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Comparison of nanoparticles effects on biogas and methane production from anaerobic digestion of cattle dung slurry

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ABSTRACT

Nanoparticles (NPs) of trace metals such as Co, Ni, Fe and Fe_3O_4 were implemented in this study to compare their effects on biogas and methane production from anaerobic digestion of livestock manure. The most effective concentrations of NPs additives were determined based on our previous studies, and were 1 mg/L Co NPs, 2 mg/L Ni NPs, 20 mg/L Fe NPs and 20 mg/L Fe_3O_4 NPs. These concentrations of NPs additives were further investigated and compared to each other in this study and were found to significantly ($p < 0.05$) increase the biogas yield by 1.7, 1.8, 1.5 and 1.7 times in comparison with control, respectively. The methane yield significantly ($p < 0.05$) increased by 2, 2.17, 1.67 and 2.16 times the methane volume of the control, respectively. The results of this study showed that the Ni NPs yielded the highest biogas and methane production compared to Co, Fe and Fe_3O_4 NPs.

1. Introduction

Anaerobic digestion (AD) can be considered as one of the most important techniques to convert organic waste into renewable energy in the form of methane byproduct as a form of fuel may reduce treatment cost. The AD is carried out by a consortium of microorganisms and depends on various factors like pH, temperature, Hydraulic Retention Time (HRT), C/N ratio, etc.; it is a relatively slow process [1]. The temperature inside the digester has a major effect on the biogas production process. AD consists of a series of microbial processes that convert organics to methane and carbon dioxide, and can take place under psychrophilic (<20 °C), mesophilic (25–40 °C) or thermophilic (50–65 °C) conditions, although biodegradation under mesophilic conditions is most common. It also enables higher loading rates than aerobic treatment and a greater destruction of pathogens [2]. The use of additives in biogas plant could improve its performance significantly. The suitability of an additive is expected to be strongly dependent on the type of substrate [3]. Trace metals as micronutrients plays a very significant role on the performance and stability of agricultural biogas digesters, which are operated with energy crops, animal excreta, crop residues, organic fraction of municipal solid wastes or any other type of organic waste [4].

Limiting the metals required by the enzymes may disturb the total process, as reported in literature [5]; Co and Ni are all involved in the biochemical process of methane production. The efficiency of the biogas production process, i.e., the methane production and the degree of degradation, has in several studies been shown possible to increase by the addition of trace elements [6]. Positive effects by trace elements addition have also been observed in specific methanogenic activity (SMA) assays with various substrates, including methanol (Co and Ni [7]), acetate and propionate (Ni, Co and Fe [8]). Bini [9], reported that nickel is an essential cofactor for Ni–Fe hydrogenases, carbon monoxide dehydrogenase, methyl *CoM* reductase, and urease. Cobalt is found mainly in coenzyme B12. At least six systems for Ni/Co uptake are known [10]. Such systems are required for the high-affinity uptake of nickel or cobalt because their environmental concentration is usually below the

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required levels.

Qiang et al. [11] mentioned that the growth of methanogenic bacteria is dependent on Fe, Co, and Ni during enzyme synthesis. Some studies have been done to determine the requirements and optimal concentration for trace metals in pure culture of methane fermentation. Trace metals are essential constituents of cofactors and enzymes and their addition to anaerobic digesters has been shown to stimulate and stabilize the biogas process performance [4,12]. Luna-del Risco et al. [13] reported that the presence of heavy metal ions (i.e., Cu, Zn, Fe, Ni, Co, Mo) during anaerobic biodegradation of organic matter is known to be fundamental for numerous reactions. However, high concentrations of these elements can inhibit the biological degradation process in anaerobic reactors. In a batch study, Demirel and Scherer [4] reviewed that the improvement of biogas production through addition of Fe was investigated using cow dung and poultry litter. For both substrates, addition of $FeSO_4$ improved biogas production and CH_4 content of biogas. Addition of $FeCl_3$ during AD of water hyacinth-cattle dung was also reported to result in an increase of more than 60% in biogas production. Furthermore, addition of $FeCl_2$ during batch experiments with swine excreta was reported to counteract the sulphide inhibition. Zhang et al. [14] mentioned that when Zerovalent Iron (ZVI) is added into an anaerobic reactor, it not only serves as an electron donor, but is also expected to help create an enhanced anaerobic environment that may improve the performance of reactors used for wastewater treatment. Zhang et al. [14] provided direct evidence that ZVI promoted the growth of methanogens, which enabled the reactor to achieve greater chemical oxygen demand (COD) removal under low temperatures and a short HRT.

