Journal of Biotechnology Research
ISSN(e): 2413-3256, ISSN(p): 2413-8878
Vol. 2, No. 8, pp: 59-67, 2016
URL: http://arpgweb.com/?ic=journal&journal=16&info=aims

The Effect of Different Zero-Valent Iron Sources on Biogas Production from Waste Sludge Anaerobic Digestion

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Abstract: Anaerobic sludge digestion is still one of the most widely sustainable applications which are used for sludge reduction and energy production. In the present study, the effect of different Zero-Valent Iron (ZVI) sources on enhancing biogas production from anaerobic sludge digestion was evaluated. Three different ZVI sources were examined, namely, ZVI powder, ZVI non-rusty scrap, and ZVI rusty scrap. The results showed that with increasing ZVI powder concentrations from 0 g/l (Control) to 15g/l, CH_4 production increased from 1.27 M CH_4/gVSS (for the control) to 2.3 M CH_4/gVSS (i.e. 82% increase). While with using 15g/l ZVI scrap (either non-rusty or rusty), CH_4 production increased up to 2.45 and 2.7 M CH_4/gVSS respectively), which Indicates the possibility of using other cheap ZVI alternatives such as Iron Scrap. The results have confirmed, by using methyl fluoride (CH_3F), that supplying anaerobic digestion process with ZVI stimulates the activity of hydrogenotrophic methanogens rather than acetolastic methanogens.

Keywords: Anaerobic digestion; Biogas production; Zero-valent iron (ZVI); Hydrogenotrophic methanogens; Scrap iron.

1. Introduction

Waste Activated Sludge produced from biological wastewater treatment processes has increased enormously in the recent years due to population growth and the continuous introduction of new wastewater treatment plants. This increase in waste activated sludge production has a potential negative impact due to its potential environmental risks, and high disposal cost is raising questions on how to develop and improve sustainable approaches for better sludge management. Anaerobic digestion is the most commonly used technique for waste activated sludge treatment because it is considered as the most energy efficient technology for waste sludge stabilization and produces a renewable bioenergy resource in the form of methane [1-3]. Anaerobic sludge digestion consists of three stages [4], of them, hydrolysis and/or poor biochemical methane production is/are known as the rate-limiting step in the anaerobic sludge digestion [3, 5].

There are a number of strategies have been developed to enhance methane production from anaerobic sludge digestion, such as thermal, chemical, and mechanical methods [5-11]. However, most of these technologies are cost effective in terms of operation and chemical additives. Therefore, alternative cost-efficient methods are desirable.

Zero-valent iron (ZVI), a cheap reducing agent, has been widely used in wastewater pretreatment, groundwater purification and soil remediation [12, 13]. Recent researches have found out that ZVI addition to biological wastewater treatment could significantly improve chemical oxygen demand (COD) removal by ca. 25% [14, 15]. ZVI may decline the oxidation-reduction potential (ORP) when added into anaerobic systems, enabling to create a more favorable environment for anaerobic biological processes [16]. In some previous works, adding ZVI in an anaerobic digestion of waste sludge was confirmed to enhance methane production [17-19].

In the current work, based on previous works, the effect of different cheap ZVI sources alternatives were examined for enhancing biogas production during anaerobic sludge digestion process. Three ZVI sources were assessed: ZVI powder, ZVI scrap (non-rusty and rusty). Also, a deeper assessment was conducted to determine the enhancement mechanism.

2. Methods

2.1. Sludge Characteristics

The sludge used in this study was collected from secondary sedimentation tank of municipal wastewater treatment. The sludge was concentrated by settling for 24 h and stored at 4 °C before use. In order to evaluate the effect of ZVI on sludge digestion, no pretreatment was conducted. The characteristics of the waste activated sludge is listed in Table 1.

