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Impact of Nanoparticles on Biogas Production from Anaerobic Digestion of Sewage Sludge

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ABSTRACT

Since anaerobic digestion (AD) is the preferred procedure for sludge treatment and disposal, it is constrained by the hydrolysis and acidogenesis stages. Nanomaterials have an impact on the AD process due to their unique properties (large specific surface areas, solubility, adsorp-tion reduction of heavy metals, degradation of organic matter, reduction of hydrogen supplied and catalytic nature) which make them advantageous in many applications due to their effective-ness in improving the AD efficiency. Magnetic Nanoparticles (MNPs) were used in the present study to improve the biogas production. The experiments were divided into two stages to evaluate the effect of adding MNPs to two types of sewage sludge (SS): attached growth process (AG) and activated sludge (AS). The first stage consists of 15 tests divided into three experiments (A, B, and C). Doses of MNPs (20, 50, 100, 200) mg/l were added to all digesters in the same experiment except for one digester (the control). Experiments A, B and C achieved the highest biogas production when 100 mg/l of MNPs was added. They were 1.9, 1.93 and 2.07 times higher than the control for A, B and C respectively. The second stage consists of 12 tests with a pretreatment for some of SS. It was divided into two experiments (D, E), where the chemical pretreatment was applied to experiment D and the thermal pretreatment was applied to experiment E except for the control. For digester D4, which had 100 mg/l of MNPs after a chemical pretreatment at pH = 12, the biogas production increased by 2.2 times higher than the control (D0) and 1.5 times higher than the untreated sludge with the addition of 100 mg/l MNPs (DN). Thermal pretreatment at 100 °C with addition of 100 mg/l MNPs (E4) achieved a biogas yield 2 times higher than the control (E0), and 1.39 times higher than untreated sludge with 100 mg/l MNPs (EN). The previous results indicate that the integration of magnetite can serve as the conductive materials, promoting inherent indirect electron transfer (IET) and direct interspecies electron transfer (DIET) between methanogens and fermentative bacteria which lead to a more energy-efficient route for interspecies electron transfer and methane productivity. This study demonstrated the positive effect of magnetite on organic biodegradation, process stability and methane productivity.

Keywords: biogas, methane, wastewater, sludge, anaerobic digestion, nanomaterial, magnetic nanoparticles

INTRODUCTION

In recent years, there has been a growing interest in the use of renewable energy. This not only reduce dependence on fossil fuels, but also mitigate the harmful impact on the environment caused by burning fossil fuels [Bamati and Raoofi 2020]. Greenhouse gases (GHGs), associated with burning fossil fuels or other sources such as natural decomposition of organic materials, have attracted public attention because of their effects on climate change [Shen et al. 2020]. The GHGs emissions from wastewater treatment plants (WWTPs) include methane (CH_4) that is emitted from sludge degradation and carbon dioxide (CO2) that is emitted during the production of the energy required for the plant operation [Campos et al. 2016]. The clean energy produced by anaerobic digestion (AD) is an effective alternative to fossil fuels [Chynoweth et al. 2001]. It is a typical biogas composition of digested sludge (CH_4, 50~70%) and (CO_2, 30~50%) [Shen et al. 2015].

Due to its efficient breakdown and low energy consumption, AD has become the preferred procedure for sludge treatment and disposal [Capodaglio and Olsson 2020]. This natural process produces biogas which is rich in CH_4. On the other hand, digested sludge is well-known as a fertilizer and a possible supply of organic compounds and nutrients (nitrogen (N_2) and phosphorus (P)) since it can effectively replace synthetic N2 and P fertilizers [Insam et al. 2015]. The pro-cess of AD is divided into four phases. These phases include hydrolysis, acidogenesis, acetogenesis, and methanogenesis [Van et al. 2020]. The hydrolysis includes breaking down higher molecular weight compounds into lower ones. Larger organic compounds such as pro-teins, carbohydrates, and lipids are hydrolyzed into smaller compounds such as amino acids, sugars, and long chain fatty acids [Wilson and Novak 2009]. Acidogenesis is termed as "fermentation", which is generally defined as an anaerobic acid-producing microbial process [Wang et al. 2021]. The amino and fatty acids resulting from hydrolysis are degraded to a number of simpler products, such as CO_2, H_2 and volatile fatty acids (VFA). During acetogenesis phase, VFA are broken down to form acetate, H_2, and CO_2. These products are also formed during the acidogenesis phase but the complete acid breakdown is achieved during acetogenesis. Acetate is the most important compound produced during the fermentation stage of the AD [Wainaina et al. 2019]. During methanogenesis, the fermentation products, such as acetate, H_2, and CO_2 are converted to CH_4 and CO2, by methanogenic organisms, which are strict obligate anaerobes. Methanogens cannot degrade complex compounds. They are dependent on the previous work of other organisms [Wainaina et al. 2019].

Although AD is a safe sludge treatment technology, but it is constrained by the hy-drolysis and acidogenesis stages, particularly the hydrolysis one [Syahri et al. 2022]. This results in low CH_4 production efficiency and energy recovery cannot be effectively achieved. Several factors affect the performance and stability the AD process, such as pH, soluble chemical oxygen demand SCOD, ammonia-nitrogen (NH_3) concentrations and microbial community [Zhou et al. 2019]. As a result, it is critical to eliminate limiting variables, and the boost methane output. So, many pretreatments and co-digestion concepts were employed in AD to en-hance biogas production and process stabilization [Zhang et al. 2014].

Since almost last two decades, the nanomaterials have found their way into sludge treatment as they can eliminate the effect of the limiting factors in the AD systems. Nanomaterials have an impact on the AD process due to their unique properties, such as the large specific surface area, solubility, adsorption reduction of heavy metals, degrada-tion of organic matter, reduction of hydrogen supplied (H_2S) and catalytic nature, which make them advantageous in many applications due to their ability to improve the AD efficiency as an electron donor [Holmes and Gu 2016]. Nanomaterials possess a capacity to penetrate through cell membranes and have high electron conductivity, which can enhance the ex-tracellular electron transport between exoelectrogenic bacteria and methanogenic archaea. This can, in turn, increase the CH_4 formation rate and reduce the lag phase [Ren et al 2020]. The positive effect of Fe_2^+ on methanogenesis is recognized since MNPs enhance methanogenic activity associated with accelerated organic degradation. MNPs have also been proved to advance methane production by promoting DIET in syntrophic methanogenesis [Li et al. 2014]. Iron oxide nanoparticles (Fe_3O_4) have been used and studied extensively over the last few years because of their super magnetic characteristics, unique electronic properties, high surface-to-volume ratios and catalytic properties [Reguera et al. 2013]. There are many other nanomaterial additives that have been studied such as cobalt and nickel, stainless steel, silver, gold, titanium, zinc oxide, cerium oxide and alumina [Choong et al. 2016, Volosova et al. 2019].

The hypothesis of this study is that using of MNPs affects positively on the biogas production since they are easy to use in practical applications. MNPs are a mixed valence magnetic mineral containing both Fe_2^+ and Fe_3^+. MNPs release ferrous or ferric irons, improve electron transport efficiency, boost enzyme activity during methanogenesis, and enhance CH_4 generation [Arya et al. 2021]. Ferrous iron and ferric iron supply nutrition to microorganisms while inhibiting sulfur irons and lowering the inhibitory action of sulfate-reducing bacteria on AD [Zhu et al. 2021]. This research aims to explore the effect of MNPs with different doses on two types of SS, tracing the different conditions to find the optimal dose that gives the best production of biogas. The present study is undertaken with the following specific objectives: to study the effect of adding MNPs to various types of SS (AS and AG) on retention time (RT) and biogas production, to compare between mesophilic and ambient temperature conditions on RT and biogas production, to examine the effect of thermal pretreatment on RT and biogas production and to study the effect of changing pH value (chemical pretreatment) on RT and biogas production of the AD.

MATERIALS AND METHODS

Raw materials

The used additive included Magnetite (Iron (IV) Oxide Nanoparticles) MNPs, (a di-ameter of 25 nm, purity N 99.5%), as reported by the manufacturer (Co., Cairo, Egypt). Two types of wastewater sludge were used in the first stage of the study. The thick-ened sludge was collected from two WWTPs located in Zagazig, Egypt. The first plant (Qenayat municipal WWTP) represents the attached growth process while the second one (Taybah municipal WWTP) represents the activated sludge process. In the second stage, the sludge used was obtained from the Qenayat municipal WWTP. The physicochemical properties of each stage are shown in Table 1.

