CHEMICAL ENGINEERING TRANSACTIONS
VOL. 106, 2023
A publication of AIDIC
The Italian Association of Chemical Engineering
Online at www.cetjournal.it
Guest Editors: Jeng Shiun Lim, Nor Alafiza Yunus, Peck Loo Kiew, Hon Huin Chin

ISBN 979-12-81206-05-2; **ISSN** 2283-9216
DOI: 10.3303/CET23106079

Effects of Electron Donor Additives to Sewage Sludge on Biomethanation of Gaseous Carbon Dioxide

Gyeongsoo Kim, Kazuyuki Oshita*, Masaki Takaoka

Department of Environmental Engineering, Kyoto University, C-1-3-463, Kyotodaigaku-Katsura, Nishikyo-ku, Kyoto, 615-8540, Japan
oshita.kazuyuki.6e@kyoto-u.ac.jp

This study investigated the effect of concentration on biomethanation in electron donor additives (zero valent iron (ZVI) 10-40 g/L, activated carbon (AC) 1.0- 4.0 g/L and Fe_3O_4 0.25-1.0 g/L) when carbon dioxide (CO_2) is the only carbon source in anaerobic digestion., and the researchers estimated the pathway of CO_2. ZVI had 43-49 % biomethanation of injected CO_2 but was not observed in AC and Fe_3O_4. It has been confirmed that the rate of hydrogen (H_2) production from ZVI depending on its size. Furthermore, when there is a shortage of CO_2, inhibition occurs due to the hydrogen generated from ZVI. The reason for the inhibition of methane (CH_4) production in AC and Fe_3O_4 was found to be due to volatile fatty acids (VFAs) accumulation. The use of additives induces the conversion of CO_2 to VFAs and inorganic carbon, which are necessary for biomethanation. The addition of ZVI increased the growth of hydrogenotrophic methanogens, thereby enabling CH_4 production without VFAs accumulation.

1. Introduction

Anaerobic digestion is used to stabilize a variety of organic wastes, including sewage sludge, animal manure, and food waste (Dong et al., 2019). Biogas from anaerobic digestion is considered a renewable energy alternative to natural gas, and biogas use may contribute to reduced greenhouse emissions (Dong et al., 2022). Raw biogas (40-70 % methane (CH_4) and 30-60 % carbon dioxide (CO_2)) must be upgraded to biomethane (>95 % CH_4) for practical use due to lower CH_4 content (Andronikou et al., 2022). Numerous recent studies have investigated the method for upgrading biogas based on hydrogen (H_2) and CO_2 usage by hydrogenotrophic methanogens by Eq(1) (Angelidaki et al., 2018).

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O \quad \Delta G^0 = -130.7\ \mathrm{kJ/mol} \tag{1}$$

This biochemical conversion of CO_2 is also carried out by homoacetegens and acetoclastic methanogens (Dong et al., 2022). Homoacetegen can use CO_2 and H_2 for acetic acid production (Eq(2)) and acetoclastic methanogens can utilize acetic acid for the generation of CO_2 and CH_4 (Eq(3)) (Vyrides et al., 2018).

$$4H_2 + 2CO_2 \rightarrow CH_3COO^- + H^+ + 2H_2O \quad \Delta G^o = -95\ \mathrm{kJ/mol} \tag{2}$$

$$CH_3COO^- + H^+ \rightarrow CH_4 + CO_2 \quad \Delta G^o = -75.7\ \mathrm{kJ/mol} \tag{3}$$

One of the typical biological methanation technologies is the use of H_2 or electron donors, and many studies have been conducted to generate H_2 and electron donors using additives (Dong et al., 2019). Zero valent Iron (ZVI, Fe^0) was mainly used in the study to generate H_2 and electron donors. ZVI has improved anaerobic digestion with H_2 generation (Eq(4)) and direct interspecies electron transfer (DIET) (Eq(5)) (Dong et al., 2022).