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Table-1. Activated Sludge Waste Characteristics

Parameters	**Values**
pH	7.04 ± 0.3
Total suspended solids (TSS - mg/l)	13530 ± 927
Volatile suspended solids (VSS - mg/l)	8237 ± 371
Total protein (TP - mg/l)	4764 ± 302
Total polysaccharides (TS - mg/l)	1159 ± 78
Total chemical oxygen demand (TCOD - mg/l)	14684 ± 1186
Soluble chemical oxygen demand (SCOD - mg/l)	574 62

2.2. Procedure

2.2.1. Zero-Valent Iron Forms

Three different sources of zero-valent iron were used; ZVI powder (diameter of 0.2 mm, BET surface area of 0.05m^2/ g, purity >98%), non-rusty scrap iron and rusty scrap iron (from machinery workshop each with an average 10mm*4mm*0.5mm in dimensions). The non-rusty scrap was obtained by pretreating the rusty scrap with acids to remove the corrosive layer.

2.2.2. Batch Experiments

Methane production from the sludge digestion using different ZVI forms and concentrations was evaluated. Different types of ZVI addition were assessed using Biochemical Methane Potential (BMP) test [17]. The Activated waste sludge was inoculated with a ratio of 8:1 with inoculum sludge collected from an anaerobic digester running in our laboratory. Two types of batch experiments were carried out (Table 2). In each test, waste activated sludge, ZVI, and the inoculum was added to serum bottles with a working volume of 250 mL. Each batch system contained 17.1 g/l MLVSS inoculum sludge and with 1.5 g/l COD. Oxygen removal from the headspace was carried out by exchanging it with nitrogen gas for 10 min, and all bottles were capped with rubber stoppers. All anaerobic experiments were performed at 35±1^{o}C for 20 days. 1.3% methyl fluoride (CH_3F) (99%; Karlsruhe, Germany, Abcr Gmbh@) was added as an inhibitor to elucidate the effect of ZVI on acetolastic methanogens, (see table 2)

Table-2. experimental set-ups

Task	**Experimental set-ups**	**Measurements**
1. Effect of ZVI Powder on Biogas production	- Sludge+innoculum+0 g/l ZVI Powder (Control) - Sludge+innoculum+1 g/l ZVI Powder - Sludge+innoculum+4 g/l ZVI Powder - Sludge+innoculum+15 g/l ZVI Powder	CH_4, CO_2, VFAs, Fe^{2+}
2. Effect of ZVI Scrap (non-rusty and rusty) on Biogas production	- Sludge+Medium+0 g/l ZVI (Control) - Sludge+innoculum+15 g/l ZVI Powder - Sludge+innoculum+15 g/l ZVI non-rusty scrap - Sludge+innoculum+15 g/l ZVI rusty scrap	CH_4, CO_2, VFAs
3. Effect of ZVI on methanogens	- Sludge+Medium+ g/l ZVI +CH_3F (Control) - Sludge+medium+15 g/l ZVI Powder +CH_3F - Sludge+medium+15 g/l ZVI non-rusty scrap +CH_3F - Sludge+medium+15 g/l ZVI rusty scrap +CH_3F	CH_4, CO_2, VFAs

2.3. Analytical Procedures

Sludge samples were analyzed for total suspended solid (TSS), volatile suspended solids (VSS), total protein and total polysaccharide. Then the samples were centrifuged at 8000 rpm for 10 min and immediately filtered through a cellulose membrane with pore size of 0.45 mm for analysis of soluble COD (SCOD), soluble protein, soluble polysaccharide, and VFAs. TSS, VSS, and SCOD were determined according to Standard Methods for the Examination of Water and Wastewater [20]. Protein and polysaccharides were measured calorimetrically following DuBois, *et al.* [21] and Lowry, *et al.* [22]. Fe^{2+} was analyzed by an adaptation of the ferrozine technique [23].

