The synergy of combined free nitrous acid and Fenton technology in enhancing anaerobic digestion of real sewage waste activated sludge

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Research article

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Abstract

Background Recently, free nitrous acid (FNA) pre-treatment of sewage waste activated sludge has been introduced as an economically attractive and environmentally friendly technique for enhancing methane production from the anaerobic digestion process. Fenton pre-treatment of sewage sludge, as an advanced oxidation process, has also been introduced as a powerful technique for methane improvement in a couple of studies. This study, for the first time, investigates the synergy of combined FNA and Fenton pre-treatment technologies in enhancing the methane production from the anaerobic digestion process and reducing waste sludge to be disposed of. Actual secondary waste activated sludge in laboratory-scale batch reactors was used to assess the synergistic effect of the pre-treatments. The mechanisms behind the methane enhancement were also put into perspective by measuring different microbial enzymes activity and solubilisation of organic matter. Result This study revealed that the combined pre-treatments release organic matter into the soluble phase significantly more than the bioreactors pre-treated with individual FNA and Fenton. For understanding the influence of pre-treatments on solubilisation of organic matter, soluble protein, soluble polysaccharide and soluble chemical oxygen demand (SCOD) were measured before and after the treatments and it was shown that they respectively increased by 973%, 33% and 353% after the treatments. Protease and cellulose activity, as the key constituents of the microbial community presenting in activated sludge, decreased considerably within the combined pre-treatments (42% and 32% respectively) and methane production enhanced by 43-69%. Furthermore, total solids and volatile solids destruction improved by 26% and 24% at the end of anaerobic digestion, which can reduce transport costs of sludge and improve the quality of sludge for application in farms and forests. Conclusions The results obtained from the experiments corroborate the synergic effect of the combined FNA and Fenton pre-treatment technologies in degrading the organic and microbial constituents in waste activated sludge, which improved methane production accordingly. This is of paramount importance because the total costs of wastewater treatment plants operation and greenhouse gas emission from sludge treatment and disposal processes would reduce considerably, which pave the way for the implementation of these technologies.

Background

Sewage Sludge treatment is the most cost-intensive process in wastewater treatment plants, which accounts for around 60% of total operating costs [1]. Anaerobic digestion of sludge is an environmental and economical friendly method for sludge management because not only it dispenses with aeration equipment and related costs, but it also produces bio-methane, from which renewable energy could be generated. In anaerobic digestion, organic matter of sludge is transformed into CH_4, CO_2 and N_2O [2]. Holistically, gasses produced in sludge treatment and disposal processes could account for 40% of greenhouse gas emissions in wastewater treatment plants [3,4]. Enhancing methane production in anaerobic digestion of waste activated sludge harnesses greenhouse gas emissions and converts them to renewable energy [5]. Enhancement of methane production also reduces the volume of sludge via degradation of higher organic matter, which leads to lower sludge disposal costs and higher quality sludge for land application [6].

Anaerobic digestion of waste activated sludge is often restricted by poor biochemical methane potential, low biodegradability and slow fermentation process [7]. In order to address these issues, various strategies have been employed in recent studies. Sólyom et al. [8] and Ennouri et al. [9] assessed the effect of physical pre-treatments of sludge on anaerobic digestion receptively with an application of microwave and thermal pre-treatments. Hallaji et al [10, 11] assessed the influence of chemical, physical and physiochemical pre-treatment of sludge on methane enhancement and organic matter degradation in anaerobic digestion. Recently, Svensson et al [12] and Campo et al [13] also propounded the methods of the post and intermediate treatments of sludge with the intent of enhancing anaerobic digestion of sludge, dewaterability and quality of sludge. These treatments disrupt cell walls and extracellular polymeric substances (EPS), which improve the solubilisation of organic matter and methane production accordingly [14].

As an environmentally friendly and economically favourable substance, free nitrous acid (HNO2) can be produced by nitration of anaerobic digestion liquor[15]. It generates hydrogen peroxide (H2), peroxynitrite (ONOO-), nitrogen dioxide (˙NO2), hydroxide ion (˙OH -) and nitric oxide (˙NO) which have inhibitory impacts on key microorganisms in wastewater treatment plants at part per billion (ppb) levels [16]. Zahedi et al. [17] demonstrated that after 5 hours of exposure time at 2.49 mg N-HN2/L, cells' viability in waste activated sludge declined by 80%, and methane production increased by 20%.