Experimental design

In the present study, 27 bio-methane potential [BMP] tests were used. Each group of five digesters was placed in the same water bath. Figure 1 shows a schematic diagram for the used model. The first stage included 15 runs while the second was 12 runs. All experiments were tested in triplicate. Stage one aimed to determine the optimal dosage of MNPs that can be added to improve biogas production, to detect the effectiveness of adding MNPs to different types of sludge, and to compare between mesophilic and am-bient temperature depending on RT and biogas production. This stage consisted of three experiments. Each experiment includes five digesters. The first experiment (A) quantified the effect of MNPs addition on AD of sludge from (AG) process using four MNPs concentrations (20, 50, 100 and 200 mg/l) at 35 °C, and the second experiment (B) quantified the effect of MNPs addition on AD of (AS)

Table 1. Characteristics of raw sludge

Parameters	Stage one		Stage two
	Experiments A and C	Experiment B	Experiments D and E
TCOD (mg/l)	20,000	27,000	24,794
SCOD (mg/l)	1,005	1,155	480
TS (mg/l)	19,263	83,330	23,950
VS (mg/l)	14,236	43,890	11,550
N (mg/l)	2,300	1,000	2,100
C (mg/l)	35,000	12,000	37,000
C/N	15.22	12	17.6
VFA (mg/l)	1,665	770	1,137
pH	5.84	6.62	7.2

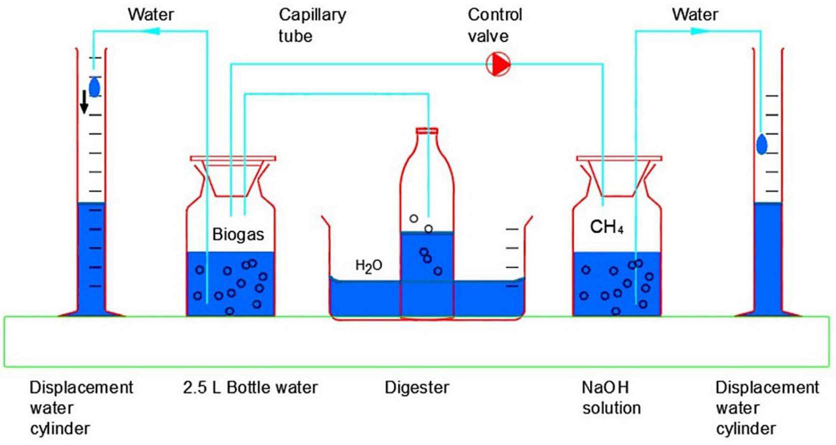

Figure 1. Schematic diagram for the used experimental model

using four MNPs concentrations (20, 50, 100 and 200 mg/l) at 35 °C. On the other hand, the third experiment (C) was the same as the experiment A, but at 25 °C as shown in Table 2. In stage two, the optimal dosage of MNPs in the first stage was used to study the effect of thermal and chemical pretreatment on gas production. This stage consisted of two experiments. Each experiment includes six digesters. The first experiment (D) determined the effect of adding the optimal dose of nanoparticles to chemical pretreated SS at different pH (9, 10, 11, 12) to produce biogas. The second experiment (E) determined the effect of adding the optimal dose of nanoparticles to thermally pretreated SS at different temperatures (50, 70, 90, 100 °C) to produce biogas as shown in Table 3. The batch experiments were conducted in 1,000 ml bottles. The MNPs was added in different concentrations to 500 ml of thickened sludge. For all digesters, except for the control one, small amounts of Na2CO3 powder, ranging from 0.10 to 0.60 g were added to prevent any critical drop in pH. Each bottle was sealed tightly, using a silicone layer. All bottles were shaken (manually) and immersed up to half of their height in a hot water path, kept at a constant temperature of 35 ± 1 °C (except for ambient temperature runs). Each bottle was connected by a capillary tube to a 2,500 ml glass bottle containing only water for biogas measurement. Each water-filled bottle (2,500 ml) was connected to another one of the same capacity (containing alkaline solution (2% NaOH) and sealed in the same way).

Table 2. Operating conditions of stage 1

Experiment	Digester	T (°C)	pH	MNPs dose (mg/l)	Sludge type
Exp. A	A0	35 ± 1	6.5 - 7.5	0.0	Attached growth process (AG)
	A1	35 ± 1	6.5 - 7.5	20.0	Attached growth process (AG)
	A2	35 ± 1	6.5 - 7.5	50.0	Attached growth process (AG)
	A3	35 ± 1	6.5 - 7.5	100.0	Attached growth process (AG)
	A4	35 ± 1	6.5 - 7.5	200.0	Attached growth process (AG)
Exp. B	B0	35 ± 1	6.5 - 7.5	0.0	Activated sludge process (AS)
	B1	35 ± 1	6.5 - 7.5	20.0	Activated sludge process (AS)
	B2	35 ± 1	6.5 - 7.5	50.0	Activated sludge process (AS)
	B3	35 ± 1	6.5 - 7.5	100.0	Activated sludge process (AS)
	B4	35 ± 1	6.5 - 7.5	200.0	Activated sludge process (AS)
Exp. C	C0	25 ± 1	6.5 - 7.5	0.0	Attached growth process (AG)
	C1	25 ± 1	6.5 - 7.5	20.0	Attached growth process (AG)
	C2	25 ± 1	6.5 - 7.5	50.0	Attached growth process (AG)
	C3	25 ± 1	6.5 - 7.5	100.0	Attached growth process (AG)
	C4	25 ± 1	6.5 - 7.5	200.0	Attached growth process (AG)

Table 3. Operating conditions of stage 2

Chemical pretreatment				
Experiment	Digester	pH	T °C	MNPs dose
Exp. D	D0	6.5-7.5	35 ± 1	Without MNPs
	DN	6.5-7.5	35 ± 1	The optimum dose of the first phase
	D1	9	35 ± 1	The optimum dose of the first phase
	D2	10	35 ± 1	
	D3	11	35 ± 1	
	D4	12	35 ± 1	

Thermal pretreatment							
Experiment	Digester	Thermal pretreatment		Cooling	Operating conditions		MNPs dose
		T °C	Time (min)	T °C	pH	T °C	
Exp. E	E0	Without pre-treatment		_	6.5–7.5	35 ± 1	Without MNPs
	EN	Without pre-treatment		_	6.5 - 7.5	35 ± 1	The optimum dose of the first phase
	E1	50	30	20	6.5 - 7.5	35 ± 1	The optimum dose of the first phase

The produced gas replaced the water in the 2,500 ml bottle, moving it to a graduated 1,000 ml bottle where the gas produced could be measured. Then, the control valve opened to pass the biogas through the capillary tube into a bottle containing a 2% NaOH solution.

All measurements were conducted according to standard methods. The pH was de-termined, using a pH meter (PHS-25, Rex, Shanghai, China). Total chemical oxygen demand (TCOD) and soluble chemical oxygen demand (SCOD) were tested, using the potassium dichromate-silver sulfate method. The biogas volume production was deter-mined using a water displacement unit. The amount of methane in biogas was measured by adding 2% NaOH to the water to dissolve CO_2. The volume of methane was measured 5 times during the experiment.

RESULTS AND DISCUSSION

Stage one

Effect of MNPs on biogas production

Figures (2, 3, 4) show the cumulative biogas production (ml) for experiments A, B and C while figures (5, 6, 7) indicate the shifts in the daily biogas yield with distinct treatments during the 30 days. In general, the range of daily biogas production fluctuated during the digestion period. The amount of cumulative biogas generated varied because of different MNPs dosages, taking the following order (from high to low): 200 mg/l (A4, B4, or C4), 100 mg/l (A3, B3, or C3), 50 mg/l (A2, B2, or C2), 20 mg/l (A1, B1, or C1), 0.0 mg/l (A0, B0, or C0). The range of cumulative biogas yields of AG sludge in experiment A varied from 2402 ml to 4721 ml as shown in Figure 2. Adding 100 mg/l of MNPs (A3) could maximize the cumulative biogas yield of AD. The cumulative biogas yield increased by 96% for A3 in comparison with the A0. However, when the dosage of MNPs increased to 200 mg/l (A4), the cumulative biogas yield was only 18.5% higher than that of the A0.

For experiment B, the cumulative biogas production in AD showed a trend of increasing, followed by a decrease with the increase in MNPs dosage as shown in Figure 3. Under different MNPs dosages, the order of cumulative biogas productions was roughly as fol-lows: 100 mg/l (B3) > 50 mg/l (B2) > 20 mg/l (B1) > 200 mg/l (B4) > 0 mg/l (B0) mg/l. The cumulative biogas yields ranged from 4740 ml to 9189 ml in experiment B, and 100 mg/l (B3) represented the optimum dosage in this experiment.

For experiment C, the range of cumulative biogas yields varied from 1927 ml to 3998 ml as shown in Figure 4. Adding 100 mg/l of MNPs (C3) could maximize the cumulative biogas yield of AD. The cumulative biogas yield increased by 107% for C3 in comparison with C0. At 200 mg/l MNPs (C4), the cumulative biogas yield

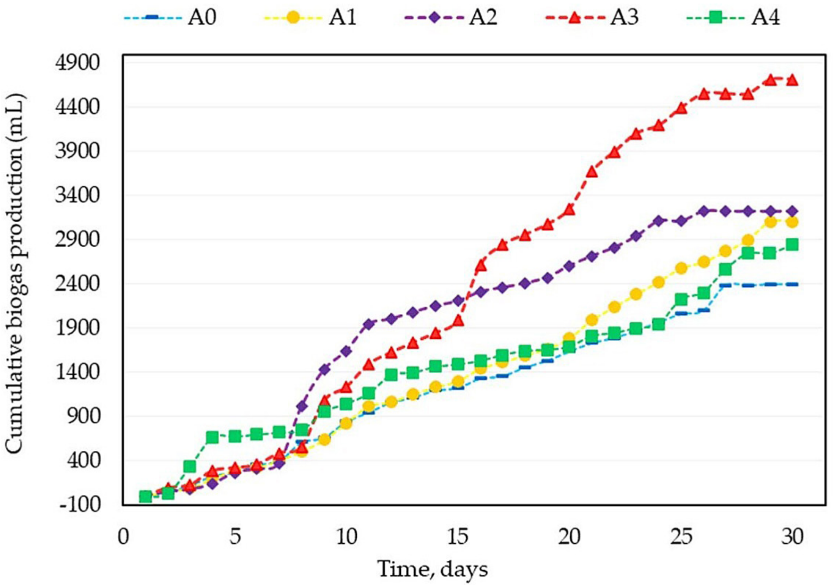

Figure 2. Cumulative biogas production (ml) for experiment A [results for sludge from trickling filter plant (at 35 °C and pH= 6.5-7.5) for: A0 (no MNPs Dose), A1 (20.0 mg/l MNPs Dose), A2 (50.0 mg/l MNPs Dose), A3 (100 mg/l MNPs Dose), and A4 (200 mg/l MNPs Dose)]