Nanotechnology is the engineering and art of manipulating matter at the nanoscale (1–100 nm); that considered as one of the most important advancements in science and technology of the last decades. Moreover, it offers the potential of new functional materials, processes and devices with unique activity toward obstinate contaminants, and enhanced mobility in environmental media. Particles in nanometric size range are termed nanoparticles (NPs). The size greatly depends on the process used for their synthesis. They can be obtained by bottom-up assembly of atoms through chemical process or, on the contrary, from top-down fragmentation of bulk material. The latter allows the synthesis of smaller particles [15]. Nano-size is the cardinal property for interaction with biological systems since it determines the ability to penetrate cell membranes, thus facilitating the passage across biological barriers, interaction with immune system, uptake, absorption, distribution and metabolism [16].

Compared to atomic or bulky counterparts, nano-sized materials owe superior physical and chemical properties due to their mesoscopic effect, small object effect, quantum size effect and surface effect. Recently, Fe_3O_4 magnetic nanoparticles (MNPs) have been intensively investigated because of their super paramagnety, high coercivity, non-toxic and biocompatible [17]. Mu et al. [18] concluded that among four metal oxide nanoparticles (nano-TiO_2, nano-Al_2O_3, nano-SiO_2 and nano-ZnO) investigated it was found that only nano-ZnO showed inhibitory effect on methane generation, and the influence of nano-ZnO was dosage dependent. Lower nano-ZnO (6 mg/g-TSS) gave no impact on methane generation.

Abdelsalam E. [19]. compared the effects of Co and Ni NPs on biogas and methane production from slurry anaerobic digestion and concluded that Ni NPs yielded the highest biogas and methane compared to Co NPs. Furthermore, the effects of Fe and Fe_3O_4 NPs on biogas and methane production were also investigated and concluded that Fe_3O_4 NPs attained the highest biogas and methane yield compared with Fe NPs. Therefore, the main objective of this study was to compare the effects of Co, Ni, Fe and Fe_3O_4 NPs on biogas and methane production using livestock manure and to determine which of these NPs will yield the highest biogas and methane production. For this purpose, we have used the biodigesters and biogas production system designed by Abdelsalam et al. [20] for carrying out the experiments in the biogas laboratory of the National Institute of Laser Enhanced Sciences at Cairo University. Such applications were hypothesized to increase the biogas yield, methane percentage and decrease the hydraulic retention time (HRT). The main objective of this study can be further elaborated as follows: (1) preparing and characterizing different trace metals nanoparticles such as Co, Ni, Fe and Fe_3O_4; (2) comparing the effects of these nanoparticles to each other and investigating their effects on biogas production (biogas yield and methane concentration) from anaerobic digestion of livestock manure compared with the control.

2. Materials and methods

2.1. Fresh manure samples

The fresh raw manure (feces and urine) was collected randomly from a livestock waste holding pen unit located in the Western Farm of the faculty of Agriculture, Cairo University, Giza City, Egypt. The slurry was obtained by adding distilled water to fresh manure (1:1 by weight) and homogenized by a mixer for 30 min.

2.2. Samples analysis

The pH and temperature of substrate were measured using a pH meter (QJS, proline B210, Oosterhout, Netherlands) equipped with long pH-electrode (QP174X, Epoxy, 300 mm). Total solids (TS), volatile solid (VS) and ash were determined every 10 days during the experiments, by the standard methods (EPA METHOD 1684, 2001) using muffle furnace (Vulcan D-550, Ney Tech, York, USA) as shown in Table 1.