$$2H_2O + \mathrm{Fe} \rightarrow H_2 + Fe^{2+} + 2OH^- \quad \Delta G^o = -5.02\ \mathrm{kJ/mol} \tag{4}$$

$$CO_2 + 4\mathrm{Fe} + 8H^+ \rightarrow CH_4 + 4Fe^{2+} + 2H_2O \quad \Delta G^o = -150.5\ \mathrm{kJ/mol} \tag{5}$$

Paper Received: 31 May 2023; Revised: 1 September 2023; Accepted: 14 September 2023
Please cite this article as: Kim G., Oshita K., Takaoka M., 2023, Effects of Electron Donor Additives to Sewage Sludge on Biomethanation of Gaseous Carbon Dioxide, Chemical Engineering Transactions, 106, 469-474 DOI:10.3303/CET23106079

Activated carbon (AC) and Fe_3O_4 were mainly used as electron donors. AC is an inexpensive material that can significantly increase CH_4 production by accelerating DIET and volatile fatty acids (VFAs) consumption (Li et al.,2019). Fe_3O_4 has the ideal potential to accelerate CH_4 production through the decomposition of complex organic matter. Fe_3O_4 can promote CH_4 production through DIET (Zhao et al., 2018).
Many studies have been conducted to improve the efficiency of anaerobic digestion using additives (Wang et al., 2016). Existing research has predominantly focused on materials that generate H_2, and there is a lack of research on electron donors in the context of CO_2 conversion to CH_4 using a single carbon source (Andronikou et al., 2022). The study focused on investigating the role of electron donors using AC, Fe_3O_4, and ZVI, as electron donors for the biochemical conversion of CO_2 to CH_4 in anaerobic digestion experiments. This study aimed to understand how the electron donors could influence the biochemical process of converting CO_2 to CH_4 and to determine the impact of electron donors on the pathways of CO_2 in biomethanation.

2. Materials and Method

2.1 Anaerobic biomethanation batch test

125 mL serum bottles with a working volume of 50 mL were used for batch lab-scale anaerobic biomethanation experiments. The working volume contains 20 mL of nutrient mineral medium, 20 mL of anaerobic inoculum, and 10 mL of HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) buffer solution. According to a prior study, nutrient mineral media for methanogens was created (Dong et al., 2019). HEPES buffers were used to maintain neutral pH (Dong et al., 2019). In a 1 L batch reactor operating at 37 °C, pH 7.1-7.3, and 150 rpm, anaerobic inoculum was cultivated with anaerobic digested sludge for 2 weeks to remove biodegradable organic matter from the sludge. In every day, generated biogas was removed and the pH was adjusted and purged with nitrogen gas (99.99 %) for 1 min. The concentration of volatile suspended solids (VSS) in the anaerobic inoculum was 5,083 ± 144 mg/L and the initial pH of the liquid including nutrient mineral medium, anaerobic inoculum, and HEPES buffer solution was 7.12 ± 0.01, before running the experiment. After pH measurement, AC (0.2-1 mm, Fujifilm wako pure chemical) concentration of 1, 2, and 4 g/L, Fe_3O_4 (50-100 nm, Sigma Aldrich) concentration of 0.25, 0.5, and 1 g/L, ZVI (<150 µm, Fujifilm wako pure chemical) concentration of 10, 20 and 40 g/L were added. Without adding materials, the control was prepared. After being sealed with silicon septa, all serum bottles underwent a one-minute nitrogen gas (99.99 %) purge. The samples, which contained nutritional mineral medium, anaerobic inoculum, HEPES buffer solution, and additives, were injected with 20 mL of CO_2 gas (99.99 %), and a push-pull method was used to extract the same amount of headspace gas. All serum bottles were placed in a water bath (BT300, Yamato scientific., Japan) at 37 °C and shaken at 140 rpm. Triplicates of each anaerobic batch experiment were carried out. During the experiment, the gas composition was analyzed, and the gas volume was measured using a syringe. After the experiment, the samples were analyzed for pH, total inorganic carbon (TIC), VFAs, and microbial community structure.