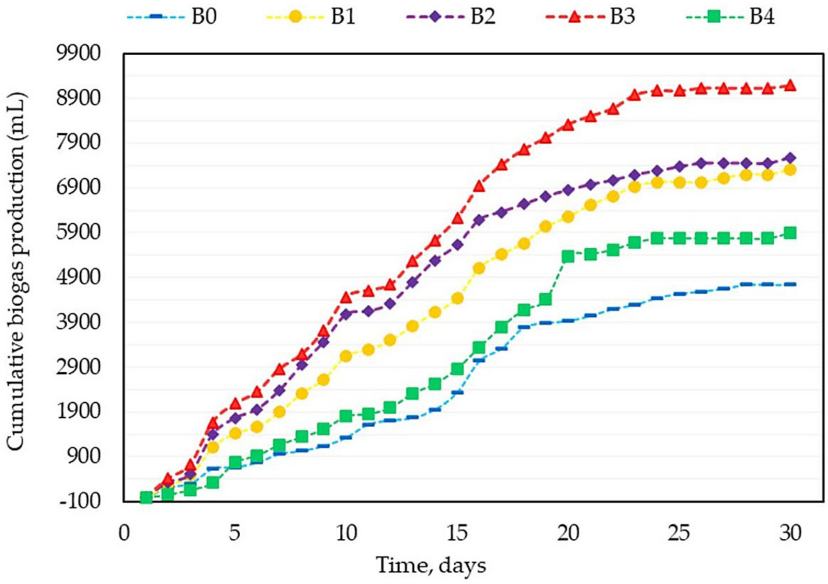

Figure 3. Cumulative biogas production (ml) for experiment B [results for sludge from activated sludge process (AS) (at 35 °C and pH= 6.5-7.5) for: B0 (no MNPs Dose), B1 (20.0 mg/l MNPs Dose), B2 (50.0 mg/l MNPs Dose), B3 (100 mg/l MNPs Dose), and B4 (200 mg/l MNPs Dose)]

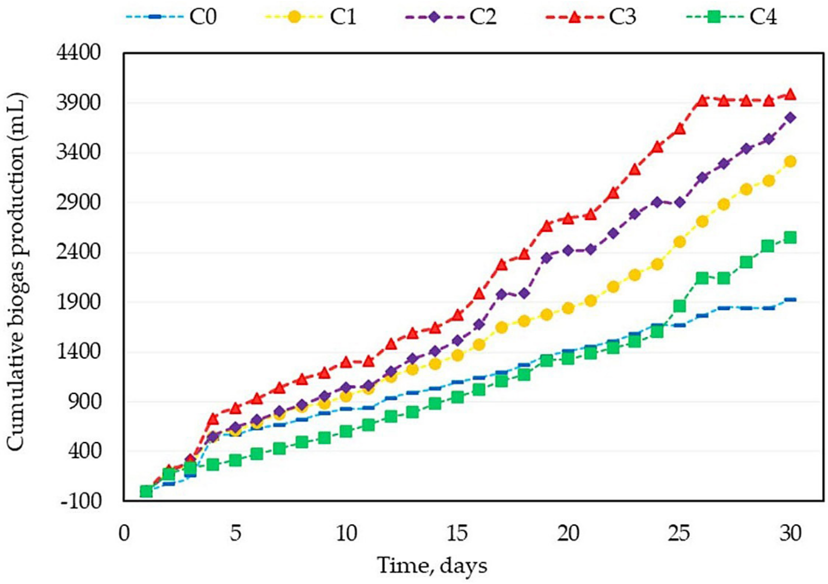

Figure 4. Cumulative biogas production (ml) for experiment C [results for sludge from trickling filter plant (at 25 °C and pH= 6.5-7.5) for: C0 (no MNPs Dose), C1 (20.0 mg/l MNPs Dose), C2 (50.0 mg/l MNPs Dose), C3 (100 mg/l MNPs Dose), and C4 (200 mg/l MNPs Dose)]

was only 32.6% higher than that of the C0. This result was consistent with earlier studies which suggested that adding MNPs could enhance biogas production; however, excessive MNPs addition can damage the integrity of cells and inhibit methane production. For example, Wu et al. reported that methane production was positively correlated with Zero valent iron (ZVI) addition [Wu et al. 2015]. At the same time, a high dosage of ZVI weakened the promotion of ZVI on the AD of swine wastewater. In the same context, some researchers observed that the methane yield rate increased by 44% due to the DIET promotions by MNPs addition [Al-Essa 2020]. In a typical experiment, when 100 mg/l of Fe_3O_4 were introduced into an anaerobic waste treatment reactor, the biogas production per gram of organic matter increased up to 180%, which is the largest improvement in biogas production [Casals 2015].

The daily biogas production curve for experiment A is presented in Figure 5. These results indicate that the production of biogas began on the second day and increased until day 16. After that, there was an abrupt decrease in biogas production until the end of the experimental period. Furthermore, all MNPs dosage reduced the time required to reach the highest biogas yields in comparison with the control sample. The maximum daily biogas yield (208 ml) for the control (A0) was achieved on day twelve while the maximum daily biogas yield for reactors A1, A2, A3 and A4 (580, 210, 620, and 324 ml respectively) was achieved on the fourth day (for A1 and A2) and on the third day (for A3 and A4).

A similar trend was observed for experiment B since the highest amount of biogas was achieved on the eight day and it was 714 ml. On the other hand, the maximum daily bi-ogas yield was 630, 880, 940, and 683 for reactors B1, B2, and B4 respectively, and it was achieved on the fourth day as shown in Figure 6.

For experiment C, the maximum biogas production for control (C0) was 107 ml, which was achieved on the thirteenth day. In contrast, the highest production for C1 was on the fourth day,

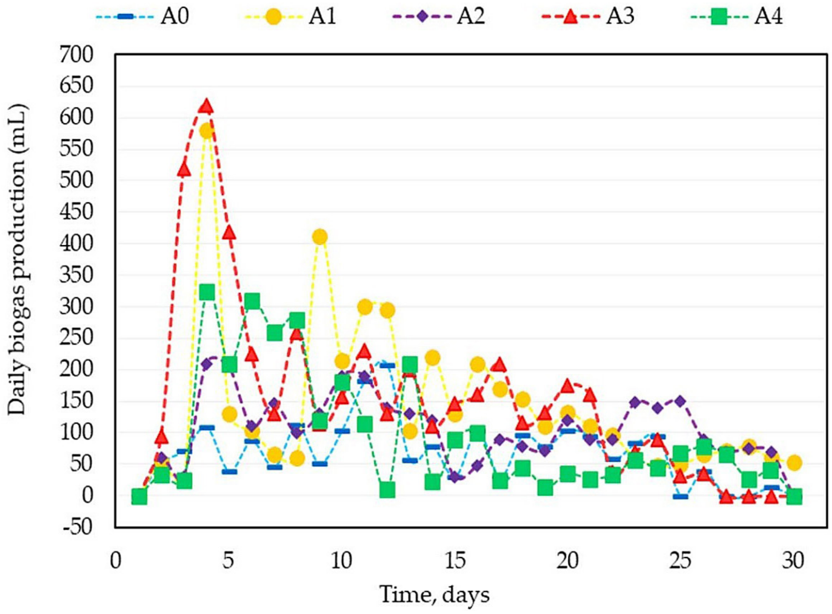

Figure 5. Daily biogas production (ml) for experiment A [results for sludge from trickling filter plant (at 35 °C and pH= 6.5-7.5) for: A0 (no MNPs Dose), A1 (20.0 mg/l MNPs Dose), A2 (50.0 mg/l MNPs Dose), A3 (100 mg/l MNPs Dose), and A4 (200 mg/l MNPs Dose)]

