2012; Van et al., 2020) However, due to the hydrogenotrophic conversion of CO_2 and H_2 to CH_4, the methane production and methane content of biogas were higher than control at all flow rates (Rittmann et al., 2015). The mixing impact of gases recirculation, on the other hand, improves substrate breakdown and boosts methane output (Singh et al., 2020). The optimum flow rate for hydrogen supply as well as recirculation of gases along with hydrogen supply, was found to be 32 ml/min.

Afterwards, upon 12 hours of feeding, the hydrogen was supplied for 15 mints in each hour at a flow rate of 32 ml/min with intervals to reduce the partial pressure of hydrogen in the reactor. The methane yield and content were increased to 500 $NmLg^{-1}VS_{added}$ and 92%, respectively (Fig. 2A,B). The intervals of hydrogen supply may diminish the partial pressure in the reactor, resulting in an increase in methane yield and content.

3.3. Effect of gases recirculation time on methane yield and quality of output gas

In phase 4 (RC+HS), after optimizing the flow rate, the gases were recirculated at the optimal flow rate for varied time durations (3, 6, 9, and 12 hours) in order to check the effect of recirculation duration on high methane yield and methane concentration (Fig. 3). When the recirculation period was raised to 12 hours from 3 hours, the methane yield increased to 897 NmL $g^{-1}VS_{added}$ from 569 NmL $g^{-1}VS_{added}$, and the methane content of biogas increased to 98.9% from 93% (Fig. 3). (Yun et al., 2017) reported the highest methane yield and methane content were achieved after 10 hours

recycling of gases during ex-situ biogas up-gradation. After 12 hours duration of recirculation, the methane concentration reached 99%, which can be used to generate energy and as a vehicle fuel (Rosa et al., 2016). In addition, the 12 hour recirculation duration is important for using sunlight as a source of energy for hydrogen production and recirculation of gases.

3.4. Comparison of different phases at optimum parameters

After optimizing the flow rate and recirculation duration, it was concluded that the gas flow rate must be 32 ml/min for a high methane yield and the recirculation duration must be 12 hours. During different phases, the methane yield and content with these optimum conditions are compared and given in (Fig. 4). In phase 2, biogas recirculation enhanced methane yield and content by 36% and 4%, respectively, as compared to control. According to Wang et al. 2017, mixing increased methane production by 77%. Mixing improves substrate liquefaction, substrate movement, and nutrient transport, resulting in higher methane production (Singh et al., 2020). Methane yield and content increased by 66 and 26%, respectively, during phase 3 (hydrogen supply) at a flow rate of 32 ml/min with intervals, compared to control (Fig. 4). In phase 4, the recirculation of gases along with hydrogen supply at a flow rate of 32 ml/min for a length of 12 hours, highest methane yield and methane content were attained, showing increase by 199% and 36%, respectively, as compare to the control (Fig. 4). The increase in methane generation is from substrate due to mixing effect (Singh et al., 2020) and hydrogenotrophic conversion of CO_2 and H_2 to methane are responsible for the rise in methane yield and content (Rittmann et al., 2015). Furthermore, recirculation of gases increases the contact time between methanogens and H_2 in the liquid phase, increasing methane yield and content (Zhu et al., 2020).