2.2 Analytical techniques

A pH meter (HM-31P, DKK-TOA Co., Japan) was used to measure the pH. VSS was measured according to the standard method from American public health association (American public health association, 1998)
The concentrations of CH_4, CO_2, and H_2 of gas samples were analyzed using a Gas Chromatograph (490 Micro GC, Agilent Technologies, USA) equipped with a Thermal Conductivity Detector (TCD). PoraPLOT Q Columns was used with helium as the carrier gas at 40 °C and 190 kPa to detect CO_2. Molsieve 5Å columns was used with argon as the carrier gas at 80 °C and 170 kPa to detect CH_4 and H_2.
Total inorganic carbon (TIC) analysis was conducted by a TOC analyzer (TOC-V_{CSH}, Shimadzu Corp., Japan). 680 °C combustion catalyst oxidation and non-dispersive infra-red (NDIR) detectors method was adopted.
The concentrations of VFAs (Lactic, Formic, Acetic, Propionic, Isobutyric, Isovaleric, Valeric, and Butyric) was analyzed by high-performance liquid chromatography (L-20AD, Shimadzu, Japan) equipped with a CDD-10AVP conductivity detector, a Shimadzu SIL-20AC autosampler, and a CTO-10AC VP column oven. Shim-pack SCR-102H column (300 mm × 8.0 mm × 7 µm) was used with 5 mM perchloric acid rate of 1.0 mL/min at 40 °C. The injection volume was 20 µL. The samples were centrifuged at 3,500 × g for 10 min and filtered with 0.45 µm syringe filters.

2.3 Microbial community structure analysis

For the microbial community structure analysis, the samples that were collected at the finish of the experiment are analyzed using polymerase chain reaction (PCR) analysis. DNA extraction was achieved using an Extrap Soil DNA Kit Plus ver.2 (NIPPON STEEL Eco-Tech Co., Japan). The amount of 16SrRNA genes was measured by the real-time PCR method. The V4-V5 region of the 16SrRNA gene was PCR amplified using the primer set U515F (18mer, 5′-GTGYCAGCMGCCGCGGTA-3′) and 926R (19mer, 5′-CCGYCAATTCMTTT-RAGTT-3′). After purifying the obtained PCR amplification products, the double strand DNA concentration was measured

using the PicoGreen dsDNA Assay Kit (Invitrogen, USA). The concentration of PCR amplification products was sequenced with MiSeq system (Illumina, USA), and about 250 bases were analyzed from each side of PCR amplification products. The obtained array data were interpreted using the quantitative insights into the microbiological ecology (QIIME2) pipeline.

3. Results and discussion

3.1 Anaerobic batch experiment: Effects of additive concentration on CO_2 biomethanation

This experiment aimed to investigate the effect of different additives concentrations on biomethanation of gaseous CO_2. The gas change in the headspace according to the concentrations of the additives is shown in Figure 1.