Fenton reagent is formed by a combination of H_2O_2 and Fe^{+2} in a constant ratio, which Fe^{+2} functions as a catalyst for generating highly oxidising radicals (·OH) [18]. When it comes to oxidation-reduction potential, Fenton reagent produces stronger radicals (+2.33 V) than hydrogen peroxide alone (+1.36 V) and ozone (+2.07 V), and it disrupts cell walls and EPS in sludge [18]. Therefore, it has good potential for enhancing methane production and degradation of organic matter in anaerobic digestion of sludge [19]. Erden and Filibeli [20] demonstrated that 4 g Fe^{+2}/kg TS and 60 g H_2O_2/kg TS with 60 minutes exposure time increased methane production by 19.4% in anaerobic digestion of waste activated sludge. Interestingly, hydrogen peroxide can be produced in wastewater treatment plants via bio-electrochemical process [21]. The Fenton reactions are shown in Equation number 1, 2 and 3 [18]. Presence of ferrous iron accelerates the decomposition of hydrogen peroxide to hydroxyl radicals and hydroxyl anions in Eq. 1 [22]. The hydroxyl radicals react with ferrous iron, which leads to Fe^{+3} and OH^- production (Eq.2). There is huge amount of organic matter (RH) in sludge. The hydroxyl radicals produced from Fenton reaction react with organic matter in sludge (RH), producing organic radicals (•R), which are highly reactive and capable of being further oxidised (Eq. 3) [19].

$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\bullet}HO + OH^-$ (1)

${}^{\bullet}OH + Fe^{2+} \rightarrow Fe^{3+} + OH^-$ (2)

$RH + {}^{\bullet}OH \rightarrow H_2O + {}^{\bullet}R$ (3)

In this study, we hypothesised that combined FNA and Fenton pre-treatment could degrade waste activated sludge more than individual FNA and Fenton pre-treatments, leading to enhanced methane production accordingly. Considering that, this study aimed for assessing the synergistic effect of combined FNA and Fenton pre-treatments on 1) methane production from anaerobic digestion of waste activated sludge, 2) solubilisation of organic matter and 3) destruction of organic matter during the digestion. For FNA pre-treatment, two concentrations (1.5 and 2.5 mg HNO2/L) at 5 hours exposure time were considered [17] and for Fenton pre-treatment, two concentrations of H2 (25 and 50 g H2/kg TS) with a constant ratio of H2/Fe+2 (1/0.067) at 1 hour exposure time were employed [20, 23–25]. Combination of these conditions was also used for assessing the synergistic effects of FNA and Fenton. To our knowledge, this is the first study, investigating the synergistic effect of combined FNA and Fenton pre-treatment on anaerobic digestion of waste activated sludge.

Results

Influences of Fenton and FNA PTs on WAS solubility and enzymes activity

Fig.1 demonstrates the effect of Fenton and FNA pre-treatments on the solubilisation of organic matter. The amount ofuble oxygen demand (SCOD) in waste activated sludge samples before and after the treatments is shown in Fig 1-a. According to the data shown, Fenton1 and Fenton2 pre-treatments increased SCOD by 0.10 and 0.23 g SCOD/g VS respectively, and FNA1 and FNA2 enhanced SCOD by 0.15 and 0.20 g SCOD/g VS respectively. However, significantly higher enhancement was achieved by combined FNA and Fenton pre-treatment in comparison to individual pre-treatments. Combined FNA2 and Fenton2 increased SCOD by 0.43 g SCOD/ g VS, as the most effective pre-treatment in SCOD improvement among the presented pre-treatments.

The microorganisms existing in sludge are compounded mainly of protein (50%) [18], so it is so important to rest assured that the organic matter solubilised by the pre-treatments is attributed to biodegradable organic matter such as soluble protein and polysaccharide. According to the measurements, soluble protein and soluble polysaccharides increased considerably in all pre-treated bioreactors (Fig 1-b and 1-c*).* The highest increase in soluble protein (0.082 g/g VS) and soluble polysaccharide (0.059 g/g VS) was obtained by FNA2+Fenton2, which corroborate the synergistic effect of combined FNA and Fenton pre-treatments on disrupting microorganisms' intracellular compounds and disintegrating organic matters than these pre-treatments alone. Individual FNA2 and Fenton2 pre-treatments increased soluble protein by 0.039 and 0.046 g/gVS and soluble polysaccharide by 0.029 and 0.032 g/g VS respectively.

Volatile sspended solid (VSS) is another scale, indicating solubilisation of organic matter. Fig. 1-d demonstrates the amount of VSS after and before the pre-treatments of waste activated sludge samples. The amount of VSS declined in the samples after pre-treatments, indicating that suspended solids transformed into soluble phase. Although the amount of reduction was slight in the control reactor with 11%, the pre-treated reactors underwent a considerably higher reduction in their VSS with up to 63% in combined FNA2 and Fenton2 pre-treatments.

Table 1 shows protease and cellulose activity, before and after PTs. Protease and cellulose activities reduced considerably after PTs. Combined FNA2+FEN2 reduced protease and cellulose activity by 42% and 32% respectively. The other combined PT (FNA1+FEN1) led to 32% and 27% reduction in protease and cellulose activity respectively. Among the individual pre-treatments, Fenton exhibited a stronger effect on the enzyme's activity in comparison to FNA, which indicates the stronger antimicrobial properties of Fenton technology.