2.3. Experimental set up

A batch anaerobic system was designed and manufactured according to the design guidelines and parameters developed by Samer [21], and implemented in this study. The experimental setup of the batch anaerobic system consists of: (1) a biodigester; 2-liter wide neck culture vessel flask (Pyrex, FV2L, Scilabware, Staffordshire, UK) plugged with tightly Teflon cap (HOME MADE), equipped with step motor (ASMO, 24 V, 90 rpm, Japan) with maximum speed 90 rpm for mixing the slurry, and gas outlet connected to biogas holder with 1/4″ connectors through 8 mm plastic hose. (2) The temperature control; a thermostatic water bath (HOME MADE, 60 L, 0–100 °C), where the software of the control unit maintained the temperature at mesophilic conditions (37 ± 0.3 °C), and the speed of the step motor was controlled to be 20 rpm for mixing the slurry in an interval of 1 min every hour. (3) Biogas measurements; the volume of biogas produced was measured on a daily basis using

Table 1
Chemical composition of fresh manure and slurry.

Parameter	Fresh manure	Slurry
TS (%)	14.8	7.16
VS (%)	11.67	5.85
VS (% from TS)	82.24	81.28
Ash (%)	2.51	1.34
Organic carbon (% from VS)	47.7	47.15
Total Nitrogen (%)	1.83	1.96
C:N ratio	26:1	24:1
pH value	6.13	5.85

ultra clear polypropylene graduated cylinder (1000 ± 10 ml, Azlon, Staffordshire, UK) connected to gas outlet by 8 mm plastic hose at its base and placed upside down in another polypropylene cylinder (2000 ml, Azlon) filled with water. Moreover, methane (CH_4) and carbon dioxide (CO_2) concentrations were measured using portable gas analyzer (Geotech, GA2000, Warwickshire, UK). CH_4 and CO_2 were measured by dual wavelength infrared cell with reference channel. The recorded data were downloaded from the gas analyzer to PC using Gas Analyzer Manager Software (GAM, version 1.4.0.12) in the form of Excel Worksheet as shown in Fig. 1. The experiments were carried out in the Biogas Laboratory at National Institute of Laser Enhanced Sciences (NILES), Cairo University.

2.4. Nanoparticles preparation

Nanoparticles (NPs) were prepared using the following chemicals: sodium borohydride ($NaBH_4$, powder 98%, Aldrich) as reducing agent, sodium carbonate (Na_2CO_3, powder 99.5%, Sigma–Aldrich), cetyltrimethyl ammonium chloride solution (CTAC, 25 wt. % in H_2O, 0.968 g/mL at 25 °C, Aldrich) as the capping agent to prevent the agglomeration of the synthesized metal nanoparticles, Cobalt chloride hexahydrate ($CoCl_2.6H_2O$, crystallized 99%, Fluka), Nickel chloride hexahydrate ($NiCl_2.6H_2O$, crystallized 99.9%, Fluka). ascorbic acid ($C_6H_8O_6$, Sigma), ferric chloride hexahydrate ($FeCl_3.6H_2O$, granulated 99%, Riedel-de Haën). Co, Ni, Fe and Fe_3O_4 NPs were prepared as reported by Abdelsalam E. [19] with average sizes of 28 ± 0.7, 17 ± 0.3, 9 ± 0.3 and 7 ± 0.2 nm, respectively.

2.5. Experimental design

The laboratory experiments were carried out using 2L biodigesters, in batch operation mode. Co, Ni, Fe and Fe_3O_4 NPs were used to study the effect of NPs on biogas production and compared control. The concentrations of NPs were 1, 2, 20 and 20 mg/L, respectively. These concentrations were selected based on our previous study Abdelsalam E. [19]. The NPs were added separately to biodigester and the operating temperature was maintained at mesophilic conditions (37 ± 0.3 °C). The biodigester performance was measured with respect to the cumulative volume of biogas produced which was corrected to the standard pressure (760 mm Hg) and temperature 0 °C. All of the treatments were carried out in triplicate.

2.6. Statistical analysis

The aim of the statistical analysis was to evaluate the effect of selected NPs on biogas production and methane concentration. Therefore, all treatments were carried out in three replicates for the statistical analysis purposes, and analyzed using Least Significant Difference (LSD, MStat-C software v.2.1.) at a significance level of $p < 0.05$, where a different superscript letter means a significant difference among the treatments.

