Evaluation of nano zero valent iron effects on fermentation of municipal anaerobic sludge and inducing biogas production

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Abstract. The application of nano size materials on wastewater is going extensive because its high reactivity compared with other materials. As a result, numerous research studies investigated the effectiveness of dosing nano zero valent iron (nZVI) or micro zero valent iron (mZVI) on anaerobic digestion (AD) of sludge and production of biogas as promising renewable energy but inconsistent outcomes have appeared. In this paper, different dosing concentrations of nZVI were applied on anaerobic activated municipal sludge to examine the impact of nZVI on sludge fermentation, biogas generation, and methane (CH₄) content stimulation. The results showed that addition 250 mg/L nZVI nanoparticles could enhance 25.23% biogas production and the methane content reached 94.05% after one week of digestion compared with 62.67% without adding iron nanoparticles.

1. Introduction
Recently, using nano zero valent iron (nZVI) in wastewater remediation is growing worldwide, this potentially might affect the anaerobic digestion (AD) of municipal sludge. AD is a biodegradation process that transforms organic matters into biogas which its main mixture is methane (CH₄) and carbon dioxide (CO₂) under controlled conditions. The anaerobic atmosphere was applied and the temperature degree must be fixed to be appropriate for occurring mesophilic stabilization. Facultative bacteria is responsible for converting part of sludge into biogas [1]-[3]. Generally, AD involves four successive steps hydrolysis, acidogenesis, acetogenesis and methanogenesis [4].

In fact, AD is deemed to be the most energy efficient method for stabilizing sludge and methane by-product as one form of fuel that is promised to reduce the treatment cost [5]. Despite a mass of studies that compared the effect of ZVI in nano-size with that particles in micro-size, different concentrations of nZVI in the literature were not extensively studied. For example, numerous researchers compared different ZVI diameters in same concentration [6] which resulted in unconvincing comparison, further researchers studied the long term of adding same concentration of one nanoparticle on the rate of AD [7], [8].

According to some reports [9], [10] nZVI has a relatively high surface area and superior reactivity, that mean it can work as an electron donor in the AD process which can improve the sludge digestion and increase the biogas yield, this may be will make it an excellent additive to the AD process. Su et al. research results showed that adding of 0.1 wt% nZVI to the AD of waste activated sludge for 17 days at the mesophilic temperature increased the methane production by 40.4% [10]. However, Yang et al.’s investigation concluded inhabitation properties of nZVI in AD bioreactors compared with mZVI particles that stimulate the methane content. According to their study, 20%, 20% and 70% decrease in methane production were observed when adding 1, 10 and 30 mM nZVI to the municipal sludge, respectively [6].
The conflicting results indicated that further investigations are still needed to assess the impact of nZVI on methane production during AD process. Therefore, the effect of the same size of nZVI with different concentrations during sludge anaerobic digestion is still not yet well documented.

In the research presented here, we study the effect of 50, 100 and 250 mg/L of laboratory-prepared nZVI on the efficiency of anaerobic digestion process by examining the enhancement in the degradation rates and the increases in the methane production.

Three nZVI laboratory scale bioreactors in addition to the control set were tested over a range of nZVI concentrations to determine the optimal nZVI dosing and anaerobic degradation was evaluated by monitoring the chemical oxygen demand (COD) concentration and the biogas production compared to the control bioreactor.

2. Materials and methods

2.1 Iron reagent synthesis

One gram nZVI stock suspension was freshly prepared by reducing five grams of ferric chloride (FeCl₃) that were dissolved in 120 mL of deionized water with 5 grams of sodium borohydride (NaBH₄) that also were dissolved in another 120 mL of deionized water as presented in Figure 1. NaBH₄ solution was added dropwise to FeCl₃ solution under the nitrogen atmosphere while the FeCl₃ solution was vigorously stirred at 250 rpm at 27-degree Celsius temperature. The prepared nZVI particles had an average size of 60 nm as they were characterized by transmission electron microscopy (TEM, JEM-ARM 200F, JEOL Co., Japan) and they were analysed by X-Ray diffraction instrument (XRD, TTR, Rigaku, Tokyo, Japan) to check its particles composition.