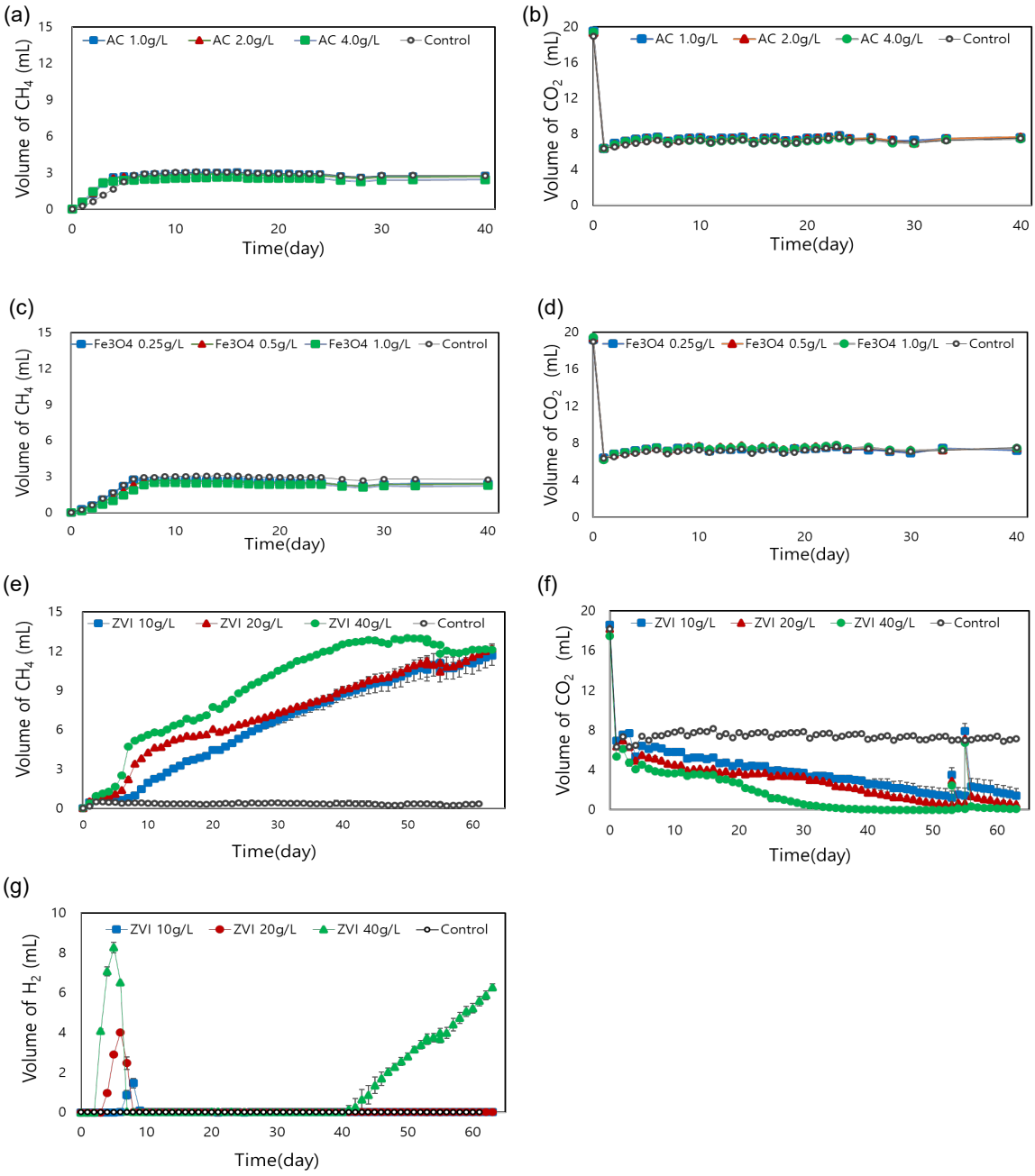

Figure 1: Gas volume of headspace with different additives concentrations: (a) CH4 with the addition of AC, (c) Fe3O4 and (e) ZVI, (b) CO2 with the addition of AC, (d) Fe3O4 and (f) ZVI, (g) H2 with the addition of ZVI