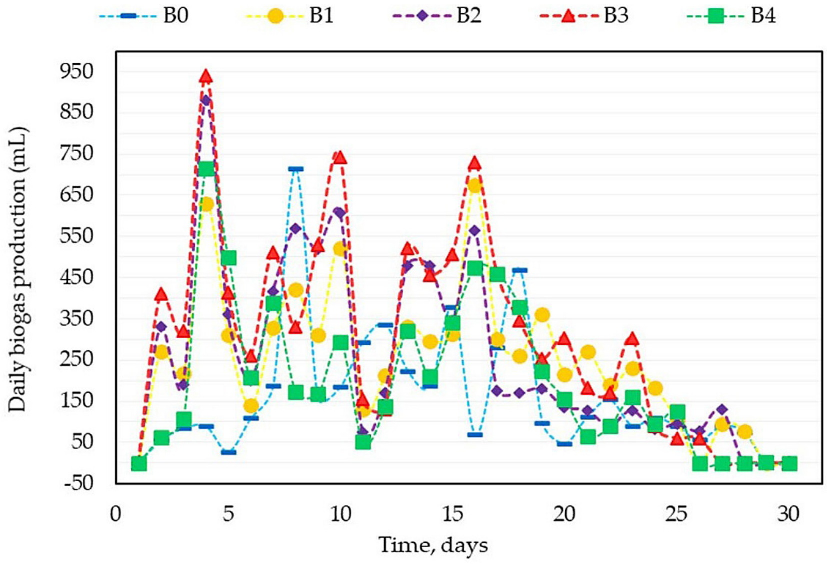

Figure 6. Daily biogas production (ml) for experiment B [results for sludge from Activated sludge process (at 35 °C and pH= 6.5-7.5) for: B0 (no MNPs Dose), B1 (20.0 mg/l MNPs Dose), B2 (50.0 mg/l MNPs Dose), B3 (100 mg/l MNPs Dose), and B4 (200 mg/l MNPs Dose)]

and reactors C2, C3 and C4 were achieved on the fifth, the third and the ninth days respectively as shown in Figure 7. These results indicated that the best concentration of MNPs was 100 mg/l, which reduced the lag phase and the time to reach the peak of biogas production. Furthermore, a positive effect on the anaerobic process was observed when 100 mg/l MNPs were added to the substrate. When comparing the results of cumulative biogas for the experiments (A, B, and C) with the addition of 100 mg/l of MNPs after 30 days, experiment B was 94% more than experiment A. The difference may be due to the different properties of the sludge [Fonts et al. 2009]. Experiment A was 18% higher than experiment C because microbial activity generally increases at higher temperature up to the optimum degree in case of methanogens. However, there are two optimum temperature ranges for intermediate conditions (32-37 °C) [Choorit and Wisarnwan 2007]. Comparing the daily production for each dose, it can be noticed that the production decreases and increases randomly and it sometimes stops completely. That is obvious within locating the result on the graph since the graphs have a lot of ups and downs.

Effect of MNPs on methane production

During AD, the primary goal is to recover methane gas. Methane is the key factor for better appreciating the AD process. Figure 8 shows the cumulative methane production (ml) for experiments A, B and C. The methane yields were (1393, 1898, 2160, 3399 and 1565) ml for (A0, A1, A2, A3 and A4), (2651, 4246, 4794, 6330 and 3012) ml for (B0, B1, B2, B3 and B4) and (1116, 2044, 2252, 2825 and 1415) for (C0, C1, C2, C3 and C4). It is worth noting that the addition of MNPs not only increased biogas production but also enhanced the methane content. Concretely, the CH4 percentage in the biogas ranged from 58% to 72% in experiment A, from 56% to 68.8% in experiment B, and from 58% to 70.6% in case of experiment C. Feng et al. reported that nano zero valent iron (NZVI) availably promoted the decomposition of two major sludge compositions: protein and total polysacharide [Feng et al. 2014]. The degradation of total polysaccharide increased by 29.6% with NZVI added in the hydrolysis-acidification experiment. The remaining biogas was mainly CO2 while H2 content was <1%. The results also show that the addition of 100 ppm MNPs for experiments A, B, and C yielded the highest methane yield (3399 ml, 6330, and 2825 ml). This is consistent with the results of Zhang et al. who reported that the optimal dosage of Fe3O4 nanoparticles was 100 mg/L as it could increase the methane yield by 58.7% in comparison with that of the control sample [Zhang et al. 2020]. Also, Ajay et al. observed an increase in methane production by 38% compared to the control sample when adding Fe_3O_4 at a concentration of 750 mg/l to the seed sludge [Ajay et al. 2020]. Farghali et al. indicated that methane yield

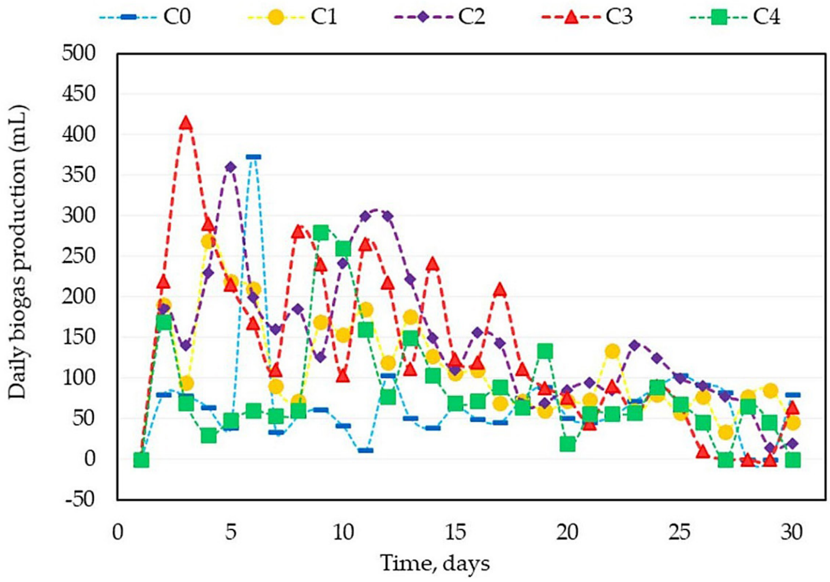

Figure 7. Daily biogas production (ml) for experiment C [results for sludge from trickling filter plant (at 25 °C and pH= 6.5-7.5) for: C0 (no MNPs Dose), C1 (20.0 mg/l MNPs Dose), C2 (50.0 mg/l MNPs Dose), C3 (100 mg/l MNPs Dose), and C4 (200 mg/l MNPs Dose)]

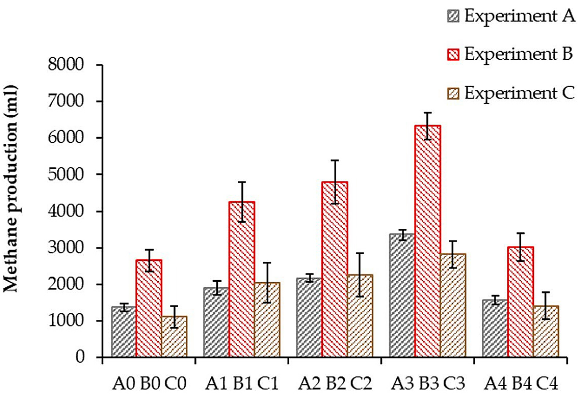

Figure 8. Cumulative methane production (ml) for experiments A, B, and C, since (A0, B0 and C0) represent sewage sludge without MNPs (control), (A1, B1 and C1) represent sewage sludge with 20 mg/l MNPs, (A2, B2 and C2) represent sewage sludge with 50 mg/l MNPs, (A3, B3 and C3) represent sewage sludge with 100 mg/l MNPs, and (A4, B4 and C4) represent sewage sludge with 200 mg/l MNPs

increased by 36.99% with 100 mg/l microscale waste iron powder addition when the substrate was dairy manure and the RT was 30 days [Farghali et al. 2020]. Al-Essa also observed that methane yield rate increased by 44% as a result of the DIET promotions by MNPs addition [Al-Essa 2020]. Also Baek et al. reported that ferric oxyhydroxide and magnetite significantly enhanced CH_4 production during AD of dairy wastewater [Baek et al. 2015]. This was likely because Fe(III) could stimulate dissimilatory iron(III) reduction combined with the oxidation of the complicated matters of sludge, which facilitated sludge destruction and hydrolytic acidification and then accelerate the CH_4 generation [Jiang et al. 2013]. The differences in the results of these studies may be attributed to differences in the sizes of NPs, substrates, and experimental conditions [Farghali et al. 2019]. The results showed that using MNPs can not only increase methane production, but can also decrease the number of days required to reach peak methane production [Hassanein et al. 2019]. The addition of MNPs could promote methanogenesis by the enhancement of the syntrophic effect of methanogen [Cheng et al. 2020]. Slonczewski et al. indicated that the homeostasis of iron is essential for all life forms, especially the archaea microorganisms [Slonczewski et al. 2009]. Iron ions (including Fe_2^+ and Fe_3^+) are fundamental in vital functions, such as power generation and DNA replication [Maiti et al. 2015]. Also, iron ions released by iron nanoparticles can infiltrate into the interior of cells and promote the synthesis of key enzymes and the growth of microorganism, especially methanogens [Maiti et al. 2015]. Due to the easiness to obtain or lose electrons, Fe_3O_4 can be used as cytochrome and ferredoxin to participate in the energy metabolism of methyl-trophic methanogens and reduce CO_2 to CH_4 by autotrophic methanogens [Zhang et al. 2019]. Although iron is essential, it is toxic at higher concentrations. So, adding an appropriate concentration of ions in the anaerobic reactor can increase biogas production. However, at a higher dosage, MNPs have a depressant effect on the AD process.

