Ex-situ biogas upgrading to methane and removal of VOCs in a system of zero valent iron and anaerobic granular sludge

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Research Article

Keywords: anaerobic granular sludge, biogas upgrading, hydrogenotrophic methanogens, zero valent iron, volatile organic compounds (VOCs), H2S removal

DOI: https://doi.org/10.21203/rs.3.rs-546207/v1

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Abstract

In the current study, a novel process of ex-situ biogas upgrading to biomethane is presented which is based on a system consisted of anaerobic sludge and zero valent iron (ZVI). The ZVI when is added in an aquatic system with anaerobic granular sludge generates $H_2$ under anaerobic abiotic conditions. Then, the $H_2$ and $CO_2$ are converted by the hydrogenotrophic methanogens to $CH_4$. Biogas upgrading to biomethane was achieved in 4 days in a system of anaerobic granular sludge, 50 g L$^{-1}$ ZVI initial pH 5 and 20 g L$^{-1}$ NaHCO$_3$. In this system when zero valent scrap iron (ZVSI) was added instead of ZVI required longer period (21 days) to achieve biogas upgrading. Volatile organic compounds (VOCs) analysis in raw biogas (in system of anaerobic sludge and ZVI) showed mainly the reduction of low mass straight- and branched-chain alkanes (C$_6$-C$_{10}$); however, no other particular trend regarding the removal of other VOCs was observed. $H_2S$ and $NH_3$ were found to be substantially reduced, when the anaerobic sludge was exposed to ZVI compared to no decrease in serum bottles free of ZVI.

1. Introduction

Biogas upgrading to biomethane not only significantly increases its calorific value, but also enables biomethane to be transported and utilized far from the anaerobic digester (Muñoz et al. 2015). Therefore, there is growing interest for biogas upgrading technologies as they can be applied closed to an anaerobic digester. Up to date, most commercial technologies used for biogas upgrading are mainly based on physicochemical processes; however, these technologies are energy intensive and present a high operational cost (Sun et al. 2015). Recently, several studies (Sun et al. 2015; Angelidaki et al. 2018) proposed an alternative option for biogas upgrading based on the injection of $H_2$ and the utilization of $CO_2$ and $H_2$ by hydrogenotrophic methanogens according to Eq. (1).

(Eq. 1) \[ 4H_2 + CO_2 \rightarrow CH_4 + 2H_2O \quad \Delta G^0 = -130.7 \text{ kJ mol}^{-1} \]

At this process, $H_2$ needs to be produced by an external source using water electrolysis that can be generated from renewable energies (solar or wind energy) (Muñoz et al. 2015; Angelidaki et al. 2018). The addition of $H_2$ can take place in-situ, in which $H_2$ is delivered inside the liquid phase of a biogas reactor or ex-situ in which $CO_2$ from external sources and $H_2$ are injected inside the liquid phase of a reactor containing hydrogenotrophic methanogens (Angelidaki et al. 2018; Angenent et al. 2018). Towards this, several challenges of this system exist; the low gas-liquid mass transfer rate of $H_2$, which limits its availability for methanogens and therefore the gas recirculation flow rate and reactor design are considered key factors for the successful biogas upgrading process (Bassani et al. 2016), as well as the relatively high cost of electrolysis (Schiebahn et al. 2015; Angenent et al. 2018).

In recent studies (Vyrides et al. 2018; Menikea et al. 2020), it was showed that $CO_2$ as a sole carbon source can be converted to $CH_4$ in a system consisted of anaerobic granular sludge (AnGrSl), zero valent
iron (ZVI) and zero valent scrap iron (ZVSI), respectively. Under anaerobic aquatic condition, ZVI released \( \text{H}_2 \) and oxidized according to Eq. (2) (Menikea et al. 2020)

(Eq. 2) \[ \text{Fe}^{(0)} + \text{HCO}_3^- + \text{H}^+ \rightarrow \text{FeCO}_3 + \text{H}_2 \quad \Delta G^0 = -79.9 \text{ kJ mol}^{-1} \]

The Gibbs free energy changes \( \Delta G^0 \) of Equation 1 in standard conditions (pH 0, all concentrations 1 M and all partial pressures 1 atm) to -79.9 kJ mol\(^{-1}\), whereas at pH 7 \( \Delta G^0 \) is -39.9 kJ mol\(^{-1}\).

Then, the \( \text{H}_2 \) from abiotic oxidation of ZVI and the \( \text{CO}_2 \) is utilized by hydrogenotrophic methanogens according to Eq. 1 for the generation of \( \text{CH}_4 \).