CH_4 production in the case of AC decreased as the AC concentrations increased (Figure 1a). The CO_2 in the case of AC was dissolved in water as in all samples and decreased rapidly, but there was no significant change with a difference of ±1.22 % from the control thereafter (Figure 1b). Fe_3O_4 had the similar tendency as CH_4 and CO_2 in the case of AC. These results suggested that CH_4 production was inhibited by all concentrations of AC and Fe_3O_4. In the case of AC, the time to reach the maximum CH_4 production was faster than control, and this acceleration indicates that the addition of AC does not inhibit microorganisms (Li et al., 2019). Fe_3O_4 can inhibit CH_4 production by using electrons necessary for CH_4 production (Zhao et al., 2018).
Figure 1e shows the CH_4 changes of the ZVI. CH_4 increased slowly for 6 d and increased sharply until 10 d, with the highest production rates of 0.69 and 0.93 mL/day at 20 and 40 gZVI/L due to H_2 generation. Since then, it has been investigated that 20 gZVI/L produced almost constant CH_4 production rate. 40 gZVI/L accelerated CH_4 production from the 22 d but gradually decreased due to decrease in CO_2. Due to the lack of CO_2 in 40 gZVI/L, 2 mL and 5 mL of CO_2 were injected into all ZVI samples on the 53 and 55 d, but CH_4 was not produced at 40 gZVI/L. CH_4 production at 10 gZVI/L began in earnest on the 8 d and was relatively constant at 0.19 mL/day until the end. Initially, CO_2 of the ZVI showed a similar rapid dissolution of CO_2 as AC and Fe_3O_4 (Figure 1f). After that, the CO_2 reduction rate had the opposite result according to the increase in CH_4, and the CO_2 increased on 53 and 55 d when CO_2 was additionally injected but decreased sharply again the next day. H_2 increased in proportion to the ZVI concentration, and all reacted within 10 d. After 10 d, H_2 has not been produced, but it has been produced at 40 gZVI/L since CO_2 became zero after 40 d. To confirm the ZVI surface corrosion according to the size of the ZVI, the ZVI was classified by size before and after the experiment (Figure 2). It decreased by 0.035 % in 70-100 μm, 2.83 % in 50-70μm and 7.16 % in 32-50 μm but increased by 10.025 % in 20-32 μm. This indicates that the smaller the ZVI size, the faster the corrosion occurs, and the reason for H_2 generation after 40 d in (Figure 1g). It is assumed that CH4 was not produced at 40 gZVI/L after 40 d due to ammonia production caused by H_2 and precipitation with Fe^{2+} dissolved in water before CH_4 production (Rocamora et al., 2023).