3. Results

3.1. Effects of NPs on biogas production

Fig. 2 illustrates the daily and cumulative production of biogas and methane when substrates were treated with 1 mg/L Co NPs, 2 mg/L Ni NPs, 20 mg/L Fe NPs and 20 mg/L Fe_3O_4 NPs additives in comparison with the control. All these additives improved the startup of biogas production and reduced the lag phase. The highest biogas startup was achieved when the substrates were treated with 2 mg/L Ni NPs and was 658 ml biogas as an average in the first five days of HRT, while 1 mg/L Co NPs, 20 mg/L Fe NPs and 20 mg/L Fe_3O_4 NPs yielded 596, 580 and 633.3 ml, respectively. However, the average production of the control during the first five days of HRT was only 159.3 ml as shown in Fig. 2a. Furthermore, the highest daily biogas yield was achieved with the addition of 2 mg/L Ni and 20 mg/L Fe_3O_4 NPs which yielded around 1300 ml biogas from day 19 to day 38 of HRT, but with 2 mg/L Ni NPs additive this high yield started two days earlier and to the end of the experiment (day 40). Moreover, the cumulative biogas production for the abovementioned additives confirmed that the addition of 2 mg/L Ni NPs attained the highest biogas yield ($p < 0.05$) through 40 days of HRT and was 47,633.3 ml biogas compared with other additives. Furthermore, the biogas yield with the addition of 1 mg/L Co NPs and 20 mg/L Fe_3O_4 NPs to the substrates found to be insignificantly different to each other ($p > 0.05$), and were 45,683.3 and 46,160 ml biogas, respectively. On the other hand, the addition of 20 mg/L Fe NPs yielded 39,406.7 ml biogas, while the control yielded the lowest biogas production ($p < 0.05$), and was 26,680 ml as shown in Fig. 2b. Furthermore, the addition of 1 mg/L Co NPs, 2 mg/L Ni NPs, 20 mg/L Fe NPs and 20 mg/L Fe_3O_4 NPs significantly increased the biogas volume ($p < 0.05$) by 1.7, 1.8, 1.5 and 1.7 times the biogas volume produced by the control, respectively. Considering methane production the maximum daily CH_4 was attained with the addition of 2 mg/L Ni and 20 mg/L Fe_3O_4 NPs. they were more than 1000 ml of CH_4 daily started from day 22–29 and from day 23 to day 30 of HRT, respectively as illustrated in Fig. 2c. Moreover, the aforementioned additives achieved the highest methane yield and were ($p > 0.05$) 28,283.2 and 28,132.6 ml CH_4, respectively in comparison with the addition of 1 mg/L Co NPs, 20 mg/L Fe NPs, which yielded 26,124 and 21,803.6 ml CH_4, respectively as shown in Fig. 2d. The addition of 1 mg/L Co NPs, 2 mg/L Ni NPs, 20 mg/L Fe NPs and 20 mg/L Fe_3O_4 NPs significantly increased the methane volume ($p < 0.05$) by 2, 2.17, 1.67 and 2.16 times the methane

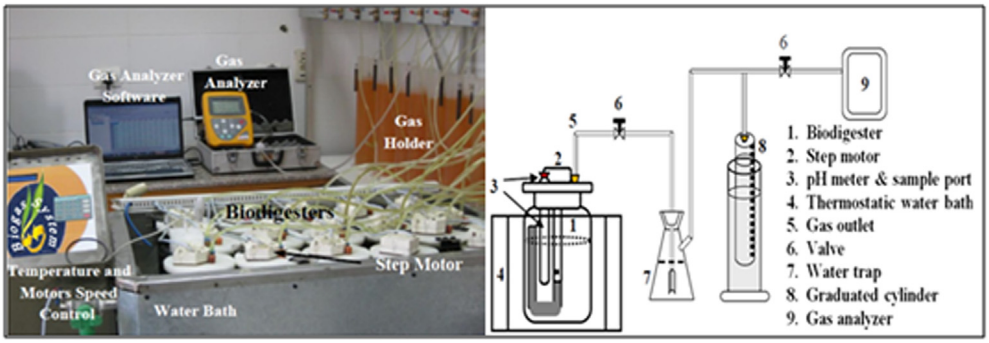

Fig. 1. Experimental setup and schematic of biogas production system [20].