Until today, this system (mix anaerobic sludge and ZVI) was tested for \( \text{CO}_2 \) conversion to \( \text{CH}_4 \) and (Vyrides et al. 2018; Menikea et al. 2020) it was not examined for ex-situ biogas upgrading under a semi-batch system. Apart from this, the use of ZVI can potentially contribute to the potential removal of volatile organic compounds (VOCs); a parameter that was also examined in the current study.

Some VOCs present low odor thresholds and even at trace levels can contribute to unpleasant smells for workers and for people who live nearby wastewater treatment plants (WWTPs). Many types of VOCs are toxic or even hazardous to humans and can be detrimental to the environment. In addition, low concentrations (ppb\(_v\) levels) of volatile sulfur compounds (VSCs) can also impact to human health causing headaches and nausea, as well as eye, respiratory, and neuropsychological symptoms (Andersson et al. 2004). They can also cause operational problems by corroding the respective equipment (e.g., compressors and pipes) used for certain tasks, such as for upgrading and cleaning the gas (Andersson et al. 2004).

Apart from VOCs, biogas often contains hydrogen sulfide (\( \text{H}_2\text{S} \)), which can severely limit its application as it is related with harmful environmental emissions and corrodes the engines of biogas purification machinery (Farghali et al. 2020). As was stated in recent studies (Ruan et al. 2017; Andriamanohiarisoamanana et al. 2018; Wei et al. 2018; Farghali et al. 2020), ZVSI could also help to prevent the emissions of \( \text{H}_2\text{S} \) via iron-sulfide (FeS) precipitation. Based on these findings, this study will examine the ex-situ biogas upgrading and \( \text{H}_2\text{S} \) removal in the same system.

The scope of this study is to examine a new proof of concept for the simultaneous ex-situ biogas upgrading to biomethane and also to examine the potential removal of VOCs, as well as \( \text{NH}_3 \) and \( \text{H}_2\text{S} \), using an aquatic system of ZVI and anaerobic granular sludge (AnGrSI).

2. Material And Methods

2.1. Biogas sources

The following sources of biogas were used:
a) A Synthetic biogas (Linde - Hadjikyriakos Gas Ltd; Cyprus) 60% CH$_4$ and 40% CO$_2$ was used at serum bottle experiments and on feeding laboratory bioreactor on day 17.

b) Raw biogas produced from a pilot anaerobic digester that was treating with food waste and operated at Moni municipal WWTP (Limassol, Cyprus) was employed. The biogas was collected using Tedlar® gas inspecting bags with Thermogreen® LB-2 septa (1L, Supelco) sampled from Moni, Limassol. The raw biogas fed laboratory batch reactor on days 1, 8 and 28. Raw biogas composition was 59 +1.5 CH$_4$ % and 41 + 1.5 CO$_2$ %, respectively.

### 2.2 Serum bottle experiments

Serum bottle anaerobic experiments were conducted in 125 mL serum bottles with a working volume of 80 mL. The anaerobic granular sludge (withdrawn from a full scale upflow anaerobic sludge blanket (UASB) reactor) was daily treated with wastewater at pH 6.8–7.3, sieved (1 mm) and washed with distilled water and used as inoculum (2 g of wet weight was added in 80 mL)). The total solids (TS) and volatile solids (VS) of granular sludge were 9.5% (wet basis) and 85.5% (of TS), respectively. ZVI (Iron ≥ 99%, reduced, powder (fine), Sigma Aldrich code 12310) was added in various concentrations of 25 g L$^{-1}$, 50 g L$^{-1}$, 75 g L$^{-1}$, 100 g L$^{-1}$. ZVSI was obtained from university machinery (lathe machines) and was approximately 2–4.0 mm in width and 0.2–0.5 mm in thickness as described in (Menikea et al. 2020). The ZVSI was added in concentrations of 100 g L$^{-1}$ and 200 g L$^{-1}$.

The remaining volume (up to 80 mL) was filled with media (Owen et al., 1979); however, the concentration of NaHCO$_3$ was increased to 20 g L$^{-1}$ in order to prevent back pressure in the serum bottle due to the consumption of CO$_2$ by methanogens and due to absorption of CO$_2$ from the liquid phase and pH increase.

The pH was monitored (pH meter, Mettler Toledo) by withdrawing around 1 mL from each serum bottle. The pH was also daily adjusted by using 2M HCl or 2M NaOH. In case where no pH adjustment was needed, the equal volume of medium was added in order to maintain the working volume.