Sludge reduction

Sludge reduction is another parameter to evaluate the performance of anaerobic digestion. Most organic matters from the sludge were decomposed and mineralized after the AD process. TCOD, TS, and VS are frequently used to characterize the sludge reduction rate. The removal of organics was consistent with the production of biogas. After 30 days of the experiment A, the effluent TCOD values for reactors A0, A1, A2, A3 and A4 were 15 035, 14 039, 13 890, 11 551 and 14 892 mg COD/L respectively as shown in Figure 9. The removal ratios of TCOD in reactors A0, A1, A2, A3 and A4 were 24.8%, 29.8%, 30.5%, 42.2% and 25.5% respectively. The effluent TS values for reactors A0, A1, A2, A3 and A4 were 14800, 12800, 12100, 11800, and 13500 mg/l respectively. Accordingly, in reactors A0, A1, A2, A3 and A4 the removal ratios of TS were 23.2%, 33.5%, 37.2%, 38.7% and 29.9 respectively, and the VS removal ratios were 19.4%, 24.2%, 26%, 35.7% and 20.9% respectively. For the experiment B, the effluent TCOD, TS and VS is shown in Figure 10. The removal efficiency of TCOD for reactors B0, B1, B2, B3 and B4 was 32.2%, 46.5%, 47.5%, 61.8% and 40.5% respectively. On the other hand, the removal efficiency of TS for reactors B0, B1, B2, B3 and B4 was 18.3%, 25.7%, 36.2%, 37.8% and 20.4% respectively, and the VS removal ratios for reactors B0, B1, B2, B3 and B4 were 13.3%, 19.7%, 23.7%, 21.5% and 16.9% respectively. For the experiment C, the effluent TCOD, TS and VS is shown in Figure 11. The removal efficiency of TCOD for reactors C0, C1, C2, C3 and C4 was 17.8%, 31.3%, 35.3%, 37.2% and 24.8% respectively. On the other hand, the removal efficiency of TS for reactors C0, C1, C2, C3 and C4 was 14.6%, 19.2%, 35.4%, 36.2% and 18.5%, and the VS

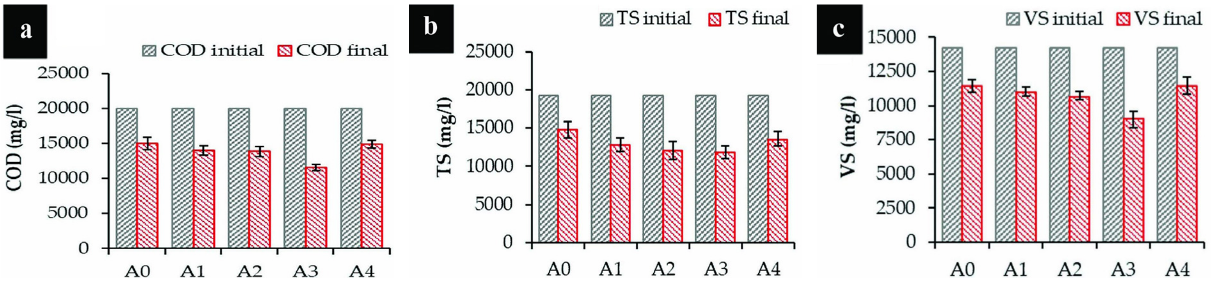

Figure 9. TCOD, TS and VS concentrations for Experiment A before and after the digestion [re-sults for sludge from trickling filter plant (at 35 °C and pH= 6.5-7.5) for: A0 (no MNPs Dose), A1 (20.0 mg/l MNPs Dose), A2 (50.0 mg/l MNPs Dose), A3 (100 mg/l MNPs Dose), and A4 (200 mg/l MNPs Dose)]

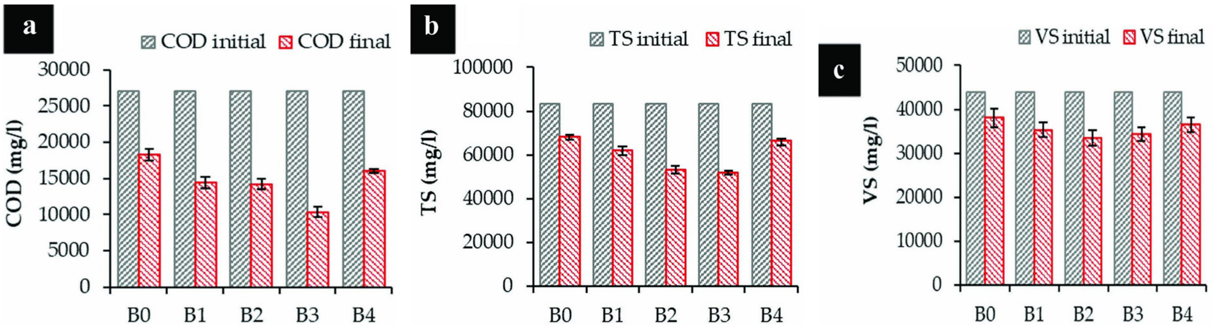

Figure 10. TCOD, TS and VS concentrations for Experiment B before and after the digestion [re-sults for sludge from activated sludge process (at 35 °C and pH= 6.5-7.5) for: B0 (no MNPs Dose), B1 (20.0 mg/l MNPs Dose), B2 (50.0 mg/l MNPs Dose), B3 (100 mg/l MNPs Dose), and B4 (200 mg/l MNPs Dose)]

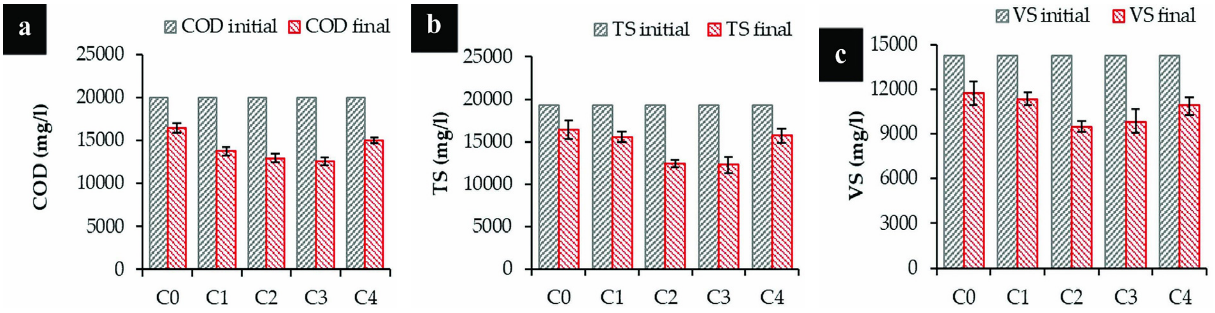

Figure 11. TCOD, TS and VS concentrations for Experiment C before and after the digestion [re-sults for sludge from trickling filter plant (at 25 °C and pH= 6.5-7.5) for: C0 (no MNPs Dose), C1 (20.0 mg/l MNPs Dose), C2 (50.0 mg/l MNPs Dose), C3 (100 mg/l MNPs Dose), and C4 (200 mg/l MNPs Dose)]

removal ratios for reactors C0, C1, C2, C3 and C4 were 17.5, 20.2%, 33.3%, 30.9% and 23.3% respectively. This finding indicated that the reactors combined with MNPs presented better organic removal and sludge reduction performance than the control reactors. The high removal rate of TCOD, TS and VS indicated that MNPs were able to promote the AD process and the anaerobic microbial activity. This contributes to the fact that MNPs can be used as an electron donor by releasing Fe_2^+ into the anaerobic system and, in turn, accelerating the hydrolysis and fermentation process [Cruz 2020].

Effect of adding MNPs on pH

For all digesters, the pH values at the end of experiments A, B and C were (7.5–7.9), (7.87–8) and (7.83–7.93) respectively, as shown Figure 12. This range is within the appropriate range for the action of methanogens (6.8–8). The digesters containing MNPs recorded higher pH values than the control. pH is a main parameter affecting the stability of anaerobic processes. It is considered a limiting factor for methanogenesis and it should be in the range 6.8–8.0 to avoid the inhibition phenomena [Siciliano et al. 2019]. The pH value

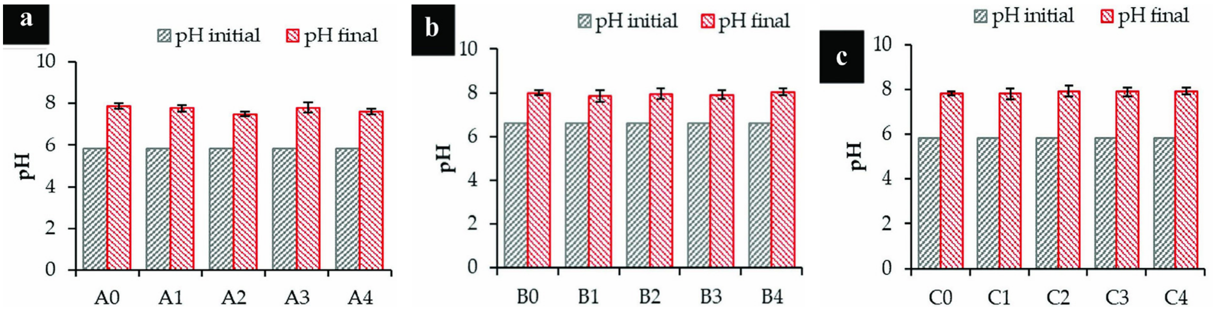

Figure 12. Effect of adding MNPs on pH values for the Experiments A, B and C, since (A0, B0 and C0) represent sewage sludge without MNPs (control), (A1, B1 and C1) represent sewage sludge with 20 mg/l MNPs, (A2, B2 and C2) represent sewage sludge with 50 mg/l MNPs, (A3, B3 and C3) represent sewage sludge with 100 mg/l MNPs, and (A4, B4 and C4) represent sewage sludge with 200 mg/l MNPs

is influenced by the nature of the substrate used and the biochemical process in the digester. Increased pH is an indication of degradation of offensive smelling VFAs [Christy et al. 2014].