Pre-treatments' effect on biochemical methane production

Cumulative methane production from the bioreactors during the digestion process is shown in Fig. 2. According to the data shown, the highest increase in methane production was achieved from combined FNA2 + Fenton2 pre-treatments, accounting for 69%, Followed by FNA2 + Fenton2 and FNA2 + Fenton1 with 61% and 57% respectively. However, methane production from individual pre-treatments did not transcend 26%, which was obtained from Fenton2 pre-treatment. FNA2 showed similar performance in comparison to Fenton2, which enhanced methane production by 25%.

Degradation of organic matters

In this study, VS and TS were measured regularly during the digestion process, and the trend of these phenomena is demonstrated in Fig. 3. During the digestion, FNA1 and FNA2 increased VS degradation by 6% and 8% respectively in comparison to the control bioreactor (Fig 3-a). Fenton1 and Fenton2, also, increased VS degradation by 4% and 9% respectively (Fig 3-b). The highest VS destruction obtained from pre-treated bioreactors with combined FNA2 + FEN2, accounting for 24% (Fig 3-c). TS degradation experienced a similar pattern, in which combined FNA and FEN pre-treatments caused the highest degradation with up to 27% and individual FNA and FEN pre-treatments increased TS degradation by up to 14% and 20% respectively (Fig 3-(d-f)).

Discussions

Soluble organic matter in waste activated sludge samples increased in all pre-treated reactors significantly, which is attributable to the disruption of cell walls and EPS by free radicals and oxidative chemicals produced in the treatment process. A slight increase of soluble organic matter in the control reactor indicates the activity of the organism in solubilising organic matter as part of the hydrolyses stage, which naturally happens in the absence of any treatment [2].

The higher efficiency of combined pre-treatments in SCOD enhancement is attributable to the synergistic effect of radicals and oxidative chemicals released by FNA and Fenton reagent simultaneously. The

SCOD enhancement from FNA pre-treatment, in this study, is in agreement with, but slightly higher than the research carried out by Zahedi et al. [17] and Wang et al. [26], which could be attributed to sludge specifications employed in this study.

The microorganisms existing in sludge are compounded mainly of protein (50%) [18], so it is so important to rest assured that the organic matter solubilised by the pre-treatments is attributed to biodegradable organic matter such as soluble protein and polysaccharide. The results achieved in this study was slightly higher than the study carried out by Wang et al. [26], in which combined FNA and thermal pre-treatments were applied to waste activated sludge and soluble protein, and soluble polysaccharide increased by up to 0.07 and 0.03 g/g VS respectively.

Protease and cellulose play a key role in the hydrolysis of organic matters and converting them to more readily biodegradable forms for anaerobic microorganisms' consumption. Protease decomposes proteins to amino acids and cellulose catalyzes the hydrolysis of polysaccharide to monoses [27]. Protease and cellulose activities were affected by the PTs substantially. The higher level of PTs resulted in the lower activity of the enzymes. This is especially evident in combined pre-treated reactors. The reduced activity can be attributed to antimicrobial properties of FNA and Fenton pre-treatments, which probably affect extracellular and intracellular constituents of the microorganisms. This effect can shorten the time needed for hydrolysis process in the AD process, which result in shorter hydraulic retention time (HRT) and smaller digesters that is of great significance from an economic and operational perspective.

The methane produced from the pre-treated bioreactors was considerably higher than that of the control bioreactor, which affirms the effectiveness of the employed pre-treatments in improving anaerobic digestion of waste activated sludge. Among the pre-treated bioreactors, those had been pre-treated with combined FNA and Fenton produced a significantly higher amount of methane, which corroborates the synergistic effect of these pre-treatments in enhancing methane production from anaerobic digestion of waste activated sludge.

Maximum methane production in pre-treated bioreactors was obtained in the fifth day of the digestion process. However, maximum methane production in the control bioreactor obtained in the second day of digestion. This difference can be attributed to overloading of the pre-treated bioreactors with soluble organic matter, which led to a delay of methane production [28]. Hallaji et al. [10] revealed that using combined FNA and Fenton pre-treatment in anaerobic digestion of mixed primary and secondary sludge enhance methane production by up to 72%, which is relatively higher than that achieved in this study. However, methane enhancement obtained in this study was considerably higher than that achieved in Wang et al. [26] with around 40%, in which they used combined FNA and thermal pre-treatments in anaerobic digestion of waste activated sludge. In Erden and Filibeli's study [20], the amount of methane enhancement from applying Fenton pre-treatments to waste activated sludge was 19.4%, which is relatively lower than that obtained in this investigation.