For creating anaerobic conditions, synthetic biogas (60% CH$_4$ and 40% CO$_2$) was introduced to each serum bottle for 5 min to remove oxygen, and then, the bottles were capped, sealed with butyl septa and aluminium crimps and stirred in a shaking incubator (100 rpm) at 30 °C during the experiment. The headspace pressure was initially regulated at 1.5–1.6 atm (the absolute pressure was measured by manometer) in order to avoid back pressure, due to CO$_2$ consumption and due to 1 mL sampling from the headspace for gas analysis.

The anaerobic batch experiments at various ZVI concentrations were carried out in duplicates (Fig. 1a), whereas the experiments regarding ZVI and ZVSI over time were repeated twice (in duplicates each time) and the standard deviation was calculated. Gaseous samples of various inorganic gases (CH$_4$, CO$_2$, H$_2$, N$_2$, and O$_2$) were analysed over time. In every experiment, controls were prepared without addition of ZVI (consisted of granular sludge only) and without addition of AnGrSl consisted only of ZVI.
2.3 Operation of laboratory bioreactor and batch biogas feeding
Semi-batch laboratory experiment took place in a bottle of 500 mL with a working volume of 350 mL and a final concentration of NaHCO$_3$ (20 g L$^{-1}$) for 31 days. The bottle was inoculated with 10 g of AnGrSl (wet weight sludge); the ZVI (Iron ≥ 99%, reduced, powder (fine)-Sigma Aldrich CAS Number 7439-89-6 and code 12310) concentration at the bioreactor was 50 g L$^{-1}$. ZVI was added only once (in the start-up phase) of bioreactor which was operated at 37 °C. 200 mL of raw biogas (59% CH$_4$ and 41% CO$_2$) was added on days 1, 8 and 28 and 200 mL of synthetic biogas was added on day 17.

2.4. VOCs sampling and analysis

The liquid samples were also analysed for their VOCs evolution using the headspace Solid Phase Microextraction-Gas Chromatography/Mass Spectrometry (HS-SPME-GC-MS) approach. In particular, the SPME 75-µm CAR/PDMS fiber (Supelco, 57344-U) was thermally desorbed in a combined GC/MS (Agilent 7890B/5977B) system. The respective fiber was exposed for 30 min at room temperature over the samples liquid phase and then exposed at the GC inlet (280 °C) and remained for 1 min to implement the VOCs desorption. The SPB-624 capillary column (60 m × 0.25 mm × 1.4 µm, Supelco) with the help of He (1.7 mL min$^{-1}$) enabled the VOCs separation. The following thermal cycle was set at the chromatographic furnace: 35 °C for 5 min, increasing at a rate of 4 °C min$^{-1}$ up to 180 °C, constant for 20 min. The MS system worked at full scan, in split mode 1:10 and from 35–350 amu. Ion source, quadrupole and transfer line temperatures were stable at 230, 150 and 250 °C, respectively. NIST17 mass spectral library contributed to chromatographic peaks identification, next to a standard solution mixture consisting of 60 VOCs (EPA 524 VOC Mix A, Supelco).

The Area Under Curve (AUC) of dimethyl sulfide (DMS) was used as a reference compound and all detected VOCs were divided by that of DMS, as follows AUC = $A_i/A_{DMS}$. If the measured AUC was from 0.1-2 is presented as + in Table 1, from 2.1–10 as ++ and higher than 10 as ++++. When the respective compound was not detected, then n.d. was placed in the Table 1.
Table 1
VOCs that were detected with HS-SPME-GC-MS in time and the concentrations of H$_2$S and NH$_3$ analysed by the respective sensors on day 7.

| VOCs | Anaerobic granular (day 2) | Anaerobic granular ZVI (day 2) | Anaerobic granular (day 7) | Anaerobic granular ZVI (day 7) |
|------|---------------------------|--------------------------------|---------------------------|--------------------------------|
|      |                           |                                |                           |                                |
| Alkanes |                      |                                |                           |                                |
| Pentane | ++                       | ++                             | +                         | +                              |
| 2-methyl-Pentane | n.d.                   | +                              | ++                        | +++                            |
| 3-methyl-Pentane  | ++                       | ++                             | +++                       | +++                            |
| 2,3-dimethyl Pentane | n.d.                   | n.d.                           | ++                        | +                              |
| n-Hexane          | +++                      | +++                            | +++                       | +++                            |
| 2-methyl-Hexane   | +++                      | +++                            | ++                        | +++                            |
| 3-methyl-Hexane   | +++                      | +++                            | ++                        | +++                            |
| 2,2,5-trimethyl-Hexane | n.d.       | n.d.                           | +                         | ++                              |
| Heptane           | +++                      | +++                            | ++                        | +                              |
| 2,5-dimethyl-Heptane | ++                  | ++                             | +                         | n.d.                           |
| 3-ethyl-2-methyl-Heptane | ++             | ++                             | +                         | n.d.                           |
| 2,6-dimethyl-Octane | +++                   | +++                            | +                         | n.d.                           |
| 4-methyl-Octane   | ++                       | ++                             | +                         | n.d.                           |
| Octane            | +++                      | +++                            | ++                        | +                              |
| Nonane            | +++                      | +++                            | +                         | n.d.                           |
| 4-methyl-Nonane   | ++                       | ++                             | +                         | n.d.                           |
| 2-methyl-Nonane   | ++                       | ++                             | n.d.                      | n.d.                           |
| 4-methyl-Decane   | +++                      | +++                            | +                         | n.d.                           |
| 2,3,8-trimethyl-Decane | n.d.             | n.d.                           | ++                        | +                              |
| Undecane          | ++                       | ++                             | +                         | n.d.                           |