Stage two

Because pretreatment suppresses methanogen activity [Angelidaki et al. 2006], the best dose of MNPs was added to the pretreated sludge in order to investigate the synergistic effect of pretreatment with MNPs on biogas production and methanogenic activity recovery.

Effect of chemical pre-treatment on the solubilization (SCOD) and sludge disintegration (DDCOD) of organic matters

The efficiency of sludge disintegration can be evaluated by a disintergration degree (DDCOD), which is calculated as Eq. (1) [Bougrier et al. 2005]. DDCOD is a key parameter to evaluate the release of soluble organics from the sludge solids to the liquid phase.

$$DD\_COD = (SCOD–SCOD\_0)/ /(TCOD–SCOD\_0)\cdot 100 \qquad (1)$$

where: SCOD0 is the SCOD of sludge before treatment (raw sludge).

Figure 13 shows the effect of chemical pretreatment on the concentration of SCOD in sludge at different pH values. SS was effectively disintegrated by the chemical pre-treatment. Both SCOD and DDCOD rapidly increased with increasing the pH value. The DDCOD and SCOD almost linearly increased within the pH range 9–12. At pH = 12, the DDCOD (%) and SCOD of sludge, after chemical pretreatment, reached 49.8% and 12 600 mg/l respectively. Previous results indicate that the chemical pretreatment had a strong effectiveness in sewage sludge disintegration.

Effect of chemical pretreatment on biogas production

In stage two, the best dose (100 mg/l) of MNPs of the first stage was used to study the effect of chemical pretreatment on gas production. The sludge was pretreated with NaOH (2% concentration) to get a pH range of (9–12). The effect of pH on the cumulative biogas yield is shown in Figure 14. The cumulative biogas values were 3326, 4382, 4704, and 4910 ml in reactorsD1, D2, D3 and D4 respectively, while the cumulative biogas values of D0 and DN were 2187 and 3233 ml. D4 recorded the highest cumulative biogas yield (4910 ml). This value was 124.5% and 52% more thanD0 and DN respectively. For the DN alone, biogas production was 47.8% more than D0. Similar results were obtained by Almokhtar et al. since they showed that alkaline pretreatment caused a significant increase in biogas production, which was highly observed at pH= 11 compared

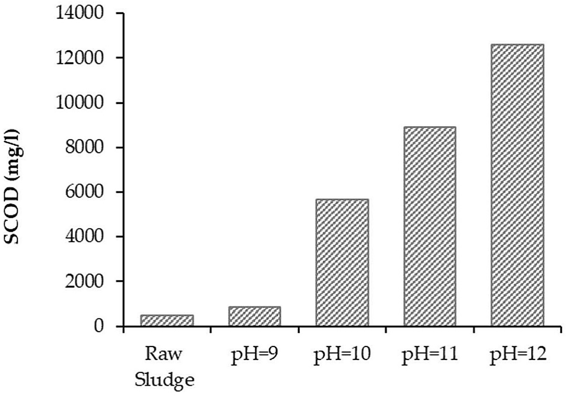

Figure 13. SCOD concentration after chemical pretreatment with different pH values

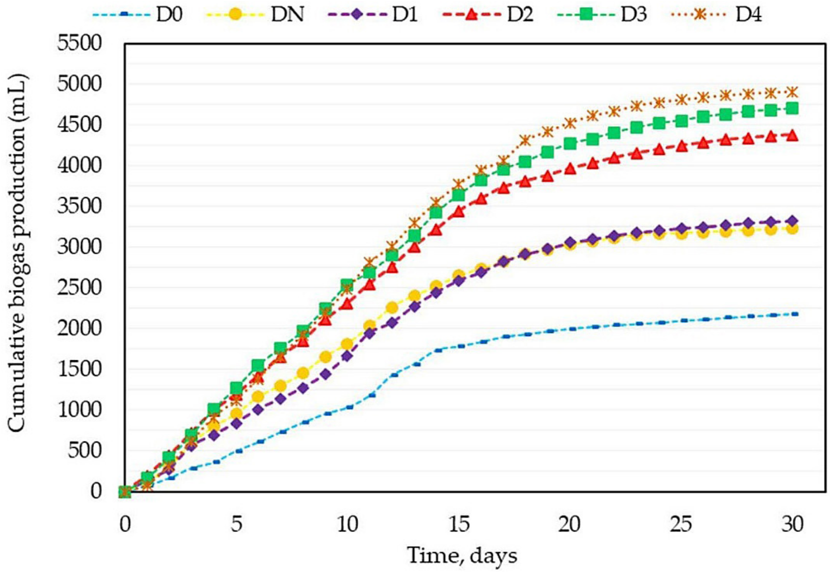

Figure 14. Cumulative biogas production for experiment D [results for sludge from trickling filter plant at 35 °C for: D0 (no MNPs dose, no pretreatment), DN (100 mg/l MNPs dose without pretreatment), D1 (100 mg/l MNPs dose with alkaline pretreatment (pH=9)), D2 (100 mg/l MNPs dose with alkaline pretreatment (pH=10)), D3 (100 mg/l MNPs dose with alkaline pretreatment (pH=11)), D4 (100 mg/l MNPs dose with alkaline pretreatment (pH=12))]

to other conditions [Almokhtar et al. 2012]. Also, Weiland found that biogas production increased with increasing pH value [Weiland 2010]. This is because the NaOH used in the pretreatment process was converted to Na^+ and OH^- and when the pH of sludge samples increased, the bacterial surfaces became increasingly negatively charged [Mbulawa 2017].

Effect of chemical pretreatment on methane production

The effect of pH on cumulative methane yield is shown in Figure 15. The methane yields were 1183, 2068, 2163, 2995, 3212 and 3561 ml for D0, DN, D1, D2, D3 and D4 re-spectively. The highest methane yield was at D4, which was 201% higher than D0. In contrast, DN achieved 74.8% more than D0. The results indicate that chemical pre-treatment with MNPs can increase the methane yield by more than two folds compared to the control sample (D0). These results also indicate that it is possible to obtain more bioenergy from the same amount of SS when MNPs are added to the pretreated sludge. In this context, Lu et al. pretreated rice straw (RS) with 1% NaOH and added 5% (w/w) of iron oxide-zeolite mixture during anaerobic digestion [Lu et al. 2017]. They found that the methane yield (394.4 ml/g VS) was higher (372.85%) than untreated RS (83.05 ml/g VS). Also, Lu et al. observed 2.16 times increase in the methane yield (302.5 ml/g VS) for cattle dung slurry at 20 mg/l of magnetite nanoparticles as compared to the control sample (140.3 ml/g VS) [Lu et al. 2018]. The combined treatment method has a significant impact on methane production compared to each type of treatment alone. The higher methane yield for pretreated sludge, using NaOH dosed with MNPs, might have been

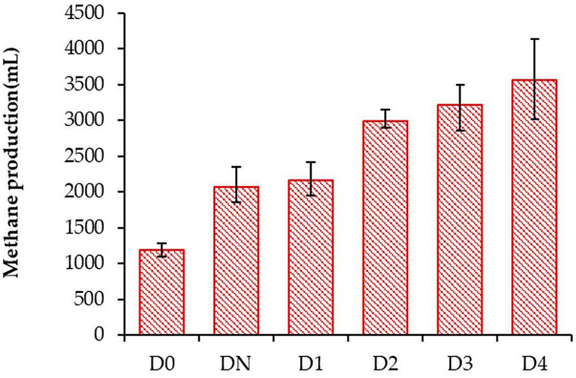

Figure 15. Effect of chemical pre-treatment with 100 mg/l MNPs on methane production for experiment D [results for sludge from trickling filter plant at 35 °C for: D0 (no MNPs Dose, no pretreatment), DN (100 mg/l MNPs dose without pretreatment), D1 (100 mg/l MNPs dose with alkaline pretreatment (pH=9)), D2 (100 mg/l MNPs dose with alkaline pretreatment (pH=10)), D3 (100 mg/l MNPs dose with alkaline pretreatment (pH=11)), D4 (100 mg/l MNPs dose with alkaline pretreatment (pH=12))]

due to the enhanced interspecies electron transfer mechanism between acetogenic and methanogenic bacteria, resulting in the increased conversion of VFAs to methane [Ahmed et al. 2022, He et al. 2021]. Iron is also very effective for H2S control within reducing sulfide toxicity to methanogens through its precipitation in the AD, which aids in enhancing the methane yield [Dykstra and Pavlostathis 2021].

Synergistic effect of alkaline pretreatment and MNPs on solids removal

Concentrations of TCOD, TS and VS in experiment D, before and after the digestion, are shown in Figure 16. After 30 day for the experiment D, the effluent TCOD, TS and VS values for D0, DN, D1, D2, D3 and D4 were (14892, 15359 and 6766), (11435, 11958 and 5060), (10048, 10843 and 4536), (8780, 7966 and 3283), (5733, 6573 and 2300), (3520, 3750 and 1740) mg /L respectively as shown figure 16. The removal ratio of TCOD, TS and VS for reactors D1, D2, D3, and D4 were (59.4, 54.7 and 60.7%), (64.5, 66.7 and 71.5%), (76.8, 72.5 and 80 %), (85.8, 84.3 and 84.9%) respectively. On the other hand, the removal per-centages of TCOD, TS, VS in DN and D0 were (53.8, 50 and 56.1%) and (39.9, 35.8 and 41.4%) respectively. These results indicate that alkaline pretreatment with MNPs removed certain parts of the organic matter from sludge [Júnior et al. 2020]. The total extraction of organic matter in alkaline may be due to the generation of strong and active oxidizing agents, such as OH and O2 swelling caused by NaOH, which increase the contact area for decomposition [Maryam et al. 2021].