3. Results and Discussions

3.1. Effect on Biogas Production Process

3.1.1. Effect on CH4 Production

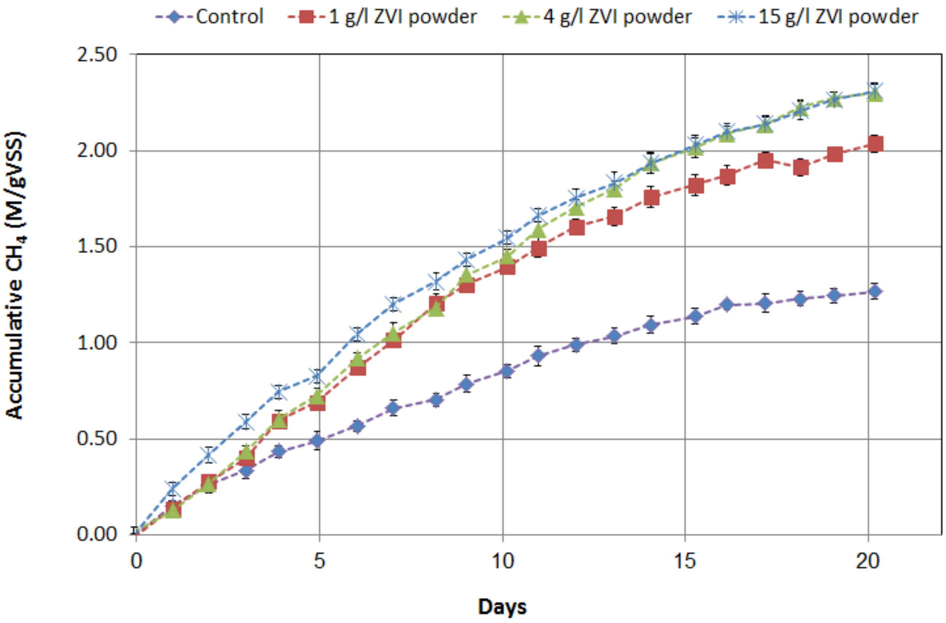
In order to evaluate the effect of ZVI on anaerobic sludge digestion, three different type of ZVI were used, ZVI powder, ZVI non-rusty and ZVI rusty scrap. Fig. 1 shows the methane accumulation profile for anaerobic batch systems supplemented with different ZVI powder concentrations during the whole experimental phase (20 days). As the figure shows, methane production in the control increased slightly up to 1.27 M CH_4/gVSS with calculated CH_4 production rate of 0.063 M CH_4/gVSS/d.

With increasing ZVI powder dose to 1 g/l ZVI powder, methane started to accumulate steadily to reach 2.04 M CH_4/gVSS at the 20th day of operation, with a CH_4 production rate of 0.102 M CH_4/gVSS/d (i.e. 61% higher than the control). With increasing ZVI powder to higher doses (4 and 15 g ZVI powder/l), CH_4 production was higher by 81% and 82% respectively. These results would indicate that CH_4 production increases with increasing ZVI dose which is in accordance with previous studies [17, 18, 24]. In general, ZVI could enhance the methane production based on three mechanisms. The first mechanisms is the is related to the enhanced production of acetate in the presence of ZVI, which providing a suitable substrate for methanogens (i.e. enhancing the activity of methanogenic bacteria) [25-27]. The second mechanisms is the ability of ZVI to serve as an electron donor for reducing CO_2 into CH_4 through methanogenesis according to the following equation [17].

$$CO_2 + 4Fe^0 + 8H^+ \rightarrow CH_4 + 4Fe^{2+} + 2H_2O \qquad (1)$$

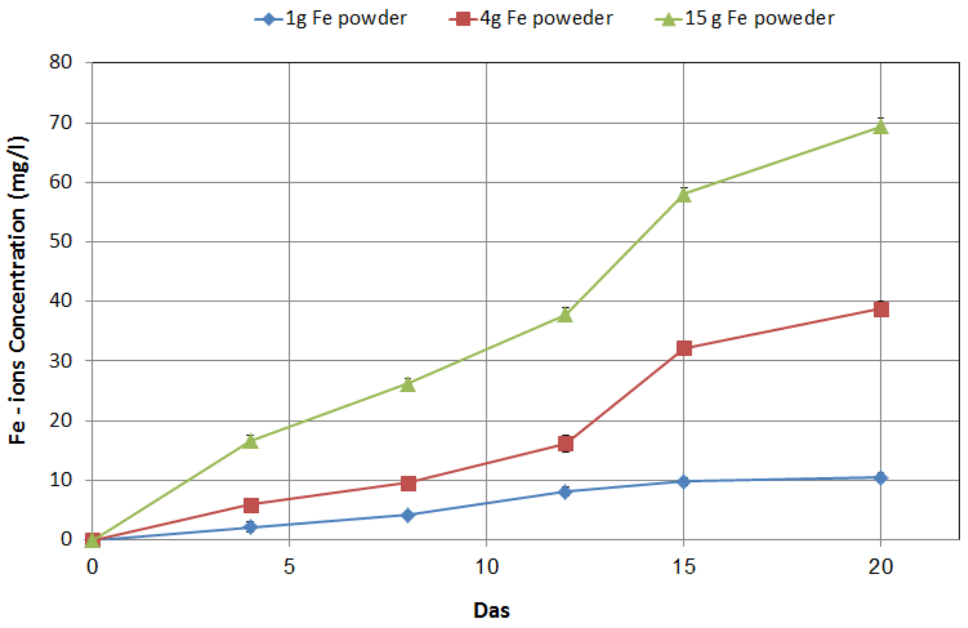
Fig. 2 shows the Fe-ions accumulation in the liquid phase indicating Fe-ions avaibality for further reduction process under anaerobic conditions verifying the second mechanism.