* Values from 0.1-2 are presented as +, 2.1–10 as ++ and higher than 10 as ++++, whereas n.d. stands for not detected. The respective values of AUC are derived from $A_i/A_{DMS}$. 
| VOCs                        | Anaerobic granular (day 2) | Anaerobic granular ZVI (day 2) | Anaerobic granular (day 7) | Anaerobic granular ZVI (day 7) |
|-----------------------------|----------------------------|--------------------------------|---------------------------|--------------------------------|
| 3-methyl-Dodecane           | +++                        | +++                            | n.d.                      | n.d.                           |
| 2,6-dimethyl-Undecane       | n.d.                       | n.d.                           | ++                        | n.d.                           |
| methyl-Cyclopentane         | ++                         | ++                             | +++                       | +++                            |
| 1,1-dimethyl-Cyclopropane   | n.d.                       | +                              | +                         | +                              |
| **Alkenes**                 |                            |                                |                           |                                |
| 2-methyl-1-Propene          | ++                         | ++                             | +                         | ++                             |
| 2,4,4-trimethyl-1-Pentene   | +                          | +                              | +                         | ++                             |
| 3,7-dimethyl-2-Octene       | +++                        | +++                            | ++                        | n.d.                           |
| 2,6-dimethyl - 4-Octene     | +++                        | +++                            | +                         | n.d.                           |
| **Aromatic hydrocarbons**   |                            |                                |                           |                                |
| Benzene                     | ++                         | ++                             | +                         | ++                             |
| 1,2,4-trimethyl-Benzene     | ++                         | +++                            | ++                        | ++                             |
| Toluene                     | +++                        | +++                            | +++                       | +++                             |
| **Terpenes**                |                            |                                |                           |                                |
| D-Limonene                  | +++                        | +++                            | ++                        | ++                             |
| .alpha.-Pinene              | +++                        | +++                            | ++                        | ++                             |
| beta.-Pinene                | +++                        | +++                            | ++                        | ++                             |
| o-Cymene                    | +++                        | +++                            | +++                       | ++                             |
| **Sulfur compounds**        |                            |                                |                           |                                |
| Methanethiol                | n.d.                       | n.d.                           | +                         | +                              |
| Dimethyl sulfide            | n.d.                       | n.d.                           | +                         | +                              |

* Values from 0.1-2 are presented as +, 2.1–10 as ++ and higher than 10 as +++; whereas n.d. stands for not detected. The respective values of AUC are derived from $A_i/A_{DMS}$. 
| VOCs            | Anaerobic granular (day 2) | Anaerobic granular ZVI (day 2) | Anaerobic granular (day 7) | Anaerobic granular ZVI (day 7) |
|-----------------|----------------------------|-------------------------------|---------------------------|-------------------------------|
| Carbon disulfide | ++                         | n.d.                          | +                         | n.d.                          |

*Sensors (ppm,v)*

|          |                |                |                |                |
|----------|----------------|----------------|----------------|----------------|
| H₂S      | 82             | 1              |                |                |
| NH₃      | 266            | 48             |                |                |

*Values from 0.1-2 are presented as +, 2.1–10 as ++ and higher than 10 as +++*, whereas n.d. stands for not detected. The respective values of AUC are derived from $A_i/A_{DMS}$.

Immediately after the HS-SPME-GC-MS analysis, Dräger X-am 8000 photo ionisation detector (PID) equipped with 4 sensors (H₂S, NH₃, CO₂, O₂), was used for measuring the respective H₂S and NH₃ gaseous emissions.