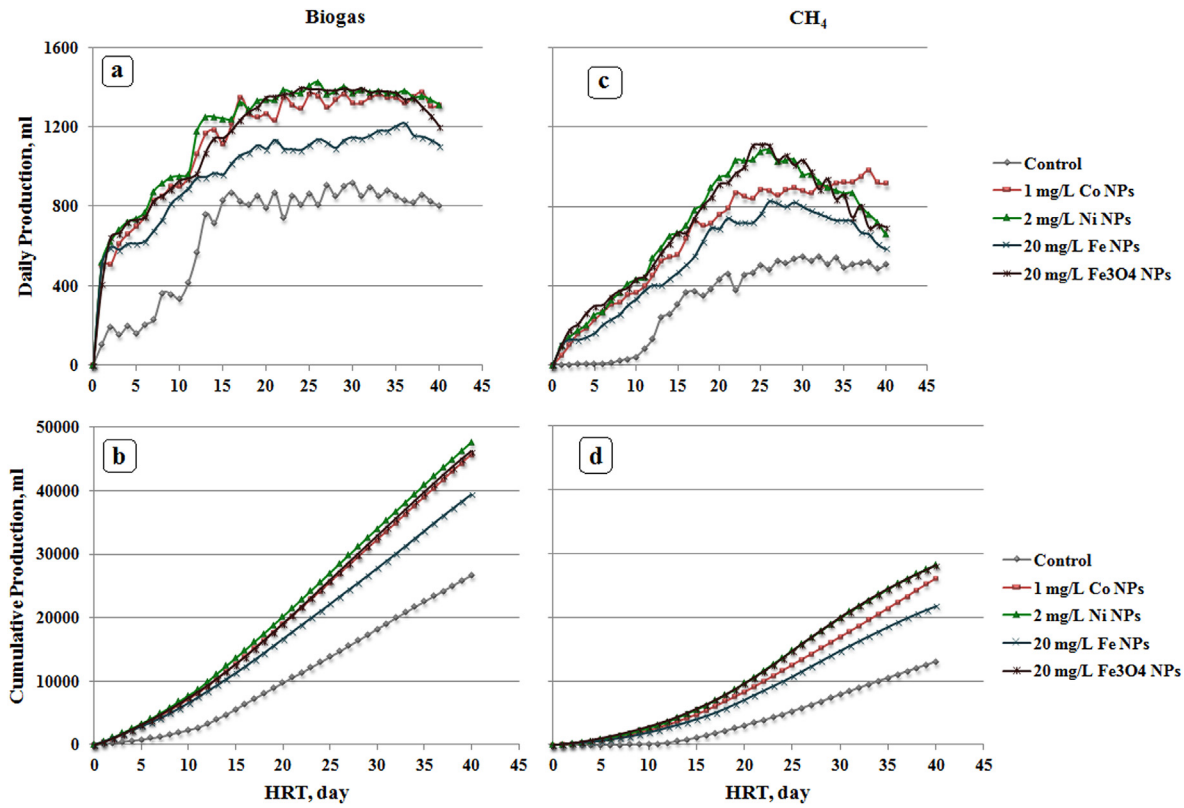

Fig. 2. Daily and cumulative productions of biogas and CH_4 affected by the addition of different NPs.

volume produced by the control, respectively.

3.2. Methane concentration

The CH_4 contents of the NPs treatments compared with the control are illustrated in Fig. 3. The substrate treated with Fe_3O_4 NPs attained the highest CH_4 contents during the startup of biogas production, and was 31.79% CH_4 ($p < 0.05$) as an average of the first 5 days of HRT. It achieved the highest peak of CH_4 contents from day 24–26 of HRT and was more than 79% of CH_4. No other additives of NPs reached so high methane concentrations.

3.3. The specific biogas and methane production

The statistical analysis of specific biogas and methane production confirmed that the substrate treated with 2 mg/L Ni NPs yielded the highest specific biogas and methane production ($p < 0.05$), and were 512.2 and 304.1 ml gas.g^{-1} VS, respectively in comparison with the other additives and the control as shown in Fig. 4. However, when the substrate treated with 20 mg/L Fe_3O_4 NPs, the specific methane production was 302.5 ml gas.g^{-1} VS which was found to be insignificant different ($p > 0.05$) when compared with the addition of 2 mg/L Ni NPs.