Fig-1. CH_4 production in anaerobic batch systems supplied with different ZVI powder concentrations.



The third mechanisms is through increasing volatile solids destruction during anaerobic digestion [28, 29]. Furthermore, in order to compare the efficiency of ZVI scrap usage as an alternative to enhance anaerobic sludge digestion and biogas production, the same concentrations of ZVI powder and ZVI scrap were tested. Fig. 3 shows the comparison between CH_4 production profile for anaerobic batch systems supplemented with ZVI non-rusty and rusty scrap on one hand, and the control (no ZVI were added) and ZVI powder at the same concentration on the other hand. According to this figure, there was a slight difference between CH_4 production using 15g/l ZVI powder, 15g/l ZVI non-rusty scrap, and 15g/l ZVI rusty scrap. Although, ZVI rusty scrap showed a slight increase in CH_4 accumulation, which was in accordance with Liu, *et al.* [18], who referred this increase to the fact that Fe^{3+} oxides on the rusty iron scrap surface could induce ferric iron reduction which enhances the degradation of complex substrates such as waste activated sludge [30]. All ZVI supplements showed higher CH_4 production compared with the control (no ZVI added).

Fig-2. Fe-ions accumulation in anaerobic batch systems supplied with different ZVI powder concentrations.



CO_2 accumulation profile for different ZVI powder doses is shown in Fig.4. According to this figure, CO_2 production for 4 and 15g/l ZVI powder reached its maximum accumulation within 2 days of operation while for the control and 1 g/l ZVI powder at CO_2 reached its maximum at the 4th day of operation. Afterwords, CO_2 production, decreased in all ZVI treatment to end up with 0.32, 0.15 and 0.23M CO_2/gVSS for 1, 4 and 15g/l ZVI powder respectively. Compared with 0.46 M CO_2/gVSS, indicating that with increasing ZVI powder dose up to 4 g/l ZVI powder, the accumulation rate of CO_2 decrease after the 4th day, while with further dose increase up to 15/g ZVI powder, CO_2 decrease rate slowed down. Similar results also were obtained with anaerobic batch systems supplemented with ZVI non-rusty and ZVI rusty scrap (Fig. 5). These results would indicate that with increasing ZVI dose, CO_2 consumption increase in the system. According to Feng, *et al.* [17], the lower CO_2 production with increasing ZVI concentration is related to the balance between the accelerated CO_2 production resulted from sludge hydrolysis and acidification process and the enhanced CO_2 consumption by hydrogen-utilizing bacteria according to the following equation:

$$4H_2+CO_2 \rightarrow CH_4+2H_2O \qquad (2)$$

Fig-3. CH_4 production in anaerobic batch systems supplied with different ZVI Scrap concentrations.