![Figure 1. Schematic diagram of nZVI synthesis reactor](image)

2.2 Sludge source

Anaerobic activated sludge was sampled from a two-stage mesophilic digester at the Mikasagawa Wastewater Purification Center, Fukuoka, Japan. Mikasagawa treatment plant treated around of 267,400 m³/day in 2016 using conventional activated process. The quality of raw sludge is listed in Table 1. The anaerobic sludge samples were kept at 4 °C to maintain its freshness prior to use.

| Parameter         | Reading/Concentration |
|-------------------|-----------------------|
| Total Solids      | 4.22%                 |
| COD               | 5,453 mg/L            |
| Total Alkalinity  | 3.53 mg/L             |
| pH                | 7.1                   |
| Organic Fraction  | 74.86 %               |
2.3 Experimental setup

The laboratory scale experimental setup of the batch anaerobic system which shown in schematic diagram in Figure 2 was consists of: (1) a bioreactor; 500 mL DURAN neck culture vessel bottle (SANSYO, Japan) plugged tightly with two nozzles perforated cap (SANSYO, Japan), (2) a thermostatic water bath (SANSYO, Japan) as a temperature control, two outlets were connected to the perforated cap, (3) first one was used for examining the sludge characterization by taking liquid sludge samples using (6) syringe while the (4) second outlet was divided into (5) two orifices, one which allows to take gas samples using (7) Hamilton syringe (Hamilton, USA) to inspect the biogas composition and CH$_4$ fraction using gas chromatography (GC, GL Sciences Inc., Japan), while the second orifice was connected to a water displacement system consisting of 300 mL (8) calibrate glass cylinder to measure the volume of produced biogas. Before the digestion, the sludge samples were flushed with nitrogen gas for 10 min to remove oxygen and to assure the anaerobic condition in the bioreactor at the beginning of experiments.

50, 100 and 250 mg/L concentrations of laboratory prepared nZVI were used to study its effect on biogas production and compared it with control bioreactor. The nZVI particles were added separately to each bioreactor and the operating temperature was maintained at mesophilic conditions 37 ± 3 °C then the bioreactors were reciprocally shaken under 150 ± 5 rpm every 12 hours for 5 minutes long. The bioreactors performance was measured with respect to the daily and cumulative volume of biogas produced and CH$_4$ yield in the course of time was analyzed. All of the treatments were carried out in duplicate to ensure the reproducitively of results.

![Schematic diagram of experimental biogas production system](image)

2.4 Analytical methods

Raw activated sludge samples were analysed for total solids (TS), total alkalinity and COD. Digested sludge samples were daily withdrawn during the batch experiments using a 10 mL syringe and were then filtered through a 0.45 µm disposable membrane filter (MILLIPORE, USA). The COD concentrations were analysed by spectrophotometer (DR 3900, Hach company, USA) using digestion method according to the standard method for examination of water and wastewater [11].

Gas composition was analyzed using the GC instrument and Argon was used as carrier gas at a flow rate of 30 mL/min. GC was equipped with capillary column with mesh range 60/80 with a molecular sieve (Molesieve 5A, 1/8). Column oven temperature was set at 60 °C whereas the injector temperature was 80 °C. The thermal conductivity detector (TCD) operating temperature was raised to 80 °C and the current was fixed at 60 mA. For analyzing the biogas composition, 1mL of generated biogas was collected using a gastight 10 mL Hamilton syringe and then injected in GC instrument. The CH$_4$ fractions were defined by analyzing the post-run results obtained from gas chromatographic measurements. All GC post-run measurements were repeated at least two times.
3. Results and discussion

3.1 Iron characterization

TEM image of the laboratory synthesized iron nanoparticles showed a largely spherical form and the single particle size was less than 100 nm, moreover, the particles distribution formed chain-like aggregate as shown in the Figure 3. The XRD spectrum of nZVI revealed apparent peak at 2θ of 44.8° indicating the presence of zero valent of synthesized iron particles and there is no major existence of iron amorphous particles Figure 4.