3.4. The average biogas and methane production

The statistical analysis of the average biogas and methane yields revealed that the most effective NPs additive was Ni NPs with a concentration of 2 mg/L, which yielded the highest biogas and

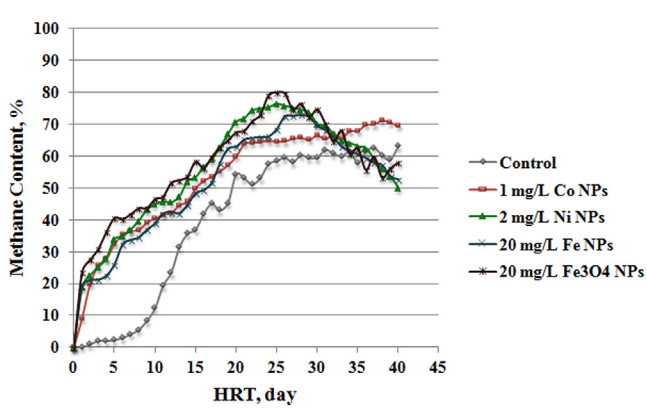

Fig. 3. Methane percentage affected by the addition of different NPs.

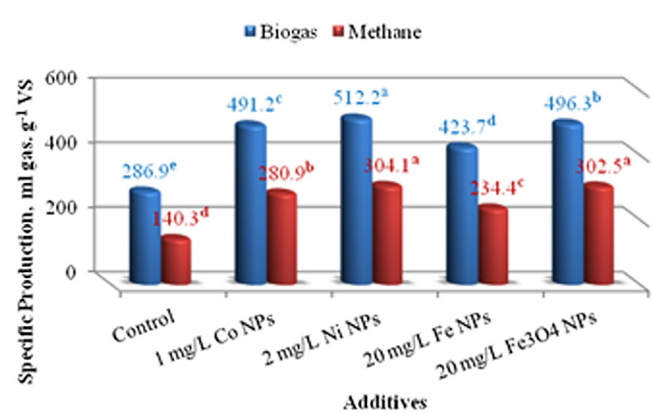

Fig. 4. Specific biogas and methane production affected by the addition of different NPs ($p < 0.05$).

methane yields through 40 days of HRT as an averages, and were 1190.8 and 707.1 ml ($LSD_{Biogas} = 14.38$, $LSD_{CH4} = 11.72$, $p < 0.05$), respectively as shown in Fig. 5. Although, the average biogas yield with the addition of 20 mg/L Fe_3O_4 NPs and 1 mg/L Co NPs, found to be insignificantly different ($LSD_{Biogas} = 14.38$, $p > 0.05$), while the average methane yield with the addition of 20 mg/L Fe_3O_4 NPs was higher than with the addition of 1 mg/L Co NPs ($LSD_{CH4} = 11.72$, $p < 0.05$). This result indicated that Fe_3O_4 NPs has a biostimulating effect on the methanogenis more than Co NPs, while Fe NPs yielded the lowest biogas and methane production. Moreover, Table 2 summarizes the overall mean of biogas and methane production affected by the addition of different NPs in comparison with the control during 8 time intervals of HRT.

3.5. Effects of NPs on organic matter

Total and volatile solids were determined every 10 days to track the decomposition of organic matter through the 40 days of the experiment. The highest decomposition of TS was observed when the substrate was treated with 2 mg/L Ni NPs and 20 mg/L Fe_3O_4 NPs up to a final TS concentration of 5.08 and 4.95%, respectively. Furthermore, the aforementioned additives achieved the highest VS decomposition which was 4.36 and 4.15%, respectively at the end of the experiment as shown in Fig. 6.