3.5. Process stability

The stability of the process is crucial for methanogenic activities. The essential metrics that suggest stability are pH, VFA buildup, and VFAs to alkalinity ratio (Hassan et al., 2020). The pH of the methanogenic reactor was in the optimum range (Table 1) for methanogenesis during all four stages (control, recirculation, hydrogen supply, and recirculation with hydrogen supply) i.e. 6.8- to 7.5 (Van et al., 2020). In the methanogenic reactor during all three phases (control, only recirculation, hydrogen supply together with recirculation), the concentration of VFAs was found to be in optimum range i.e. at or below 600mg/L (Table 1) (Musa et al., 2018). While during phase 3 (HS), the VFAs accumulation in methanogenic reactor increased to 1200 mg/L due to high partial pressure of H_2, which is the main limitation of *in-sit* biogas up-gradation with H_2 supply (Zhu et al., 2020a). On the other hand, the pH shift caused by VFA accumulation and inhibitory level depends on the reactor's buffering capacity (Singh et al., 2020; Wang et al., 2017). The VFAs concentrations were lesser than 2 gL^{-1}, which is the inhibitory level for methanogenic activities (Jain & Mattiasson, 1998). With recirculation, a slight increase in alkalinity was seen during phases 2 and 4, which is attributable to increased substrate breakdown (Singh et al., 2020). The change in reactor pH is influenced by the VFAs to alkalinity ratio and is regarded as a significant measure for monitoring digester stability (Calabr et al., 2018). The VFAs to alkalinity ratio optimal during all four phases (C, RC, HS, and RC+HS). For high biogas production, the VFAs to alkalinity ratio should be 0.4 or below (Li et al., 2018).

3.6. Taxonomic distribution of the archaea

To evaluate the effect of recirculation, hydrogen supply and hydrogen supply along with recirculation on distribution and change in the methanogenic community, samples for metagenomic analysis were taken during steady state of each phase. The relative proportion of different groups was presented on OTUs level and compared during different phases (Fig. 5). The

287 results showed that *Methanobactereium* was predominant in the control phase. Mostly, in
288 anaerobic digesters *Methanobacterium* is dominant which can produce methane by both
289 acetoclastic and hydrogenotrophic pathways (Wu et al., 2021). In phase 2, (recirculation of biogas
290 in R2) increase in the *Methanosarcina* population was noted (Fig. 5). (Saha et al., 2021) reported
291 *Methanosarcina* were flourished in high acetate concentration and is syntrophic acetate utilizing
292 methanogens. During phase 3, when of hydrogen was supplied from external source, the
293 *Methanobacterium* population was reduced while increase in *Methanosarcinales,*
294 *Methanofastidiosum* and *Methanosarcina* had occurred. A similar shift in the hydrogenotrophic
295 methanogens was reported by (Agneessens et al., 2017; Luo et al., 2012). In phase 4 hydrogen
296 supply along with recirculation further decreased in methanobacterium with increased abundance
297 of *Methanomicrobiales*, *Methanobacteriales* and *Methanosarcinales* (Fig. 5). Due to recirculation
298 increase in the population of *Bathyarchaeia* (Fig. 5) was noted that utilizes the inorganic carbon
299 in the form of CO_2 to produce acetate (Evans et al., 2015). (Maus et al., 2018) characterized the
300 metabolic features of *Bathyarchaeia* and reported the acetate and lactate as end products. Due to
301 their acidogenic and hydrolytic activities *Bathyarchaeia* have very important role in anaerobic
302 digestion (Maus et al., 2018). The increase in population of *Bathyarchaeia* confirms that increase
303 in methane production due to recirculation of biogas is due to two reasons. Firstly conversion of
304 CO_2 and H_2 into methane by hydrogenotrophic and secondly increase in methane production from
305 partially degraded organics present in the methanogenic reactor. Due to hydrogen supply reduction
306 of *Methanobactereium* is reported which produces methane by both acetoclastic and
307 hydrogenotrophic pathways (Wu et al., 2021).

308 **4. Conclusions**

The study concludes that the recirculation of biogas along with hydrogen supply in the methanogenic reactor during two-stage anaerobic digestion enhances the methane yield and the output-gas quality, without compromising the stability of the process. The gas recirculation for 12 hours daily at flow rate of 32mL/min led to improved output-gas quality and biogas with 99% CH_4 content. The microbial profile analysis showed the dominancy of hydrogenotrophic methanogens like *Methanomicrobiales, Methanobacteriales* and *Methanosarcinales* during the *in-situ* up-gradation process. The recirculation may also enhance the degradation of biomass left undigested in the hydrolytic reactor.