Effect of mild thermal pretreatment on the solubilization (SCOD) of organic matters in sludge.

Figure 17 shows the effect of mild thermal pretreatment on the concentration of SCOD at different temperatures. It was observed that the SCOD concentration increased gradually with increasing pretreatment temperature, which indicated that more particulate organic matters in sludge become soluble with the increase of mild thermal pre-treatment. However, the amount of SCOD generated at different pretreatment tempera-tures was quite different. It was noticed that the concentration of SCOD at 100 °C was much higher than those at other temperatures investigated. For example, the SCOD con-centration at 50 °C was 640 mg/l, and increased to 6560 mg/l at 100 °C. As the initial TCOD of sludge at different temperatures was identical, the higher pretreatment tem-perature benefited the ratio of SCOD to TCOD. The mild thermal pretreatment accelerated the solubilization of SS used in the present study. In the same line, Eskicioglu et al. investigated the characterization of soluble organic matter of waste activated sludge (WAS) before and after thermal pretreatment [Eskicioglu et al. 2006]. They found that pretreatment at 96 °C successfully disrupted the complex WAS floc structure and accelerated the releases of extra- and intra-cellular biopolymers, such as protein and sugars, from activated sludge flocs into the soluble phase along with the solubilization of particulate COD. Appels et al. investigated the use of moderate temperature thermal hydrolysis (70, 80, and 90 °C) as a pretreatment for WAS before anaerobic digestion [Appels et al. 2010]. They noticed that organic and inorganic components successfully solubilized

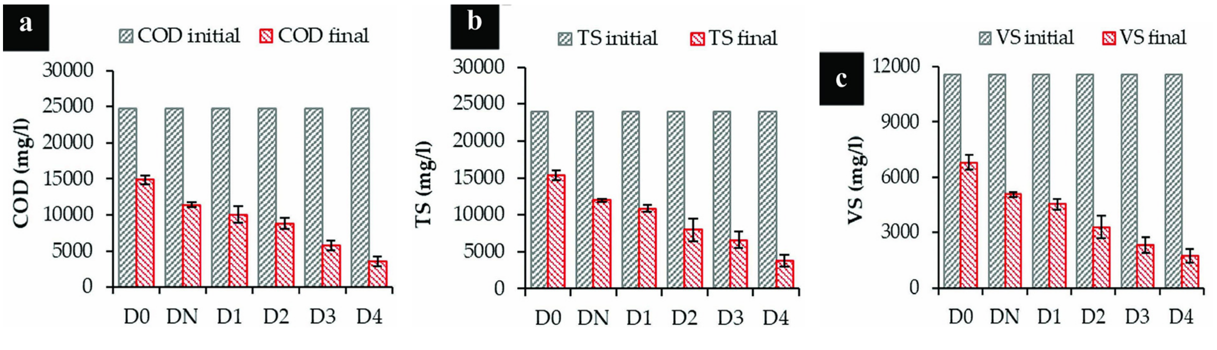

Figure 16. TCOD, TS and VS concentrations for Experiment D before and after the digestion, [results for sludge from trickling filter plant at 35 °C for: D0 (no MNPs dose, no pretreatment), DN (100 mg/l MNPs dose without pretreatment), D1 (100 mg/l MNPs dose with alkaline pretreatment (pH=9)), D2 (100 mg/l MNPs dose with alkaline pretreatment (pH=10)), D3 (100 mg/l MNPs dose with alkaline pretreatment (pH=11)), D4 (100 mg/l MNPs dose with alkaline pretreatment (pH=12))]

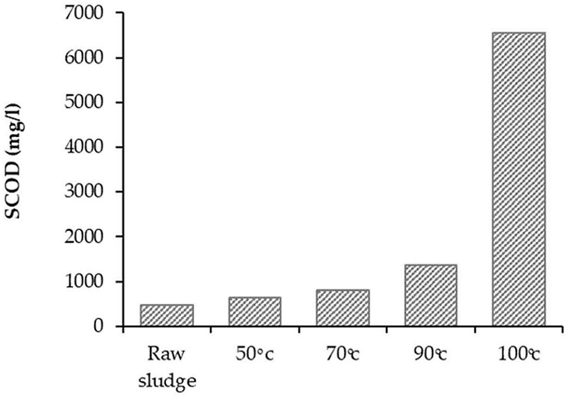

Figure 17. SCOD concentration at different temperatures of thermal pretreatment

during thermal treatment (with solubilization rates of 0.72 and 12.3% for sludge at temperatures of 70 and 90 °C respectively) after 30 minutes of treatment. In the current study, the solubilization rates of sludge after pretreatment for 30-minutes at 100 °C was 24.5% ((6560–480)/24794 = 24.5%). The sludge solubilization rates of the current study differed from those obtained by Appels et al. [2010]. This can be attributed to the different organic contents of the sludge used in this study.

Effect of thermal pretreatment with MNPs on biogas production

The daily biogas production for reactors is shown in Figure 18 while the cumulative biogas production is shown in Figure 19. The maximum biogas production was 350 mL obtained at E4 in the fourth day while the minimum value was recorded for the E0 (193 mL) on the thirteenth day. It is noticed that, in general, the production rate increases with the increase in temperature. The cumulative biogas values for reactors E1, E2, E3, and E4 were 3423, 2877, 3759, and 4505 ml respectively. For EN, the biogas production was 3233 ml, and 2187 ml for E0. Biogas production observed for EN was 47.8% higher compared with E0. For the E4, the increase in biogas production was 39% and 106% compared with the EN and the E0 respectively. Climent et al. evaluated the biogas production of the thermally treated sludge in batch tests at mesophilic temperatures and observed the increase in biogas production reached 45% compared with the untreated sludge [Climent et al. 2007]. In the study of Appels et al. it was observed that the biogas production increased significantly at higher pretreatment temperatures [Appels et al. 2010]. Chislett et al. reported that heat pretreatment could rupture bacteria's cell walls and cell membranes in the waste sludge [Chislett et al. 2020]. It caused the complex organic molecules, such as proteins, carbohydrates, lipids, and nucleic acids, to be released from the cells.

Effect of thermal pretreatment with MNPs on methane production

The values of methane for E0, EN, E1, E2, E3, and E4 were 1184, 1813, 2072, 1785, 2425 and

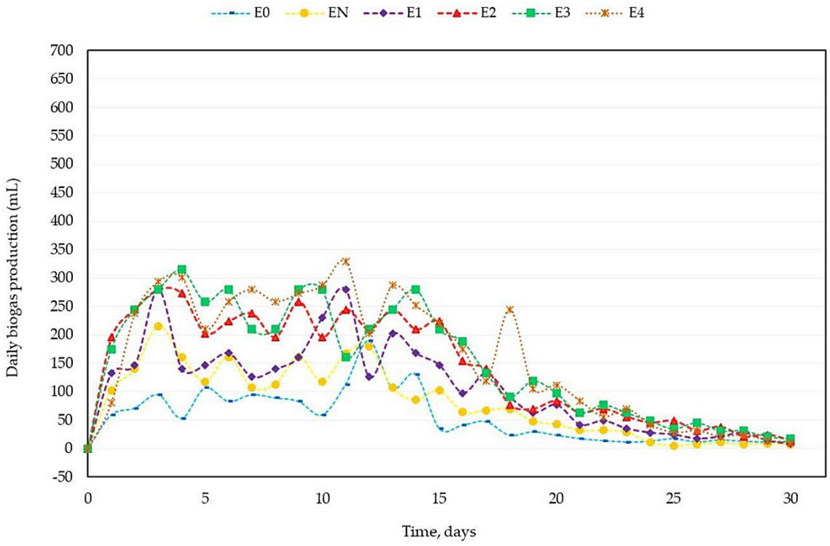

Figure 18. Daily biogas production (ml) for experiment E [results for sludge from trickling filter plant at 35 °C for: E0 (no MNPs dose, no pretreatment), EN (100 mg/l MNPs dose without pre-treatment), E1 (100 mg/l MNPs dose with thermal pretreatment (50 °C)), E2 (100 mg/l MNPs dose with thermal pretreatment (70 °C)), E3 (100 mg/l MNPs dose with thermal pretreatment (90 °C)), E4 (100 mg/l MNPs dose with thermal pretreatment (100 °C))]