**2.5. Analysis of gas composition**

Gas composition of H₂, O₂, N₂, CH₄ and CO₂ from the batch experiments was measured via gas chromatography (Agilent technologies 7820A GC system, Wilmington, DE) coupled to a thermal conductivity detector (GC-TCD), with argon as a carrier gas (as described by Vyrides et al. 2018). The coefficient of variation for 10 identical samples was ± 2%.

**3. Results**

**3.1. Ex-situ biogas upgrading by anaerobic granular sludge and ZVI**

Anaerobic granular sludge (AnGrSL) was independently inoculated with 25, 50, 75 and 100 g L⁻¹ ZVI and fed with synthetic biogas (60% CH₄ and 40% CO₂) at initial pH 6 and 20 g L⁻¹ NaHCO₃ in the media. The headspace after 6 days showed that the exposure to 50 and 75 g L⁻¹ ZVI resulted in 97% CH₄ in the headspace whereas, the exposure to 25 g L⁻¹ and 100 g L⁻¹ ZVI resulted in 85% and 81% CH₄, respectively (Fig. 1a). The CH₄ (%) in the control remained stable after 6 days.

Figure 1b shows the gas composition over time for AnGrSL exposed to 50 and 75 g L⁻¹ ZVI at initial pH 6 and 20 g L⁻¹ NaHCO₃ in the media. For both ZVI concentrations, from the beginning of the experiment until day 2, CO₂ gradually minimized whereas H₂ gradually increased. Then, from day 2 to 7, the H₂ is progressively decreased and the CH₄ steadily increased to 97% for both ZVI concentrations. The initial increase of H₂ is due to abiotic anaerobic oxidation of ZVI whereas, the reduction of H₂ and CO₂ is mainly attributed to hydrogenotrophic methanogens (Eq. 1). Part of the CO₂ was absorbed, solubilized in the
media and formed siderite according to Eq. 2. That explains its absence from the headspace after day 2 and the simultaneous generation of CH₄ from the days 2 to 7.

At the next experiment, the initial pH was set to 5 since the lower initial pH can increase the H₂ production rate from ZVI (Menikea et al. 2020). In addition due to ZVI anaerobic oxidation (Eq. 2), the pH rapidly increases up to 8.3 (Vyrides et al. 2018) and therefore the methanogens can function well in the range 6.8–7.8 during the anaerobic ZVI oxidation. At initial pH 5, the following initial concentrations of NaHCO₃ was varied in the media 2.3 g L⁻¹, 20 g L⁻¹, 35 g L⁻¹, and 50 g L⁻¹. As can be seen from Fig. 2a, at serum bottle with 20 g L⁻¹ NaHCO₃ resulted in 95% CH₄ composition after 4 days. At concentrations 35 g L⁻¹, and 50 g L⁻¹ the biogas upgrading took place at a slower rate. This could be due the inhibition of hydrogenotrophic methanogens by sodium (at 35 and 50 g L⁻¹) (Vyrides and Stuckey 2009), as results of H₂ (%) in the headspace at these concentrations are in higher level compared to the serum bottles that the NaHCO₃ concentrations was lower (Fig. 2b). At concentration of 2.3 g L⁻¹, NaHCO₃ a back pressure is created due to simultaneous consumption of CO₂ by methanogens and due to absorption of CO₂ to liquid, when pH increases (Eq. 2); therefore, the optimum concentration for this system was 20 g L⁻¹ NaHCO₃ since no back pressure was formed and the higher production of CH₄ was detected.

Anaerobic bioreactor inoculated with AnGrSl and 50 g L⁻¹ ZVI received real biogas (200 mL each time) at days 1, 8 and 28, as well as synthetic biogas at day 17. As shown in Fig. 3, the bioreactor inoculated with 50 g L⁻¹ ZVI during the first biogas fed-batch increased the CH₄ composition from 63% (day 1) to 75.5% (day 8), whereas the H₂ detected on day 8 was 21%. At the 2nd biogas fed-batch (day 8–17), the CH₄ reached 80.7% (day 15) and then due to increase of N₂ the CH₄ decreased to 75.3%. The increase of N₂ was likely due to the creation of under pressure, because of pH increase to 8.9 and the subsequent CO₂ absorption. On day 17, synthetic biogas was injected and the CH₄ increased from 60% to 92.7% in one day (day 18) and then increased to 95.1% on day 19. On day 28, a raw biogas was injected to bioreactor and the CH₄ composition in the headspace of the bioreactor increased from 67.8% to 90.5% after a day and then reached 94.7% on day 31. Noteworthy, during this period (17–31) the pH raised around 8.5 and then was daily regulated to pH 7.5. Therefore, part of the CO₂ reduction was due to CO₂ adsorption in the media and not due to the consumption by hydrogenotrophic methanogens. In addition, part of the soluble CO₂ formed siderite according to Eq. 2 and previous findings (Menikea et al. 2020).