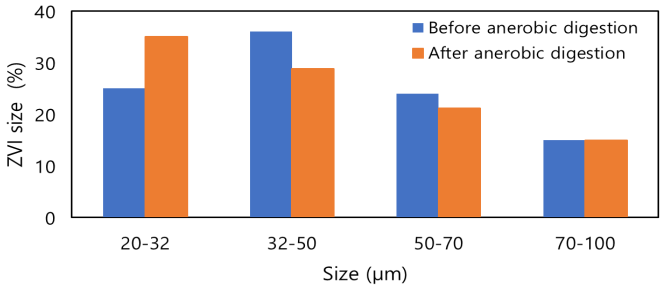

Figure 2: Changes in ZVI size before and after anaerobic digestion

3.2 Effects of additives concentration on the VFAs concentrations and pathway of CO_2

The TIC and VFAs concentration were analyzed at the finish of the experiment, and Table 1 displays the TIC and VFAs concentrations as well as CO_2 conversion rates. Only propionic acid and isobutyric acid were identified as a result of VFAs analysis. The pH of control, AC, and Fe_3O_4 was in a narrow range of 7.29-7.34, slightly increased from the initial 7.12. The pH of the ZVI was 7.68-7.77, which was increased by the reaction of H_2. The TIC and VFAs concentrations of the controls were analyzed at 7.39 mg/L and 35 mg/L. The higher the concentration of ZVI, the lower the concentration of TIC and VFAs, indicating that biomethanation progresses rapidly at high concentrations of ZVI. Considering that AC and Fe_3O_4 have higher VFAs concentration than control and ZVI at all concentrations, VFAs are accumulating. The accumulation of VFAs caused microbial inhibition in AC and Fe_3O_4, leading to the inhibition of CO_2 use and CH_4 production (Zhang et al., 2018). Additional AC addition is considered because a study investigated that adding more than 5 g/L of AC is effective in preventing VFAs inhibition (Johnravindar et al., 2020). Electron exchange by electron generated in AC and Fe_3O_4 induces CO_2 availability, resulting in higher TIC and VFAs concentrations than control (Vyrides et al., 2018).
CO_2 excluding CH_4, VFAs, and TIC was calculated as unidentified CO_2. Unidentified CO_2 can be caused by the production of by-products such as ethanol in control, precipitation of Fe^{2+} in ZVI and Fe_3O_4, and CO_2 adsorption in AC (Wu et al., 2021). Unidentified CO_2 increased with lower concentrations of CH_4 production, TIC and VFAs concentrations. In Control, CO_2 loss was the highest at 84.51 %, and in the case of ZVI, high CH_4 production reduced unidentified CO_2 to 51.21-52.68 %. ZVI was optimized with 4.8-8.1 % lower CO_2 to VFAs and 10 % higher CO_2 to CH_4 compared to a previous study (Dong et al., 2022). Subsequently, it had unidentified CO_2 of 71.58-77.37 % in AC with high TIC and VFAs concentrations, and Fe_3O_4 had unidentified CO_2 of 76.93-80.69 %,

the highest except for control. Unidentified CO_2 was lower than the control in all conditions, suggesting that the use of additives induces the conversion of CO_2 to VFAs and inorganic carbon, which are necessary for biomethanation.

Table 1: TIC and VFAs concentration and CO_2 conversion ratio

	Control	ZVI (g/L)			AC (g/L)			Fe_3O_4 (g/L)		
	-	10	20	40	1	2	4	0.25	0.5	1
VFAs concentration (mg/L)	38.5±1	19.5±13	7.5±0.7	0	39.5±9	53.5±4	49.5±1	46.5±8	50.5±28	68.5±5
TIC (mg/L)	7.39	16.87	15.00	4.47	34.02	23.76	42.92	5.35	5.29	30.14
CO_2 to CH_4 (%)	0	43.72	44.62	47.91	0	0	0	0	0	0
CO_2 to VFAs (%)	13.9	3.43	1.27	0	15.42	21.27	19.34	18.18	19.83	16.72
Dissolved CO_2 (%)	1.59	1.64	1.43	0.43	7.21	5.11	9.08	1.13	1.12	6.35
Unidentified CO_2 (%)	84.51	51.21	52.68	51.66	77.37	73.62	71.58	80.69	79.05	76.93

3.3 Microbial community structure analysis

PCR analysis was conducted to investigate the effect of ZVI addition on microbial change (Figure 3). Various groups of methanogens were found depending on the type, such as hydrogenotrophic methanogens (Methanobrevibacter, Methanobacterium, Methanoculleus, and Methanolinea), acetoclastic methanogens (Methanosaeta and Methanosarcina) and methyltropic methanogens (Candidatus Methanofastidiosum and Methanomassiliicoccus) were found. The addition of ZVI had a positive effect on the diffusion of hydrogenotrophic and methyltropic methanogens and acetoclastic methanogens decreased accordingly.