VS and TS disintegration are the most important indexes for organic matter destruction of sludge and anaerobic digestion efficiency [29]. As can be observed, the initial amount of VS and TS in pre-treated reactors is lesser than the control. This can be attributed to the effect of pre-treatments on declining VS and TS during the treatments' exposure time. The increase in the level of pre-treatments resulted in VS and TS degradation. Fenton pre-treatment demonstrated a slightly stronger effect on VS and TS destruction than FNA in higher concentrations. Analogous patterns were achieved by Pilli et al. [30] who pre-treated secondary sludge with Fenton reagent for enhancing anaerobic digestion.

Having taken all aspects into account, both FNA and Fenton improved methane production from waste activated sludge. This is mainly due to the release of more readily biodegradable organic matters into the soluble phase, caused by the pre-treatments (Fig. 1). Combined pre-treatments caused considerably higher methane production than FNA and Fenton pre-treatments individually, which is attributable to different radicals and oxidative chemicals, released by each of these pre-treatments. Methane production enhancement leads to higher bioenergy generation in wastewater treatment plants and lower greenhouse gas emission from sludge management process, which is of paramount importance from an environmental perspective. Furthermore, the improved TS and VS of the digested sludge pave the way for safer sludge reusing in farmlands and forests as an environmentally friendly and economically attractive technique.

Despite the significant implications achieved in this investigation, using new methods and technologies in wastewater treatment plants entails a long-term assessment of technical, economic and environmental aspects. Therefore, in prospective studies, these phenomena are recommended to be taken into consideration, to pave the way for the implementation of these pre-treatments in big-scale wastewater treatment plants.

Conclusion

This study revealed that combined FNA and Fenton pre-treatments significantly enhance solubilisation of organic matter, microbial degradation, methane production, and organic degradation of waste activated sludge during anaerobic digestion, compared with these pre-treatments individually. Methane production enhanced by 69% in the bioreactors pre-treated with combined FNA2 + Fenton2, which is attributable to the synergistic effect of the pre-treatments and higher solubilisation of organic matter caused in these reactors. Importantly, in combined pre-treated rectors soluble protein, as readily biodegradable organic matter, increased substantially and key enzyme's activity reduced considerably, which corroborate the synergistic effect of the pre-treatments on disrupting cell walls and EPS in waste activated sludge. Besides, TS and VS degradation was enhanced by 26% and 24% respectively, compared with the control bioreactor at the end of the digestion process, which is of great importance from the operation and environmental perspective. This study clearly revealed the potential of combined FNA and Fenton pre-treatments in improving anaerobic digestion of waste activated sludge.

Methods

Sludge characterisation

The South Wastewater Treatment Plant of Tehran is the biggest wastewater treatment plant in the Middle East, playing a vital role in collecting and treating wastewater of Tehran's population. Built-in 8 phases, the plant treats wastewater of 4,200,000 people, living in the central and southern districts of Tehran. There are 6 mesophilic anaerobic digesters having 53,400 m3 capacity in total, producing around 36,000 m3/day biogas. The digesters treat combined primary and waste activated sludge with a ratio of 40%/60%. All sludge samples used in this study were collected from this wastewater plant.

The thickened waste activated sludge was collected from belt thickener in this plant. After collecting, the sludge was immediately transferred to the University's laboratory and after measurements, it was kept at 4 ℃ and low pH. Characteristics of the waste activated sludge was as follows, which are achieved from triplicate tests with standard error: pH 6.45 ± 0.00, l solid (TS) 40.10 ± 1.56 g/L, volatile solid (VS) 32.00 ± 0.91 g/L, otal suspended solids (TSS) 36.20 ± 1.03 g/L, volatile suspended solids (VSS) 29.40 ± 0.83 g/L, chemical oxygen demand (COD) 49.20 ± 1.12 g/L and SCOD 3.92 ± 0.10 g/L.

The inoculum used in this research for biochemical methane potential tests was harvested from the mesophilic digesters of the plant. Characteristics of the inoculum were as follows, which are obtained from triplicate measurements with standard error: pH 7.58 ± 0.00, TS 31.75 ± 0.93 g/L, VS 24.75 ± 0.86 g/L, TSS 27.75 ± 1.02 g/L, VSS 22.45 ± 0.79 g/L, TCOD 37.30 ± 0.63 g/L and SCOD 3.43 ± 0.15 g/L.

Fenton and FNA methodology

For conducting FNA pre-treatment, first, the pH of waste activated sludge samples was reduced to 5.5 with 1 M HCl solution. Then, 4 M nitrite salt ($NaNO_2$) was added to the mixtures, to provide the designated FNA concentrations in the sludge environment (table.2). In the last stage, the mixtures were shacked with a shaker at 150 rpm for 5 hours. FNA concentration was computed by the equations $N\text{-}HNO_2 = (S_{N\text{-}NO2})/(Ka \times 10^{\,pH})$ and $Ka = e^{-2300/(273+°C)}$, in which °C is the temperature of the room (23 °C in this experiment), Ka is a constant which is dependent on the temperature and $S_{N\text{-}NO2}$ is the nitrite salt concentration [31].