3.2 Ex-situ biogas upgrading by anaerobic granular at 100 g L⁻¹ and 200 g L⁻¹ zero valent scrap iron

The use of zero valent scrap iron (ZVSI) was also tested in current system as a strategy for synthetic biogas upgrading to biomethane. Figure 4 shows that at inoculation of 100 g L⁻¹ and 200 g L⁻¹ of ZVSI in AnGrSl resulted in CH₄ composition of 90.7% and 95.5%, respectively after 21 days. Interestingly, very little H₂ was detected in the headspace and this could be explained due to the slow release of H₂ by ZVSI followed by immediate consumption of hydrogenotrophic methanogens (Menikea et al. 2020). The longer period for biogas upgrading by ZVSI compared to ZVI (in the presence of AnGrSl) is counterbalance by
the lower price of ZVI, which is estimated 5 times lower (Cheng et al. 2020). In a recent study, Charalambous and Vyrides (2020) independently added ZVI and ZVSI in anaerobic sludge for the treatment of cheese whey; they found substantial increase in CH$_4$ production, biogas upgrading and that ZVI and ZVSI can provide alkalinity in the system. Under these conditions, in a desktop scale study it was found that only ZVSI was economically feasible process for the anaerobic treatment of cheese whey (Charalambous and Vyrides 2020).

3.3. VOCs removal by anaerobic granular and ZVI

Serum bottles inoculated with AnGrSl, 50 g L$^{-1}$ ZVI and AnGrSl (without ZVI) were fed with raw biogas that was generated from a pilot anaerobic digester treated with food waste and VOCs were analysed on days 2 and 7. The serum bottle in which ZVI was added in AnGrSl upgraded the biogas to approximately 95% CH$_4$ after 7 days. The inorganic gas composition (CH$_4$, H$_2$, CO$_2$) followed the same trend, as was previously shown at Fig. 1a (using synthetic biogas).

The VOCs analysis over time for samples exposed to ZVI plus anaerobic sludge and anaerobic sludge only are shown at Table 1. These samples were fed with raw biogas from a pilot anaerobic digester fed with food waste. The main VOCs detected can be separated in five main groups: (a) alkanes, (b) alkenes, (c) aromatic hydrocarbons, (d) terpenes and (e) organic sulfur compounds (Table 1). Similar compounds were also detected by other studies (Salazar Gómez et al., 2016; Paolini et al., 2018). The main linear and branched alkanes that were detected were pentane, 3-methyl-pentane, hexane, heptane, methyl-cyclopentane and 3-methyl-dodecane. The main aromatic hydrocarbons that were identified was benzene, toluene, and 1,2,4-trimethyl-benzene. The key terpene was D-limonene and the main sulfur compounds were DMS and carbon disulfide. The addition of ZVI in AnGrSl reduced mainly the low mass straight-chain and branched alkanes such as heptane, branched heptanes, octane and branched octanes, nonane, branched nonane as well as 3-methyl-dodecane. However, the removal of other VOCs and VSCs has not revealed any particular pattern by this system (AnGrSl and ZVI).

Noteworthy, in the first days several compounds were not present in the headspace gas, but as the experiments were in progress more VOCs were released (e.g. 2,2,5-trimethyl-hexane, methanethiol, DMS, see Table 1); this could be attributed to the auto-biodegradation of the anaerobic sludge and the release of these compounds. In addition, these compounds could be initially absorbed from raw biogas in the liquid phase and then were gradually released in the gas phase.

Interestingly, the H$_2$S and NH$_3$ were significantly reduced in the serum bottle that anaerobic granular sludge was inoculated with ZVI. This process highlights a great potential for simultaneous biogas upgrading and H$_2$S removal, however as the analysis of H$_2$S was performed with an electrochemical sensor, further experiments need to be contacted to verify this. The chromatographs on day 7 for the both conditions can be seen at Figs. 5a and 5b, respectively.

Similar experiment was contacted using ZVSI (200 g L$^{-1}$) and AnGrSl and the serum bottles were fed with raw biogas. In this experiment, not a particular trend was detected as several of these compounds were
decreased, whereas other were increased over time. However, as in the previous experiment with ZVI, using the respective electrochemical sensors, the H$_2$S and NH$_3$ were significantly lower for the AnGrSl that ZVSI was added compared to AnGrSl without ZVSI.