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Captions of Figures

Fig. 1. Methane yield and methane contents at different phases mentioned (control, recirculation of gases, hydrogen supply, and hydrogen supply along with recirculation) during *in-situ* biogas up-gradation. Columns with the *** showed that these are significantly different ($p<0.05$) as compared to control.

Fig. 2. Effect of flow rate on (A) methane yield (B) methane contents of biogas during hydrogen supply and hydrogen supply along with recirculation. Columns with the *** showed that these are significantly different ($p<0.05$) as compared to control. (3 with intervals: After 12 hours of feeding, the hydrogen was supplied for during of 12 hours with 1 hour interval at flow rate 32 mL/min)

Fig.3. Effect of duration of recirculation of gases with supplied with hydrogen on methane yield methane content of biogas when hydrogen supplied from external along with recirculation of gases into the reactor. Columns with the *** show the significant difference ($p<0.05$). The recirculation was carried out for respective duration during the last period of 24 hours. 3 with intervals: After 12 hours of feeding, the gasses were recirculated for 15 mints in each hour.

Fig. 4. Comparison of methane yield methane contents of biogas during different phases (control, recirculation, hydrogen supply and hydrogen supply along with recirculation) at optimized conditions (flow rate of hydrogen supply:32 ml/min, flow rate of gases recirculation: 32 ml/min, for 12 hours). Columns with the *** showed that these are significantly different ($p<0.05$) as compared to control.

Fig. 5 Taxonomic distribution of the methanogens during different phases (control, recirculation, hydrogen supply and hydrogen supply along with recirculation) in methanogenic reactor.

Legends of Tables

Table. 1. Process stability parameter at different phases (control, recirculation, hydrogen supply and hydrogen supply along with recirculation).