For conducting Fenton reaction, first, waste activated sludge samples were put into 1 L bottles, then their pH was decreased to 3 with H_2SO_4 solution (99%). In the next stage, iron salt ($FeSO_4$) was added to the mixture, to produce the designated Fe^{+2} in the sludge environment (table.2). Then, the designated hydrogen peroxide concentrations were added to the reaction. The ratio of H2/Fe+2 was set at 1/0.067 according to the literature [20, 23–25]. The mixtures were finally shacked with a shaker at 150 rpm for 1 hour at ambient temperature, so that the Fenton reaction would be approximately terminated [20, 24].

For combined FNA and Fenton pre-treatments, first, FNA pre-treatment was applied to waste activated sludge at 5 hours exposure time (pH = 5.5), then Fenton pre-treatment was applied at 1 hour (pH = 3). The combined conditions are shown in Table2.

Biochemical methane potential tests

In order to measure the volume of methane production from waste activated sludge, 27 batch reactors in addition to blank were carried out in 1000 mL glass bottles with a working volume of 500 mL (Additional file 1). The schematic of the reactor is shown in Figure 4. The ratio of inoculum and substrate (I/S) was adjusted to 2, based upon dry volatile solid [32]. The pH of treated waste activated sludge samples was adjusted to 7 and their temperature was increased to 37 °C prior to mixing with the inoculum, so as to prevent any temperature and pH shock to the inoculum. After mixing, the bottles were flushed with N2 gas for 1 minute (1 L/min), then they were put into the water bath, whose temperature was controlled at 36 ± 1 °C by automatic heaters. The bottles were permanently stirred by magnetic stirrers at 100 rpm, to provide adequate mixing and uniform temperature distribution. All tests were carried out in triplicate. The digestion process lasted for 44 days when biogas generation was approximately terminated.

Analytical methods

Routine experiments on sludge quality such as TS, TSS, VS, VSS, COD and SCOD were measured according to standard methods for the examination of water and wastewater[33]. For measuring soluble proteins before and after the treatments, Folin Phenol reagent was used according to Lowry's method [34]. Soluble polysaccharides were also measured with phenol and sulfuric acid, according to Dubois's method [35]. In order to separate soluble matter from suspended solids, 10 minutes centrifuge at 15000 rpm was first implemented, then the solution was passed through 0.45 μm pore size glass fibre filter, using Buchner funnel, and vacuum equipment.

The biogas volume was measured according to the liquid displacement method with an acidified liquid barrier (pH = 2) that was saturated with NaCl for minimizing the solubility of biogas [36]. Methane production was measured by gas chromatography (GC), using a Thermal Conductivity Detector (TCD). The temperature of the column and TCD was set respectively at 75 °C and 104 °C. In each measurement, 0.05 cc sample was injected to the equipment, and 1 minute exposure time was considered for each measurement. In this study, biogas and methane production were measured once daily and once every fifth day, respectively during the digestion process.

Key hydrolytic enzymes (Protease and cellulose) activity was measured according to well agar diffusion method in our previous study [37–39]. For carrying out the enzymatic tests, methanogenic organisms should be first eliminated so as to decline potential errors [40]. For eliminating methanogens from the samples, heat treatment at 102 °C for 30 minutes and BESA (2-bromoethanesulfonic acid) were applied to the BMP reactors [41, 42]. The samples then were maintained at 37 °C for 72 hours before being assessed for enzymatic activity.

Abbreviations

BMP: biochemical methane potential; COD: chemical oxygen demand; SCOD: soluble chemical oxygen demand; TS: total solid; TSS: total suspended solid; VS: volatile solid; VSS: volatile suspended solid; GC: gas chromatography; TCD: thermal conductivity detector; FNA: free nitrous acid; FEN: Fenton.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

All data generated during this study are included in this published article and its additional file.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

Conceptualization, R.K and S. M.H; Data curation, R.K and S. M.H; Formal analysis, S. M.H, and R.K; Funding acquisition, S. M.H; Investigation, R.K and S. M.H; Methodology, S. M.H and R.K; Project administration, S. M.H; Supervision, S. M.H; Validation, R.k and S. M.H; Writing—original draft, R.K; Writing—review & editing, R.K and S. M. H.

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Tables

Table. 1 Key enzymes activity in WAS before and after the pre-treatments

	before PT	After PT			
Reactors	Control	FNA1	FEN1	FNA1+FEN1	FNA2+FEN2
Protease	320.9	280.7	241.1	21.70.8	18.50.2
Cellulose	371	330.3	28.70.5	270.8	250.4

Enzyme activity is based on activity zone diameter (mm) in well agar diffusion method. The data is average of triplicate tests (with standard errors).