4. Discussion

The main technologies for biogas upgrading are based on several physicochemical processes such as cryogenic separation, membrane separation, pressure swing adsorption, high-pressure water scrubbing and amine scrubbing. These physicochemical processes are considered very efficient but expensive technologies requiring complex technical equipment and enabled methane losses (Muñoz et al. 2015; Zabranska and Pokorna 2018). In the last decade, it was pointed out that biogas upgrading can also be implemented by power to gas technology using hydrogenotrophic methanogens that convert the CO$_2$ and externally provided H$_2$ to CH$_4$. However, the water electrolysis can increase the operational and capital cost (Schiebahn et al. 2015; Angenent et al. 2018). As a strategy to reduce the operational cost and to increase the efficiency, many studies directly submerged the cathode into the biomethanation system; this system is called microbial electrosynthesis. However, as reported in recent studies (Jiang et al. 2019; Bajracharya 2020; Prévost et al. 2020) high capital and operating/maintenance costs and particularly the cost of the proton exchange membrane are the key limitations in the industrialization of microbial electrosynthesis. According to recent review (Fu et al. 2020) biological biogas upgrading by CO$_2$-to-CH$_4$ bioconversion depends on an accessible, available, and cost-effective H$_2$. Despite the environmental benefits currently in situ biological biogas upgrading by bioconversion of CO$_2$ into CH$_4$ seems to be more expensive than physicochemical biogas upgrading and more research need to take place for optimization of this process (Fu et al. 2020).

On the other hand, as was shown in this study, using ZVI and anaerobic granular sludge, the biogas can be upgraded to CH$_4$ without the complicate configuration of electrolysis (electrodes, connection with power supply and proton exchange membrane in case of microbial electrosynthesis). The present proof of concept study, indicates that the addition of ZVI to anaerobic granular sludge, apart from biogas upgrading to biomethane, can substantially decrease H$_2$S and NH$_3$, as well. Regarding H$_2$S removal, this is in line with the findings of other studies (Liu et al. 2015; Ruan et al. 2017; Andriamanohiarisoamanana et al. 2018; Wei et al. 2018; Farghali et al. 2020), whereas the current study also found that the presence of ZVI reduced mainly the low mass straight-chain and branched alkanes.

This is the first laboratory study experimentally demonstrated the ex-situ biogas upgrading under these conditions. Briefly, H$_2$ generated by the abiotic anaerobic oxidation of ZVI (Eq. 2) and then is consumed along with soluble CO$_2$ by hydrogenotrophic methanogens for CH$_4$ production (Eq. 1). However, part of the CO$_2$ is precipitated as siderite as shown in Eq. 2) (Menikea et al. 2020, Samanides et al. 2020). In addition, homoacetogens can also utilized H$_2$ and CO$_2$ however they are supressed by hydrogenotrophic methanogens as was found by Vyrides et al. (2018) in a similar system. Based on the aforementioned reactions, the overall equation of this system shown at Eq. 3:

\[
\text{(Section of the equation)}
\]
4Fe(0) + CO₂ + 4HCO₃⁻ + 4H⁺ ↔ 4 FeCO₃ + CH₄ + 2H₂O (Eq. 3)

Clearly, further research will be needed regarding the biogas upgrading in this system under operation at continuous mode, demonstration at a larger scale for longer period, as well as performance and economic comparison with power-to-gas technology. Recent studies (Menikea et al., 2020; Samanides et al., 2020), found that under these conditions the main oxidized product in the outer surface from ZVI is siderite. Siderite can decrease the H₂ released from ZVI (Menikea et al., 2020) and this can hinder the sustainability of this process. Based on this, several approaches can be examined to increase the competitiveness of this process such as: (a) the conversion of siderite to ZVI; however, this is not an easy process and considerable amount of energy need to be consumed, (b) the selective removal of the oxidized layer after ZVI and anaerobic sludge separation can be another process for the sustainability of this process, (c) the formation of nano ZVI from plant extract, however, can be used for reducing the capital cost of the process; although this process can be limited due to the formation of mixture of other iron such as iron oxides of iron hydroxides (Mystrioti et al., 2015). The results of this study pointed out that ZVSI can also be used for biogas upgrading; however, this process is approximately 3 times slower compare with the same process using ZVI. Nevertheless, ZVSI is cheaper than ZVI and both factors need to be considered for larger scale studies.