![Figure 3. Morphology graphs of aggregates of nZVI particles](image)

![Figure 4. X-ray diffraction results of the synthesized iron nanoparticles](image)

3.2 Biogas production and methane content in the presence of nanoparticles

The cumulatively produced biogas volume for each bioreactor was daily recorded along the study period as shown in Figure 5. It was noted that the biogas started generation at the beginning of digestion duration and at the first four days, the biogas production in all bioreactors had an almost similar tendency. Afterward, the cumulative biogas production increased greatly with nZVI bioreactors. The daily biogas volume reached the maximum on the day 6th and high concentration of nZVI produced more biogas if compared with low concentrations. By the end of digestion period, 250 mg/L nZVI generated 273 mL of biogas, 100 mg/L nZVI generated 219 mL of biogas and 50 mg/L nZVI generated 192 mL of biogas compared with 218 mL that control generated it. Comparing the produced biogas volume at the day 14th, the biogas volume increased by 25.23% with dosing 250 mg/L of nZVI and this increment did not depict with the concentration of 50 mg/L and 100 mg/L nZVI. Figure 6 shows the methane fractions in the generated biogas from the municipal sludge with the addition of nZVI. The addition of 250 mg/L nZVI resulted in an increase in the fraction of methane from 22.73% at the second day of digestion to 68.65% in the day 7th and after that, the methane content fractions did not significantly increase and almost remained constant with different nZVI concentrations. Increasing the methane fractions by the addition of nZVI revealed that nZVI have a clear stimulating effect on methanogenic activity during the anaerobic digestion process in comparison with the control.

Based on the consideration above, it was indicated that adding of relatively high concentration of nZVI in anaerobic activated sludge was likely to improve the methanogenic activity and as result increase the biogas production and CH₄ yield which confirmed the positive effects of iron on AD[12].
3.3 COD changes during anaerobic digestion

The soluble COD was used as an indicator for the change in dissolved/soluble organic matter concentration in the anaerobic sludge [13].

The soluble COD in all reactors was increased through the 14 days of digestion for all treatments indicating that the sludge fermentation process particularly hydrolysis and acidogenesis were took place and the decreasing in COD reading was not depicted due to the long period needed to start decreasing the COD values as reported by Yang et al. 2013a [14].

Figure 7 shows the changes of COD during the digestion, with the increase of nZVI dosage the COD concentration increased, which was in accordance with the enhancement of sludge fermentation. These results were in agreement with Feng et al., study that proved the addition of ZVI enhanced the formation of acetate and therefore raised in COD concentrations [5]. The COD concentration was 5453 mg/L before the digestion, and after the 14 days of digestion, its concentration increased to 8722, 8681, 9404 and 11309 at the nZVI of 0, 50, 100 and 250 mg/L, respectively. It pointed out that the sludge fermentation increased significantly with increasing the nZVI concentrations.

According to Figure 7, both of the 50 and 100 mg/L nZVI concentration increased the COD concentrations lower than those obtained by 250 mg/L under same conditions during the digestion duration process means that the reactivity of nZVI on AD could be improved significantly at relatively high dosing concentrations.
4. Conclusion
From the results obtained in this study, it can be concluded that the addition of nZVI to anaerobic activated sludge led to an increase in the fraction of methane in all produced biogas samples through AD. This can be explained by the increase in the production of enzymes by microorganisms that are often detected in the anaerobic digestion environment.
Among the different nZVI concentrations, the relatively high additive was the best one for enhancing the biogas production from municipal activated sludge, which has reached 25.23% higher than the control group.
The addition of nZVI proved to be an interesting alternative for improving energy production from municipal activated sludge which not only may enhance activated sludge fermentation, but also could save a significant portion of wastewater treatment plants operation cost.

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