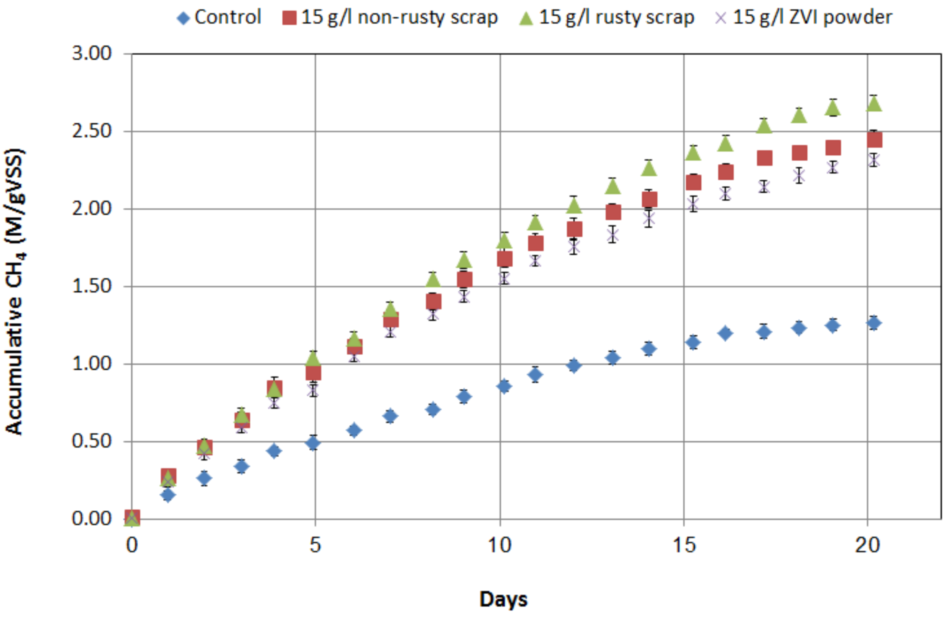


Fig-4. CO_2 production profile in anaerobic batch systems supplied with different ZVI powder concentrations.

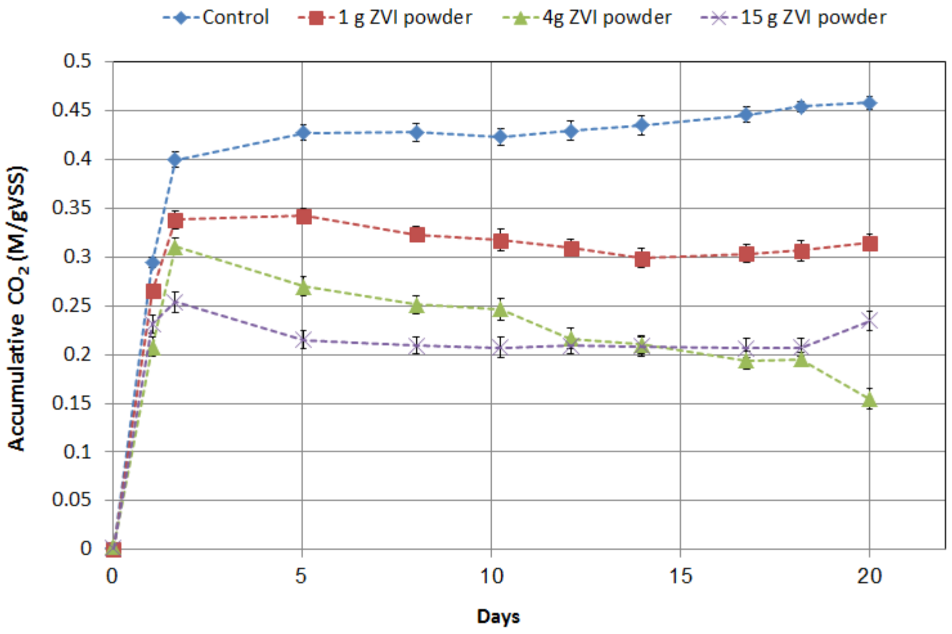
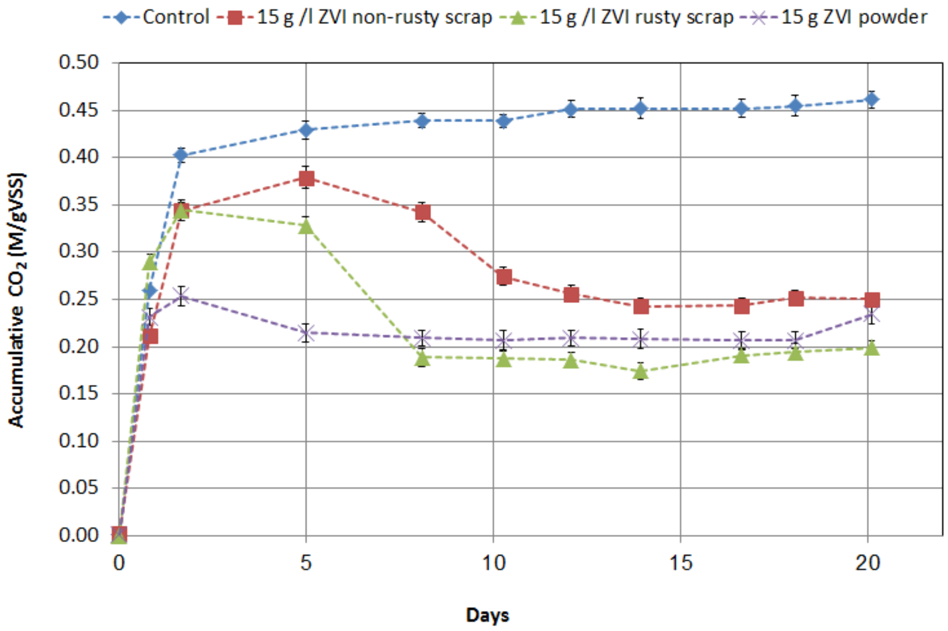