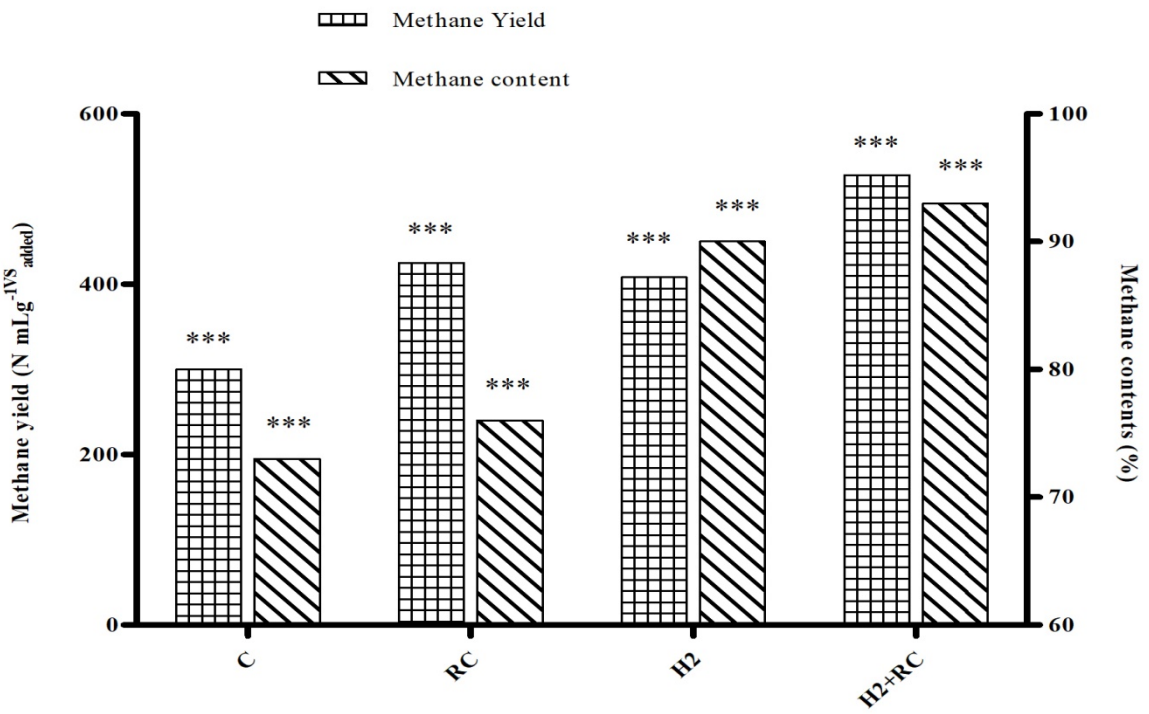
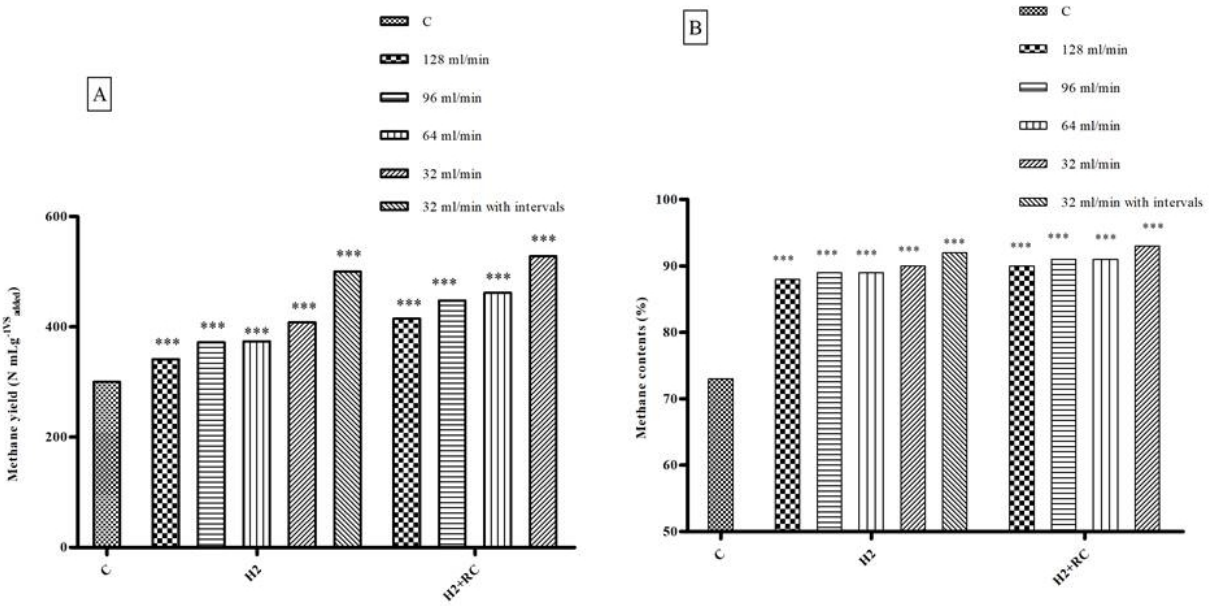

Methane Yield
Methane content
Methane yield (N mL.g-1VSadded)
Methane contents (%)
600
400
200
0
100
90
80
70
60
***
C
RC
H2
H2+RC

Fig. 1.