Table 2. Pre-treatment conditions used in this research

Pre-treatment	FNA (mg /L)	Fenton mg /g TS	Fe+2 Mg Fe+2/g TS
Control	0	0	0
FNA1	1.5	0	0
FNA2	2.5	0	0
FEN1	0	25	1.68
FEN2	0	50	3.35
FNA1+FEN1	1.5	25	1.68
FNA2+FEN2	2.5	50	3.35
FNA2+FEN1	2.5	25	1.68
FNA1+FEN2	1.5	50	3.35

Figures

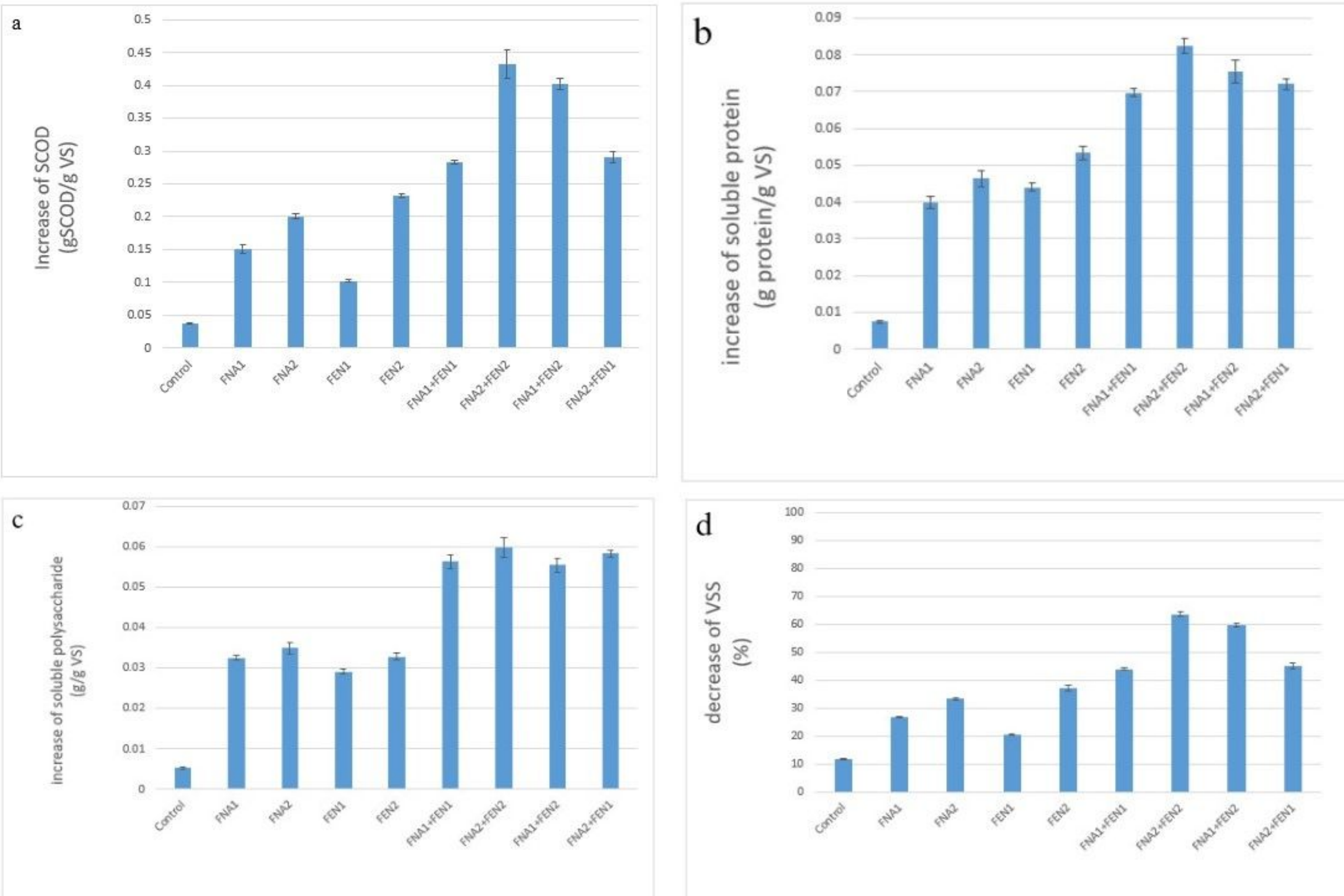

Figure 1

Biomass specific production of (a) SCOD, (b) soluble proteins, (c) soluble polysaccharides and (d) VSS after pre-treatment. Error bars represent standard error from triplicate measurements.