Fig-5. CO_2 production profile in anaerobic batch systems supplied with different ZVI sources at 15 g/l concentration.



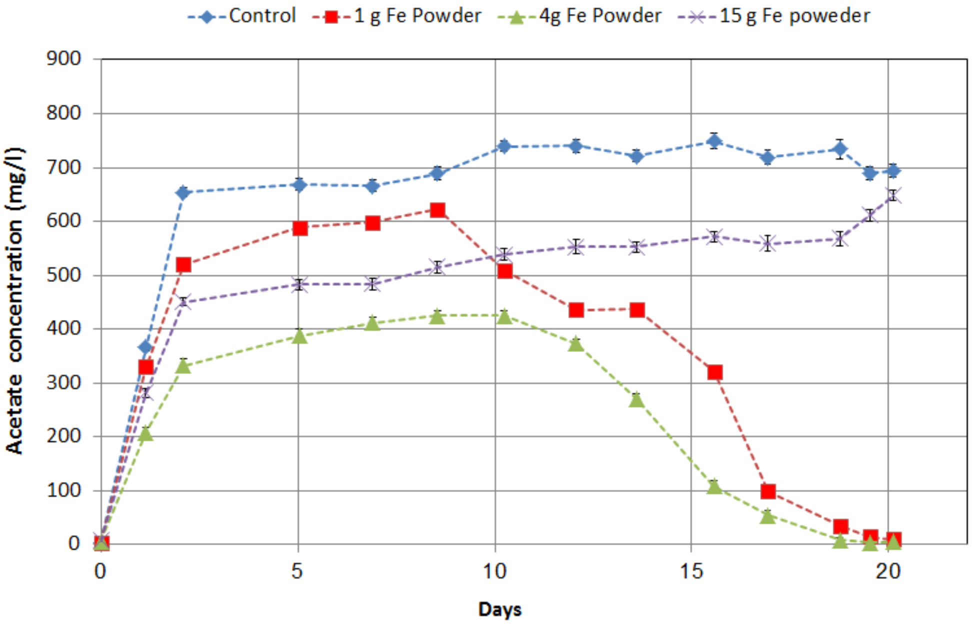
pH values were also monitored during the experiment since the pH has a significant effect on anaerobic digestion process and specifically methanogenesis. At the beginning, pH was adjusted for all anaerobic batch set-ups at 7.3±0.1 using 0.1M HCl. After running the experimental set-up to assess the effect of ZVI on biogas production, the pH values recorded were 6.4±0.3, 7.6±0.1, 8.2±0.1 and 8.3±0.2 for 0 (control), 1, 4 and 15 g/l ZVI powder respectively.

pH observed in anaerobic systems supplied with 15g/l ZVI powder was lower than that of 4g/l. The pH increase pattern was in correlation with the ZVI powder doses reflecting a positive relationship between pH and Fe^{+2} concentrations. This can be related to H-ions consumption during methanogenesis for CO_2 reduction to CH_4 according to equation (2) [31].