523

524 Fig.2.

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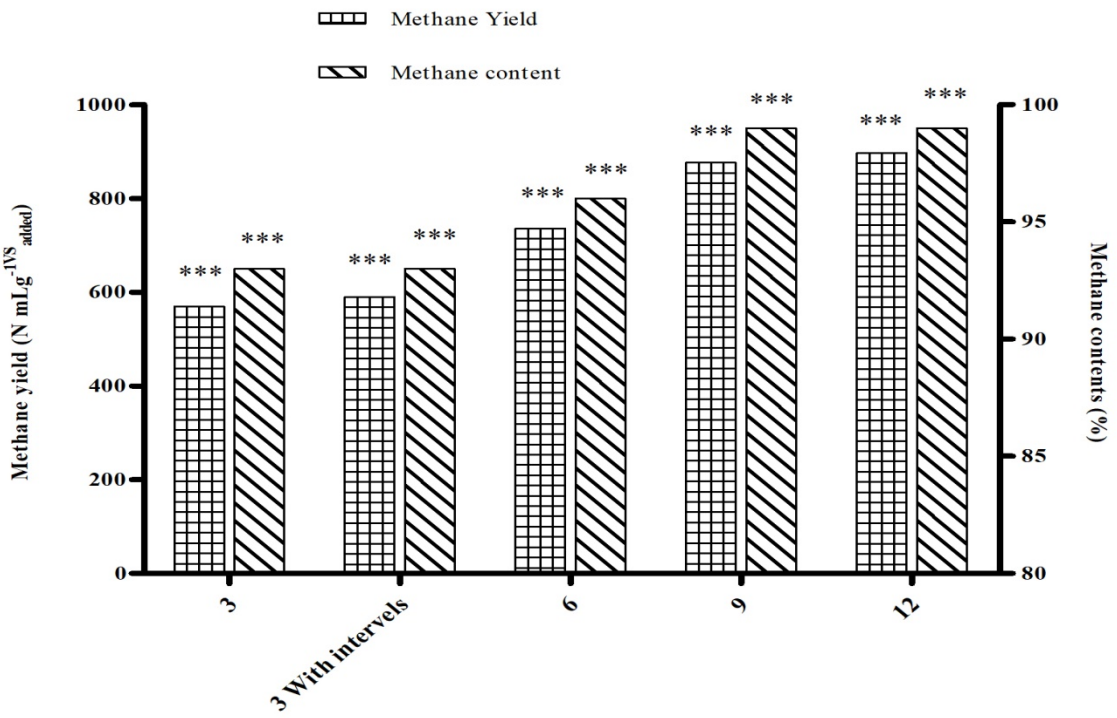

Methane Yield
Methane content
Methane yield (N mL.g-1VSadded)
Methane contents (%)
1000
800
600
400
200
0
100
95
90
85
80
***
3
3 With intervels
6
9
12

Fig.3.