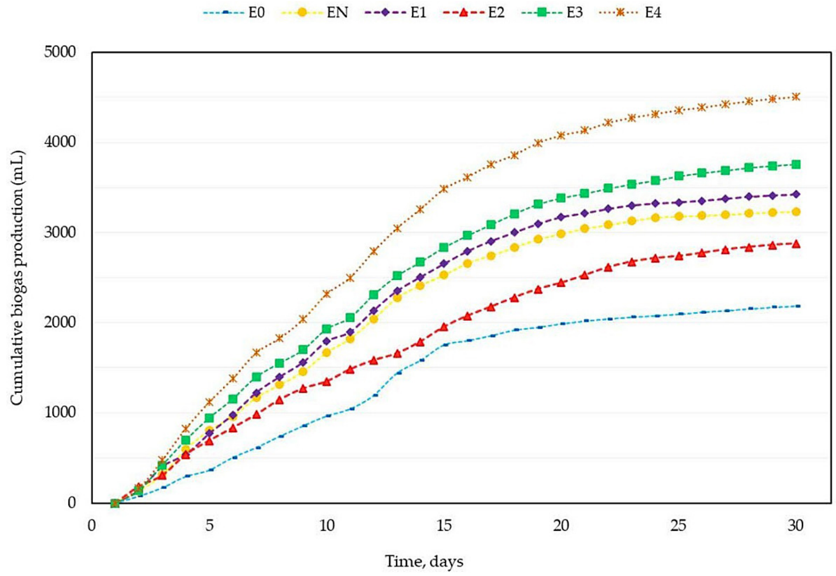

Figure 19. Cumulative biogas production (ml) for experiment E [results for sludge from trickling filter plant at 35 °C for: E0 (no MNPs dose, no pretreatment), EN (100 mg/l MNPs dose without pretreatment)), E1 (100 mg/l MNPs dose with thermal pretreatment (50 °C)), E2 (100 mg/l MNPs dose with thermal pretreatment (70 °C)), E3 (100 mg/l MNPs dose with thermal pretreatment (90 °C)), E4 (100 mg/l MNPs dose with thermal pretreatment (100 °C))]

3089 ml respectively as shown in Figure 20. The highest value of methane pro-duction was at E4, as compared to E0 and EN, was 161% and 70.4% respectively. Almukhtar et al. evaluated the effects of thermal pretreatment (25, 50 and 70 °C) on the biodegradability of dewatered sludge and found that the higher SCOD after the pre-treatment did not necessarily imply an increase in methane production [Almukhtar et al. 2012]. Although the initial biodegradability rate improved, i.e. a great improvement in SCOD concentration (up to 27%), but the methane production increased by 8% only. According to the study of Almukhtar et al. the pretreatment of sludge by 60 °C resulted in lower biogas ac-cumulation (3643 ml) as compared to 3749 ml at 80 °C pretreatment [Almukhtar et al. 2013]. Thermal pretreatment can improve the organic solubilization (acceleration of the hydrolysis step through digestion) in addition to enhancement of sludge dewaterability. Yan et al. investi-gated the effect of mild thermal pretreatment (50–120 °C) on the solubilization and me-thane potential of excess sludge with a low concentration of organic matters and it turned out that the concentration of soluble organic matters increased gradually with temperature [Yan et al. 2013]. The potential of methane production from excess sludge was greatly en-hanced by mild thermal pretreatment (at 100 °C pretreatment and 20 days digestion time; the methane yield was 142.6 ± 2.5 mL/g of VS) [Zhang et al. 2019]. In another study conducted by Rafique et al. the maximum increase in methane production after thermal pretreatment (at 100 °C) in comparison to untreated sludge was 28% after 29 days [Rafique et al. 2010]. This result is consistent with that of the current study, which showed that the production of methane increased with the

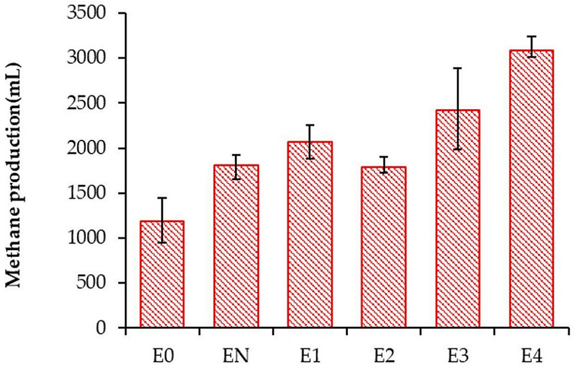

Figure 20. Effect of thermal pre-treatment on methane production for experiment E [results for sludge from trickling filter plant at 35 °C for: E0 (no MNPs dose, no pretreatment), EN (100 mg/l MNPs dose without pretreatment)), E1 (100 mg/l MNPs dose with thermal pretreatment (50 °C)), E2 (100 mg/l MNPs dose with thermal pretreatment (70 °C)), E3 (100 mg/l MNPs dose with thermal pretreatment (90 °C)), E4 (100 mg/l MNPs dose with thermal pretreatment (100 °C))]

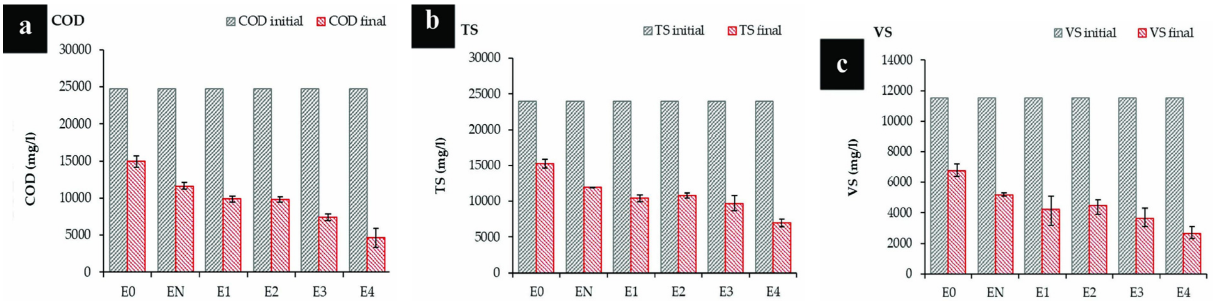

Figure 21. TCOD, TS and VS concentrations for Experiment E [results for sludge from trickling filter plant at 35 °C for: E0 (no MNPs dose, no pretreatment), EN (100 mg/l MNPs dose without pretreatment)), E1 (100 mg/l MNPs dose with thermal pretreatment (50 °C)), E2 (100 mg/l MNPs dose with thermal pretreatment (70 °C)), E3 (100 mg/l MNPs dose with thermal pretreatment (90 °C)), E4 (100 mg/l MNPs dose with thermal pretreatment (100 °C))]

increase of the pretreatment temperature from 50 to 100 °C and the highest production of methane was achieved at 100 °C.

Synergistic effect of thermal pre-treatment and MNPs on solids removal

After 30 days digestion for experiment E, the effluent TCOD, TS and VS for E0, EN, E1, E2, E3 and E4 were (14925, 15276 and 6766), (11585, 11941 and 5160), (9905, 10446 and 4229), (9789, 10825 and 4456), (7412, 9676 and 3660) and (4653, 6984 and 2647) mg/L respectively as shown in Figure 21. The removal ratios ofTCOD, TS and VS in reactors E0, EN, E1, E2, E3 and E4 were (39.8, 36.2 and 41.4%), (54, 50.1 and 55.3), (60, 56.4 and 63.4%), (60.5, 54,8 and 61.4%), (70.1, 59.6 and 68.3%) and (81.2, 70.8 and 77%) respectively. The efficiency of organic matter removal has been considered as a critical indicator for assessing the biodegradation process of AD [Ponsá S. 2011].

CONCLUSIONS

Major pollution problems are associated with sewage sludge. Also, because tradi-tional sewage sludge treatment techniques are not sufficient and taking too long retention time. So, further research in the field of sewage sludge treatment should be focused on to maximize the biogas production of this technology as well as minimize the impact on the environment. The results revealed that the addition of iron-based additives to AD significantly enhanced the biogas production of sewage sludge. In the first stage of this research, the use of 20 to 200 mg/l MNPs improved the system stability and the sludge biodegradation. Biogas and methane production increased for all MNPs doses compared to the control sample but the dose of 100 mg/l was the optimal one for all experiments as it achieved the highest yield of biogas and methane. The cumulative yield of methane at a dose of 100 mg/l MNPs for experiments A (AG at 350 c), B (AS at 350 c) and C (AG at ambinet temperature) was 140, 138.8 and 154% higher than each one of the controls re-spectively. The addition of magnetite to the SS in the anaerobic process created a good conductive environment for electroactive microorganisms and methanogens to increase the abundance and enhance the activity, facilitating the IET and DIET during CH_4 pro-duction. However, high doses of magnetite would restrict the electron exchange between different species by covering the cell surface, resulting in the inhibition of methanogen activity. In the second stage of the study, addition of MNPs to anaerobic sludge digesters could efficiently recover the methanogenic activity by heat pre-treatment or alkaline conditions. Pretreated sludge at 100 ℃ with addition MNPs increased methane production by 161% for E4 (experiment was done with 100 mg/l MNPs and pretreated at 100 °C) compared to the control sample (E0), and the VS removal percentage increased from 41.4% for the control (E0) to 77% for E4. For pretreated sludge at pH=12, methane production increased with the addition of MNPs (D4) to 201% compared to the control (D0), and the VS removal ratio increased from 39.9% for the control (D0) to 84.9% for D4 (experiment was done at 35 °C with 100 mg/l MNPs and pretreated at pH=12). The effi-ciency of microbial electron transfer is fundamental for determining the performance of fermentative hydrogen/methane production.

The present study suggested that MNPs can efficiently promote methanogenesis to improve the anaerobic sludge even if methanogens are completely removed or inhibited in the pretreatment process. The present work can be extended to further studies. The future scope has to use the optimum MNP dose and pH and/or temperature to be tested on other types or sources of sludges.

Acknowledgements

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