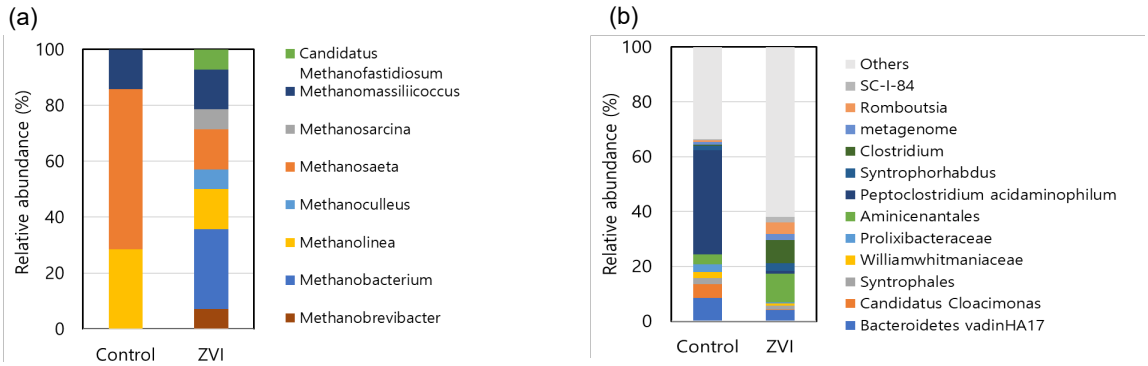

Figure 3: The microbial community in control and 40 gZVI/L, the amounts of microbial genus of (a) archaea and (b) bacteria

Control had a simpler classification than ZVI, dominated by acetoclastic methanogens, and on the contrary, ZVI produced a variety of archaea and changed its dominant structure from acetoclastic methanogens to hydrogenotrophic methanogens. In the control group, Methanosaeta was dominant, but since Methanosaeta can only utilize acetic acid, CH_4 was not produced in the control group where only propionic and isobutyric acid were present (Mori et al., 2012). In ZVI, Methanobacterium was dominant, and this can convert propionic acid into biogas (Han et al., 2020). In bacteria, Peptoclostridium acidaminophilum was dominant in control and clostridium and aminicenantales were dominant in ZVI. Clostridium is one of the typical genera contained in homoacetegens, which can utilize H_2 and CO_2 to produce VFAs (Dong et al., 2022). Aminicenantales is a functional bacterial product associated with hydrolysis and acidification and has the potential to decompose inhibitory compounds (Fan et al., 2022). Unlike previous studies where acetic acid was the predominant VFAs, the presence of propionic and isobutyric acids led to the proliferation of Methanobacterium rather than Methanolinea (Andronikou et al., 2022). Additionally, various archaea species were observed to grow instead of being limited to one or two species (Dong et al., 2022). The addition of ZVI enabled the degradation of various VFAs by growing various archaea, resulting in the biomethanation of CO_2.

4. Conclusion

This study centered on exploring the function of electron donors in the biochemical conversion of CO_2 to CH_4. This study reconfirmed the biomethanation of gaseous CO_2 through ZVI, but the addition of AC and Fe_3O_4 did

not lead to biomethane generation due to the accumulation of VFAs. The addition of AC and Fe_3O_4 increased the concentrations of VFAs and TIC, both of which are essential for biomethanation. This serves as evidence that the addition of AC and Fe_3O_4 supports the potential for biomethanation. The addition of ZVI facilitated the growth of various microbial species, resulting in the production of biomethane. The study found that ZVI exhibited varying H_2 generation rates depending on its size, and under conditions of CO_2 deficiency, inhibition occurred due to the H_2 produced by ZVI. Maintaining adequate CO_2 supply is essential to prevent this inhibition caused by H_2 generated from ZVI. While this study demonstrated the potential of electron donors for the biomethanation of CO_2, a significant portion of the CO_2 remains unidentified for, indicating the need for broader analyses and in-depth investigations to ascertain the pathways of CO_2.

References

American Public Health Association., 1998, Standard methods for the examination of water and wastewater: Washington. DC, American Public Health Association, American Water Works Association, and Water Environment Federation, 3-37.

Andronikou M., Adamou V., Koutsokeras L., Constantinides G., Vyrides I., 2022, Magnesium ribbon and anaerobic granular sludge for conversion of CO_2 to CH_4 or biogas upgrading, Chemical Engineering Journal, 435, 134888.

Angelidaki I., Treu L., Tsapekos P., Luo G., Campanaro S., Wenzel H., Kougias P.G., 2018, Biogas upgrading and utilization: Current status and perspectives. Biotechnology Advances, 36(2), 452-466.