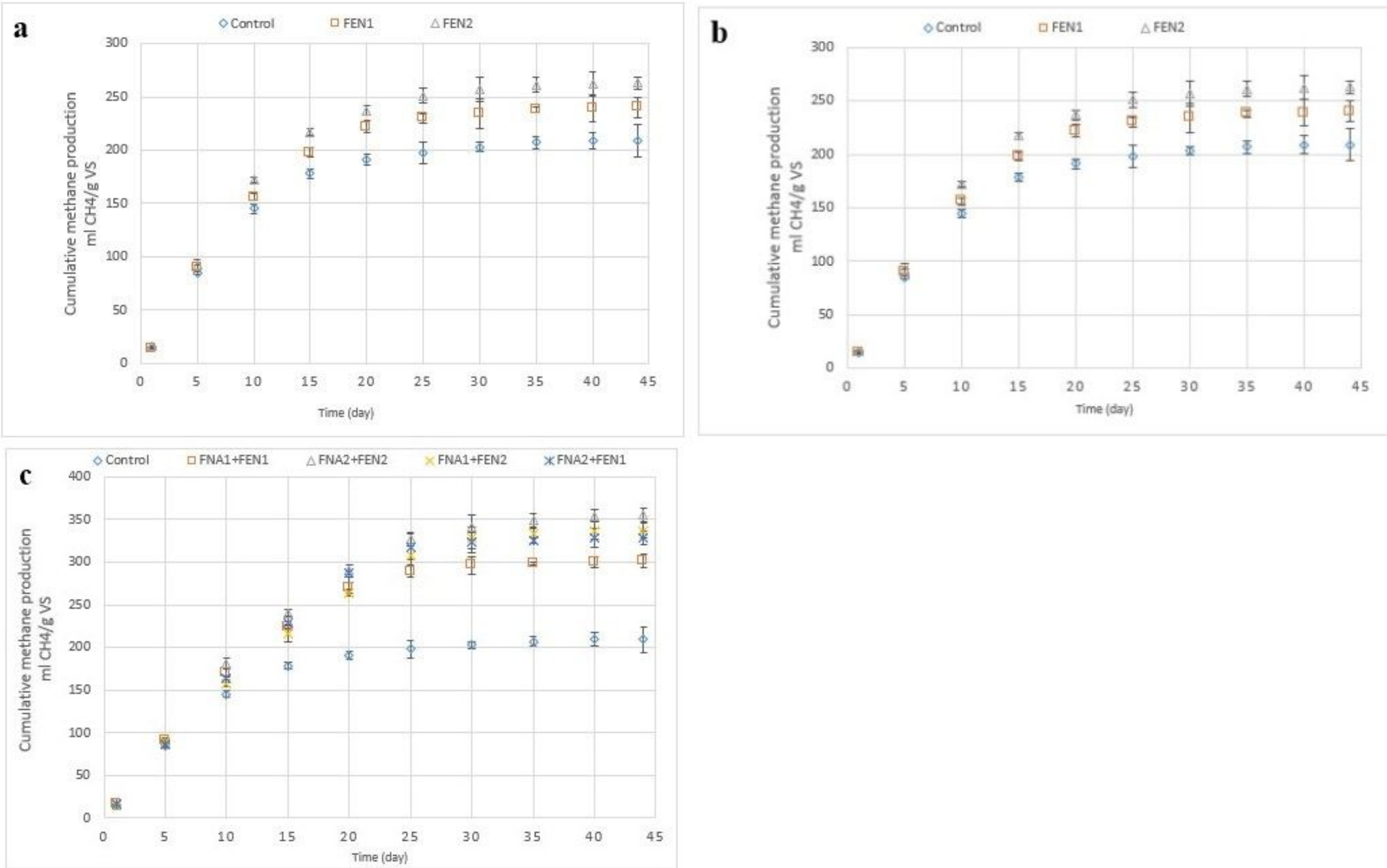

Figure 2

Cumulative methane generation from waste activated sludge with a) FNA, b) Fenton and c) combined FNA and Fenton pre-treatments. Error bars represent standard error from triplicate tests.