3.1.2. Effect of ZVMn on Volatile Fatty Acids (VFAs) accumulation:

Acetate and propionate concentrations in the liquid phase were monitored during the whole experimental phase. Acetate accumulated rapidly in all batch systems in the first 5 days of operation, while for propionate in the 6th days. According to Fig. 6, acetate accumulation varied with ZVI powder concentrations. Acetate concentration in the control reached 739 mg/l by the 7th day and remained steady around this value until the end of the experiment. Moreover, with increasing ZVI dose to 1 and 4 g/l ZVI powder concentration, acetate concentration started to decrease after reaching its maximum concentration (622.6 and 424.8 respectively) at the 9th day of operation. At higher ZVI concentration (15 g/l ZVI powder), acetate concentration continued to increase in the system to reach 648.2 mg/l at day 20. This would indicate that with increasing ZVI powder dose up to 4 g/l ZVI powder, acetate consumption by acetolastic methanogens increases (98 and 99% respectively compared to the control), while further increase up to 15g/l ZVI powder resulted in a reduction in acetate consumption. This was in agreement with the previous study which showed that with the addition of ZVI acetate production increased by 20 % in sucrose anaerobic digestion [32].

Fig-6. Acetate accumulation profile in anaerobic batch systems supplied with different ZVI powder concentration.



Also, for propionate, according to fig. 7, increasing ZVI dose to concentrations higher than 1 g/l ZVI powder resulted in a decrease in propionate accumulation profile by 42 and 25.5% compared with the control. According to Meng, *et al.* [33], reported that propionate con- version rate increased from 43 to 77% to 67 to 89% by ZVI addition. It might be one reason for the increase of acetate and decrease of propionate, which might provide a favorable substrate form for methanogenesis.

Moreover, for 15 g/l ZVI non-rusty and rusty scrap, both acetate and propionate concentrations were similar to what was observed with 15 g/l ZVI powder indicating that there was no significant difference between the three ZVI types regarding VFA's production.

3.1.3. ZVI Enhances Hydrogenotrophic Methanogens

According to Demirel and Scherer [34], 25 to 60% of the degraded organic matter is converted into CO_2 during acetogenesis and acetoclastic methanogenesis. Only 30% of the CO_2 produced during acetogenesis and acetoclastic methanogenesis can be reduced to CH_4 via hydrogenotrophic methanogens using H_2 as an electron donor. This is mainly due to the limited H_2 production during the anaerobic digestion process [35]. In-situ microbiological metals corrosion can supply hydrogenotrophic methanogenesis process with H_2 and enhances the methane production [36]. In order To examine this hypotheses which assumes that hydrogenotrophic CH_4 production can be improved through the consumption of H_2 generated from manganese corrosion, methyl fluoride (CH_3F), an acetolastic methanogens inhibitor was used to block acetolastic CH_4 generation [19, 37, 38].

Fig. 8 shows CH_4 production comparison between CH_3F-added batch systems, as an acetolastic inhibitor, to the digestion medium and batch systems with no CH_3F addition. CH_4 production was reduced in all treatments supplied with CH_3F, which indicates the inhibition of acetolastic methanogenesis. For the control set, where only CH_3F was added without ZVI supplements, CH_4 production was reduced to more than 63% compared to control (with no CH_3F addition).

Fig-7. Propionate accumulation profile in anaerobic batch systems supplied with different ZVI powder concentration.