Dong D., Choi O.K., Lee J.W., 2022, Influence of the continuous addition of zero valent iron (ZVI) and nano-scaled zero valent iron (nZVI) on the anaerobic biomethanation of carbon dioxide, Chemical Engineering Journal, 430, 132233.

Dong D., Aleta P., Zhao X., Choi O.K., Kim S., Lee J.W., 2019, Effects of nanoscale zero valent iron (nZVI) concentration on the biochemical conversion of gaseous carbon dioxide (CO_2) into methane (CH_4). Bioresource Technology, 275, 314-320.

Fan Q., Fan X., Fu P., Sun Y., Li Y., Long S., Guo T., Zheng L., Yang K., Hua D., 2022, Microbial community evolution, interaction, and functional genes prediction during anaerobic digestion in the presence of refractory organics. Journal of Environmental Chemical Engineering, 10(3), 107789.

Fu L., Ren Z., Si W., Ma Q., Huang W., Liao K., Huang Z., Wang Y., Li J., Xu P., 2022, Research progress on CO2 capture and utilization technology. Journal of CO_2 Utilization, 66, 102260.

Han Y., Green H., Tao W., 2020, Reversibility of propionic acid inhibition to anaerobic digestion: inhibition kinetics and microbial mechanism. Chemosphere, 255, 126840.

Johnravindar D., Liang B., Fu R., Luo G., Meruvu H., Yang S., Yuan B., Fei Q., 2020, Supplementing granular activated carbon for enhanced methane production in anaerobic co-digestion of post-consumer substrates. Biomass and Bioenergy, 136, 105543.

Li D., Song L., Fang H., Li P., Teng Y., Li Y. Y., Liu R., Niu Q., 2019, Accelerated bio-methane production rate in thermophilic digestion of cardboard with appropriate biochar: dose-response kinetic assays, hybrid synergistic mechanism, and microbial networks analysis. Bioresource Technology, 290, 121782.

Menikea K. K., Kyprianou A., Samanides C. G., Georgiou S. G., Koutsokeras L., Constantinides G., Vyrides I., 2020, Anaerobic granular sludge and zero valent scrap iron (ZVSI) pre-treated with green tea as a sustainable system for conversion of CO_2 to CH_4. Journal of Cleaner Production, 268, 121860.

Mori K., Iino T., Suzuki K. I., Yamaguchi K., Kamagata Y., 2012, Aceticlastic and NaCl-requiring methanogen “Methanosaeta pelagica” sp. nov., isolated from marine tidal flat sediment. Applied and Environmental Microbiology, 78(9), 3416-3423.

Rocamora I., Wagland S. T., Hassard F., Villa R., Peces M., Simpson E. W., Fernández O., Bajón-Fernández Y., 2023, Inhibitory mechanisms on dry anaerobic digestion: Ammonia, hydrogen and propionic acid relationship. Waste Management, 161, 29-42.

Vyrides I., Andronikou M., Kyprianou A., Modic A., Filippeti A., Yiakoumis C., Samanides C. G., 2018, CO_2 conversion to CH_4 using Zero Valent Iron (ZVI) and anaerobic granular sludge: Optimum batch conditions and microbial pathways. Journal of CO_2 Utilization, 27, 415-422.

Wang T., Zhang D., Dai L., Chen Y., Dai X., 2016, Effects of metal nanoparticles on methane production from waste-activated sludge and microorganism community shift in anaerobic granular sludge. Scientific Reports, 6(1), 25857.

Wu L., Wei W., Song L., Woźniak-Karczewska M., Chrzanowski Ł., Ni B. J., 2021, Upgrading biogas produced in anaerobic digestion: Biological removal and bioconversion of CO_2 in biogas. Renewable and Sustainable Energy Reviews, 150, 111448.

Zhao Z., Zhang Y., Li Y., Quan X., Zhao Z., 2018, Comparing the mechanisms of ZVI and Fe_3O_4 for promoting waste-activated sludge digestion. Water Research, 144, 126-133.