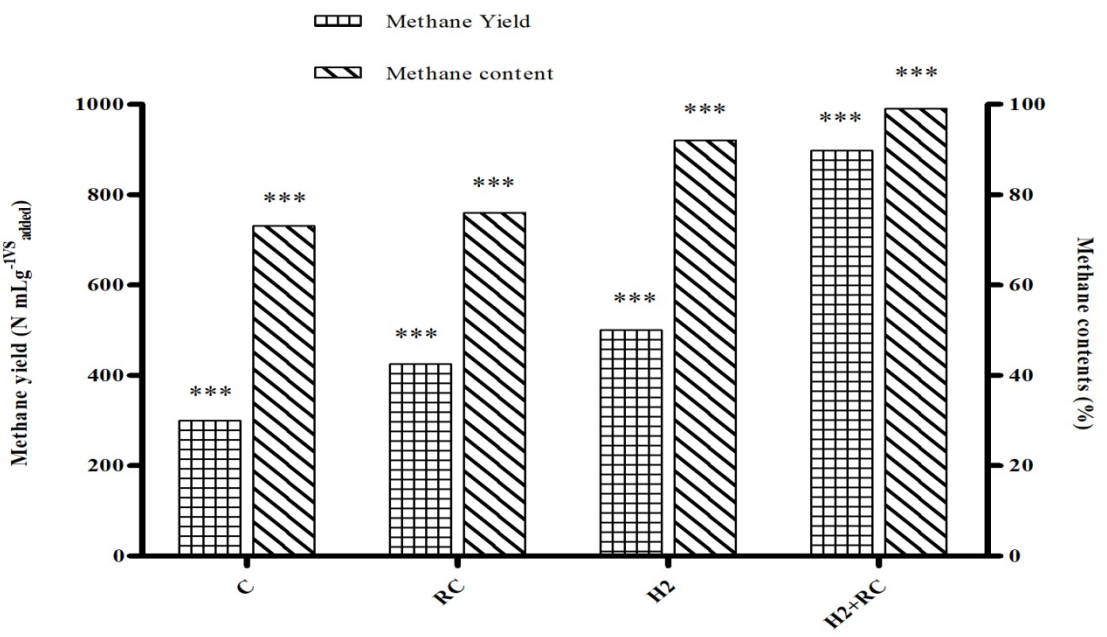
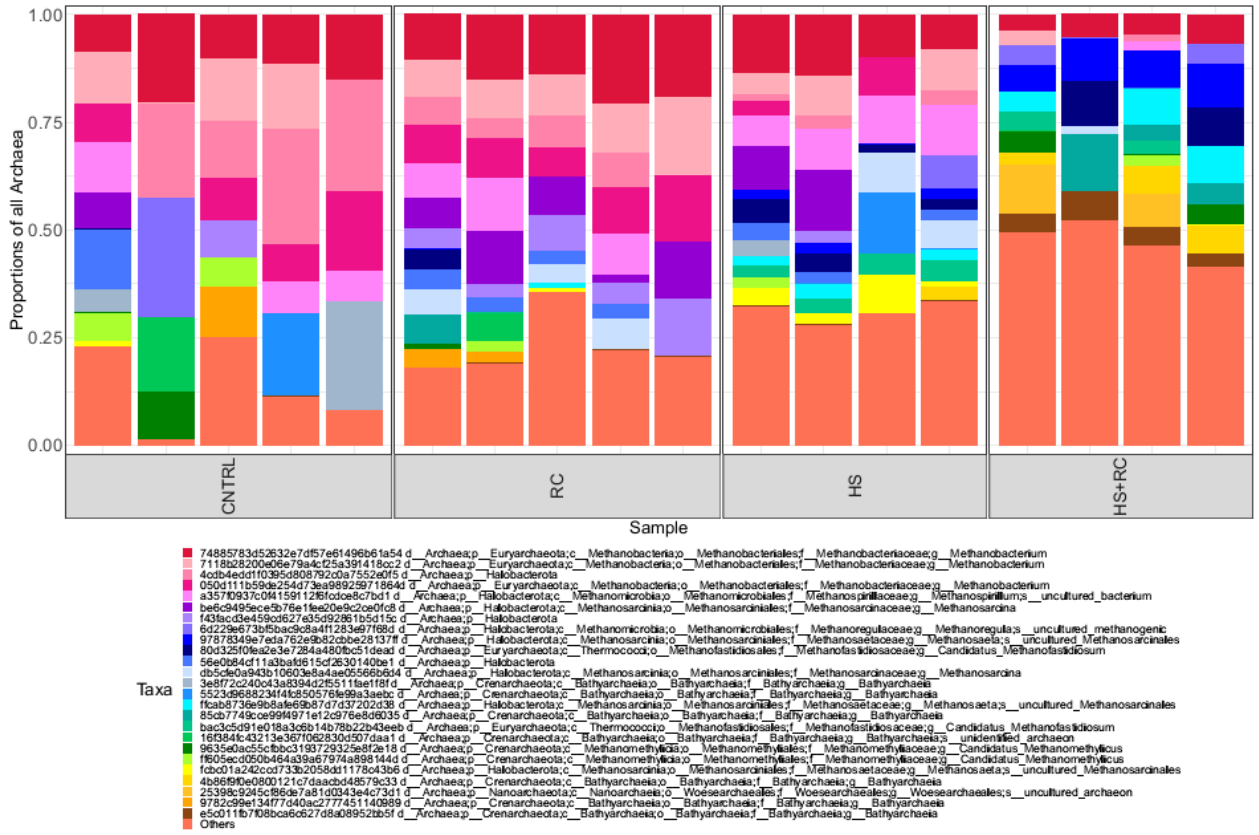

Methane Yield
Methane content
Methane yield (N mLg-1VSadded)
Methane contents (%)
1000
800
600
400
200
0
100
80
60
40
20
0
***
C
RC
H2
H2+RC

Fig. 4.