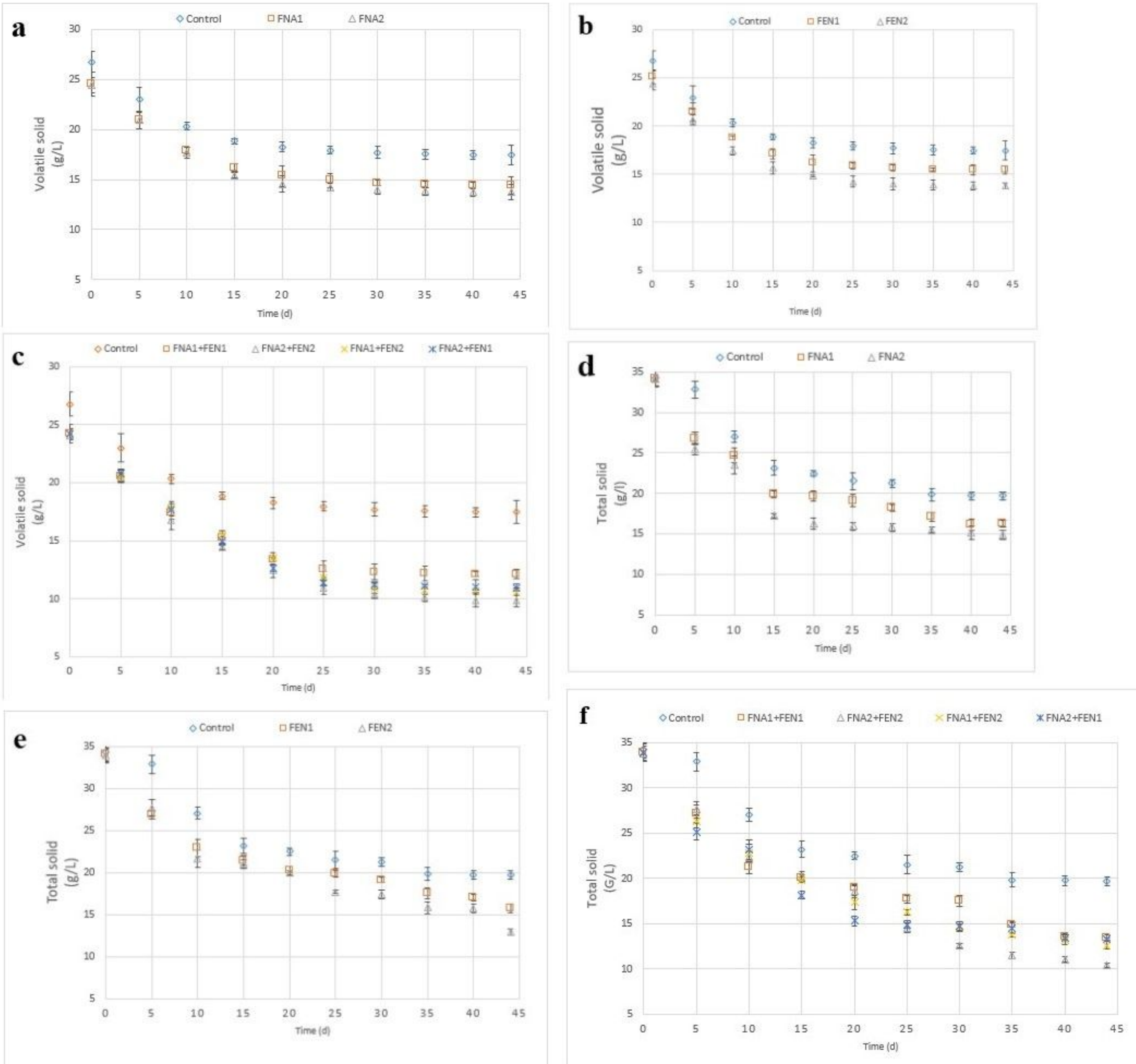

Figure 3

Sludge degradation during AD. Note: a, b and c demonstrate volatile solid degradation. d, e and f demonstrate total solid degradation. Error bars represent standard error from triplicate measurements.

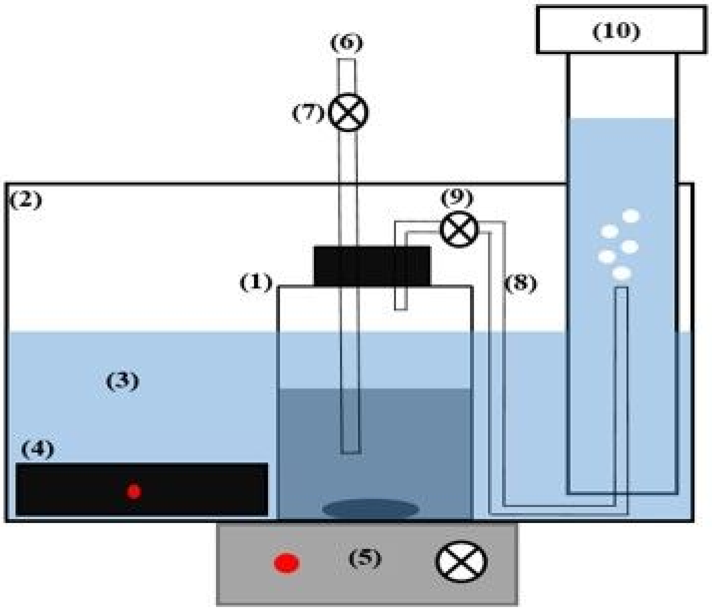

Figure 4

Schematic of the experimental system. 1) BMP reactor, 2) aquarium 3) saturated and acidified water, 4) automatic heater, 5) magnetic stirrer, 6) sampling pipe, 7) sampling control valve, 8) biogas collecting pipe, 9) biogas control valve, 10) graduated cylinder [10].

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