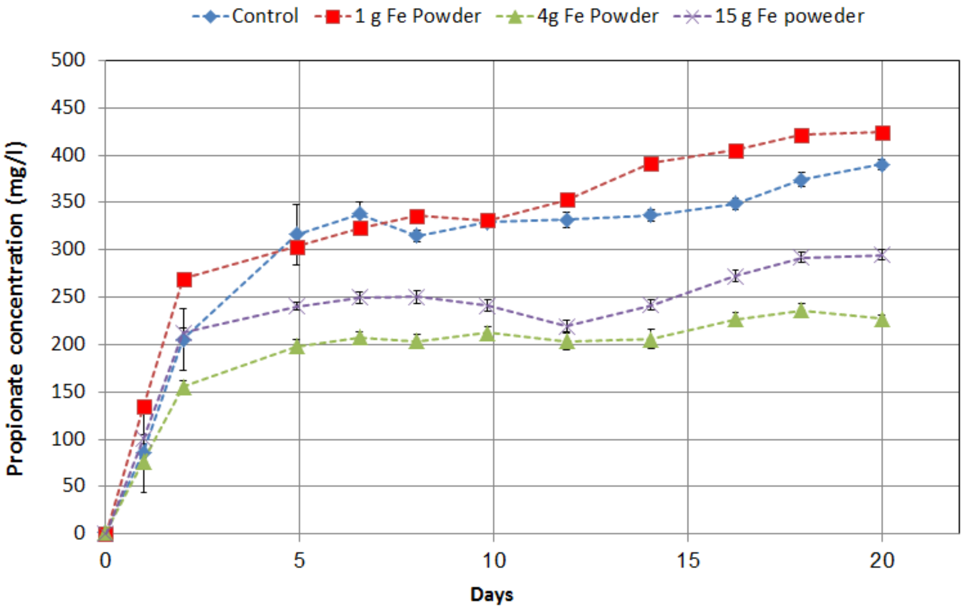
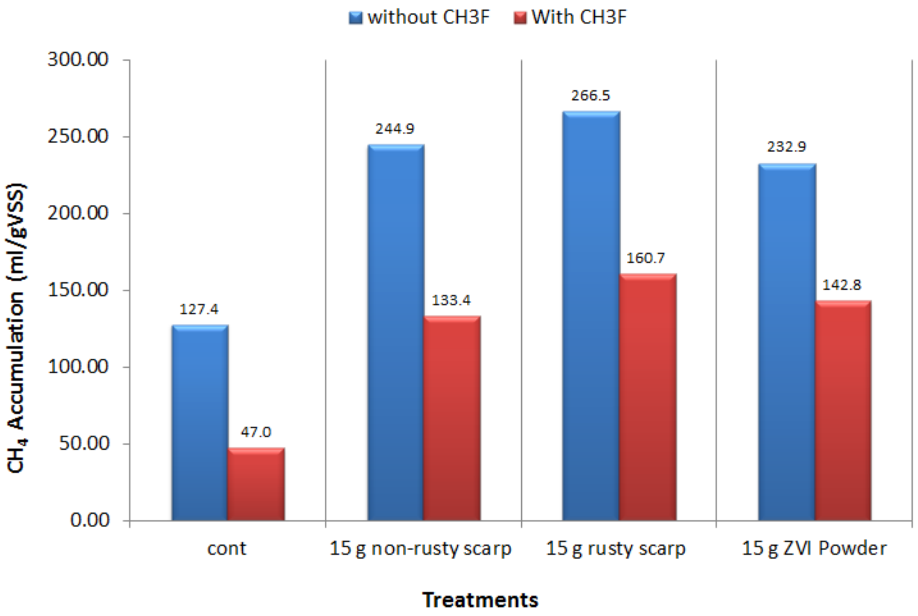


Fig-8. The effect of CH_3F on CH_4 production with different ZVI sources.



This would suggest that in the absence of ZVI source, 63% of CH_4 was produced through acetolastic methanogenesis pathway and only 36% through hydrogenotrophic methanogenesis. However, adding ZVI improved the hydrogenotrophic methanogenesis contribution to the overall CH_4 production. Adding 1, 4 and 15 g/l of ZVI powder to CH_3F treated batch sets, increased the contribution of hydrogenotrophic methanogenesis to 54.5, 60.3 and 61.3% respectively. Accordingly, these results would suggest that ZVI enhances hydrogenotrophic methanogens rather than acetolastic methanogens and this increase in hydrogenotrophic methanogens activity was mainly related to ZVI corrosion under anaerobic conditions.

4. Conclusion

Anaerobic sludge digestion is still one of the most widely sustainable applications which are used for sludge reduction and energy production. Improving energy production can significantly reduce the operation cost of wastewater treatment plants. In the current study, it has been shown that CH_4 production can be enhanced using ZVI through exogenous H_2 supply. ZVI had a positive effect on hydrogenotrophic methanogens, in which their activity for methane production increased up to 61%. This would indicate that using in-situ metal bio-corrosion could be a cost-effective approach for exogenous H_2 supply and improves CH_4 production. Besides, low-cost ZVI source such as iron scrap (either rusty or non-rusty) can be used effectively to enhance anaerobic sludge digestion and biogas production. Therefore, the incorporation of such a low-cost alternative can significantly improve the process cost-efficiency.

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