1.00
0.75
0.50
0.25
0.00
Proportions of all Archaea
CNTRL
RC
HS
HS+RC
Sample
Taxa
74885783d52632e7df57e61496b61a54 d__Archaea;p__Euryarchaeota;c__Methanobacteria;o__Methanobacteriales;f__Methanobacteriaceae;g__Methanobacterium
7118b28200e06e79a4cf25a391418cc2 d__Archaea;p__Euryarchaeota;c__Methanobacteria;o__Methanobacteriales;f__Methanobacteriaceae;g__Methanobacterium
4cdb4edd1f0395d808792c0a7552e0f5 d__Archaea;p__Halobacterota
050d111b59de254d73ea98925971864d d__Archaea;p__Euryarchaeota;c__Methanobacteria;o__Methanobacteriales;f__Methanobacteriaceae;g__Methanobacterium
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6d229e673bf5bac9c8a4f1283e97f68d d__Archaea;p__Halobacterota;c__Methanomicrobia;o__Methanomicrobiales;f__Methanoregulaceae;g__Methanoregula;s__uncultured_methanogenic
97878349e7eda762e9b82cbbe28137ff d__Archaea;p__Halobacterota;c__Methanosarcinia;o__Methanosarcinales;f__Methanosaetaceae;g__Methanosaeta;s__uncultured_Methanosarcinales
80d325f0fea2e3e7284a480fbc51dead d__Archaea;p__Euryarchaeota;c__Thermococci;o__Methanofastidiosales;f__Methanofastidiosaceae;g__Candidatus_Methanofastidiosum
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ffcab8736e9b8afe69b87d7d37202d38 d__Archaea;p__Halobacterota;c__Methanosarcinia;o__Methanosarcinales;f__Methanosaetaceae;g__Methanosaeta;s__uncultured_Methanosarcinales
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16f384fc43213e3671062830d507daa1 d__Archaea;p__Crenarchaeota;c__Bathyarchaeia;o__Bathyarchaeia;f__Bathyarchaeia;g__Bathyarchaeia;s__unidentified_archaeon
9635e0ac55cfcbc3195729325e8f2e18 d__Archaea;p__Crenarchaeota;c__Methanomethylicia;o__Methanomethylicales;f__Methanomethylicaceae;g__Candidatus_Methanomethylicus
ff605ecd050b464a39a67974a898144d d__Archaea;p__Crenarchaeota;c__Methanomethylicia;o__Methanomethylicales;f__Methanomethylicaceae;g__Candidatus_Methanomethylicus
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9782c99ef134ff77d40ac27774511408989 d__Archaea;p__Crenarchaeota;c__Bathyarchaeia;o__Bathyarchaeia;f__Bathyarchaeia;g__Bathyarchaeia
e5c011fb7f08bca6c627d8a08952bb5f d__Archaea;p__Crenarchaeota;c__Bathyarchaeia;o__Bathyarchaeia;f__Bathyarchaeia;g__Bathyarchaeia
Others

Fig. 5.

574 Table. 1. Process stability parameter at different phases (control, recirculation, hydrogen supply
575 and hydrogen supply along with recirculation).

Different phases	pH	VFAs accumulation (mgL^{-1})	Alkalinity (mgL^{-1})	VFAs to alkalinity ratio
C	7.2	500	2500	0.20
RC	7.3	350	2800	0.13
HS	7.0	1200	2600	0.31
HS+RC	7.2	400	2700	0.15

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577 Highlights

578 • Hydrogenotrophic methanogens enriched during *in situ* biogas upgradation
579 • 99% methane content was archived at optimized conditions.
580 • Methane yield increased by two folds during *in situ* biogas upgradation
581 • Gases recirculation minimized the adverse effect of H_2 on microbial diversity

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