5. Conclusion

The ex-situ biogas upgrading to biomethane in a system of anaerobic granular sludge and ZVI is exhibited for the first time. The ZVI when is added in an aquatic system with anaerobic granular sludge produces H₂ under anaerobic conditions. Then, the H₂ and CO₂ are utilized by the hydrogenotrophic methanogens to CH₄. The biogas upgrading to biomethane can be achieved in 4 days at a batch system of anaerobic granular sludge, 50 g L⁻¹ ZVI, initial pH 5 and 20 g L⁻¹ NaHCO₃. The presence of ZVSI instead of ZVI resulted in slower rate of biogas, upgrading probably due to slower release of H₂ by ZVSI compared to ZVI. Raw biogas (from a pilot anaerobic digester treated with food waste) was fed in a system of anaerobic sludge and ZVI and VOCs composition was analyzed by HS-SPME-GC-MS over time. The presence of ZVI in anaerobic sludge mainly decreased the low molecular weight straight-chain and branched alkanes, but other chemical categories such as alkenes, aromatic hydrocarbons, terpenes and organic sulfur compounds did not exhibited any particular trend regarding their reduction. In addition, gaseous H₂S and NH₃ measurements were found to be substantially decreased when the anaerobic sludge was exposed to ZVI compared to no decrease in serum bottle free of ZVI.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication
Not applicable.

**Availability of data and materials**

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

**Competing interests**

The authors declare that they have no competing interests.

**Funding**

This work was funded by the Cyprus University of Technology

**Authors Contribution**

**Nikolaos Lytras**: Writing- Original draft preparation, data curation, validation, **Maria Andronikou**: Writing- Original draft preparation, data curation, Validation, **Georgia Chrysanthou**: Data curation, **Marinos Stylianou**: Data curation Writing - Review & Editing, Methodology, **Agapios Agapiou**: Supervision, Writing - Review & Editing, Methodology, **Ioannis Vyrides**: Conceptualization, Methodology, Supervision, Writing - Review & Editing

**Acknowledgments**

I.V. would like to thank Cyprus University of Technology for the internal financial support.

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**Figures**

**Figure 1**

a. CH4 (%) after 6 days for AnGrSl fed-batch with synthetic biogas (60 % CH4 and 40 % CO2) and inoculated with 0, 25, 50, 75 and 100 g L⁻¹ ZVI (initial pH 6). b. Gas composition (%) over time for AnGrSl fed-batch with synthetic biogas (60 % CH4 and 40 % CO2) and inoculated with to 50 and 75 g L⁻¹ ZVI.

**Figure 2**
a CH4 (%) over time for AnGrSl fed-batch with synthetic biogas (60 % CH4 and 40 % CO2) and exposed to 2.6 g L-1 NaHCO3, 20 g L-1 NaHCO3, 35 g L-1 NaHCO3 and 50 g L-1 NaHCO3. b. H2 (%) over time for AnGrSl fed-batch with synthetic biogas (60 % CH4 and 40 % CO2) and exposed to 2.6 g L-1 NaHCO3, 20 g L-1 NaHCO3, 35 g L-1 NaHCO3 and 50 g L-1 NaHCO3.

Figure 3

Gas composition over time for bioreactor inoculated with AnGrSl and 50 g L-1 ZVI. Raw biogas was fed in the bioreactor on days 1, 8 and 28, whereas synthetic was fed only on day 17.

Figure 4

Gas composition (%) over time for AnGrSl fed-batch with synthetic biogas (60 % CH4 and 40 % CO2) and inoculated with 100 and 200 g L-1 ZVSI.
Figure 5

a shows the VOCs that were detected with HS-SPME-GC-MS on day 7 for samples that received raw biogas and were inoculated with AnGrSl. The numbered compounds are: 1= Carbon disulfide, 2= 2-methyl-Pentane, 3= 3-methyl-Pentane, 4= n-Hexane, 5= methyl-Cyclopentane, 6= 2-methyl-Hexane, 7= 3-methyl-Hexane, 8= Heptane, 9= Toluene, 10= alpha – pinene, 11= 1,2,4, trimethyl benzene, 12= D-Limonene, 13= o-Cymene. b shows the VOCs that were detected with HS-SPME-GC-MS on day 7 for samples that received raw biogas and were inoculated with AnGrSl and zero valent iron (ZVI). The numbered compounds are: 1= Pentane, 2= Dimethyl sulfide, 3= 2-methyl-Pentane, 4= 3-methyl-Pentane, 5= n-Hexane, 6= methyl-Cyclopentane, 7= 2-methyl-Hexane, 8= 3-methyl-Hexane, 9= Heptane, 10= 2,6-dimethyl-Octane, 11= Toluene, 12= D-Limonene.