4. Discussion

Our study confirmed that Ni and Fe_3O_4 NPs yielded the highest biogas and methane production compared to Co and Fe NPs, where the statistical analysis showed insignificant difference of methane yield with the addition of 2 mg/L Ni NPs and 20 mg/L Fe_3O_4. This is in agreement with Uemura [22] who reported that nickel is the most important trace element for the anaerobic digestion of the organic fraction of municipal solid waste. Ni NPs additive implemented in this study enhanced various biological processes related to biogas and methane production. This observation accords with Bożym et al. [23] and Gustavsson et al. [12] who stated that nickel concentrations in digester substrates improve biogas production by increasing methane yield and maintaining process stability. Bożym et al. [23] mentioned that the toxic threshold for nickel in fermented wastes is 10 mg dm^{-3} (equal to 10 mg/L). According to Schattauer et al. [24], nickel concentrations of 0.005–0.5 mg/L in substrate contribute to biogas production. Demirel and Scherer [4] observed that feedstock containing 0.11–0.25 mg Ni kg^{-1} stimulates biogas generation. However, our results disagree with Zhang et al. (2003) [25] who mentioned that the process related to

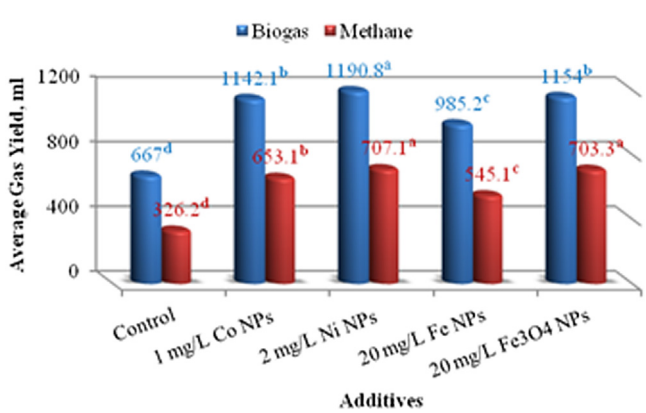

Fig. 5. The average biogas and methane yield affected by the addition of different NPs ($p < 0.05$).

methane production is inhibited when nickel concentrations exceed 1.2 mg Ni/L. Altas [26] and Wu et al. [27] reported that Ni dose of 0.5–16 mg/L increased the cumulative CH_4 production, which is in agreement with our results which confirmed that Ni NPs with concentrations of 2 mg/L increased the cumulative CH_4 production by 100.17% compared with the control. These results agree with Demirel and Scherer [4] who stated that the optimum or stimulatory concentrations of Ni for batch cultures of methanogens range between 0.012 and 5 mg/L. On the other hand, Fe_3O_4 magnetic NPs additive was proved to enhance biogas and methane production with maximum concentration of 20 mg/L, where this is in agreement with Ni et al. [28] who indicated that the adverse effects during the exposure of 50 mg/L magnetic NPs on bacterial activity were insignificant, and concluded that magnetic NPs seemed to be non-toxic during long-term contact, and only exhibited mild toxicity to bacteria at initial stage. In contrast, our results indicated that the addition of 20 mg/L Fe_3O_4 magnetic NPs enhanced the bacterial activity during the start up and through 40 days of HRT of biogas production. Furthermore, our results agree with Krongthamchat et al. [29] who stated that the addition of iron, as a trace metal, had the capacity to reduce the lag time of mixed culture. Our results indicated that the highest methane productivity with 20 mg/L Fe_3O_4 magnetic NPs increased by 100.16%, which is higher than the methane yielded by Feng et al. [1] at the dosage of 20 g/L increased by 43.5%. These results indicated that Fe_3O_4 magnetic nanoparticles led to enhanced anaerobic digestion, and consequently to higher methane production and organic matter degradation. The improved performance is due to the presence of Fe^{+2}/Fe^{+3} ions, introduced into the reactor in the form of nanoparticles which could be adsorbed as the growth element of anaerobic microorganisms. Considering other metal oxides NPs such as CuO and ZnO, our results disagree with Luna-del Risco et al. [13] who found that biogas and methane production were severely affected at concentrations of bulk and nanoparticles over 120 and 15 mg/L for CuO and 240 and 120 mg/L for ZnO, respectively. However, this confirms that the excessive concentration of the essential metals can significantly inhibit the bacterial activities. We assumed that particle size of trace metal can biostimulate or inhibit the AD depending on its concentration. Our results indicated that the addition of 20 mg/L Fe_3O_4 magnetic NPs was the most effective concentration for biogas and methane production, where this is in agreement with Liu et al. [30] who reported that the release of iron ions from the dissolution of magnetic NPs can also be responsible for the increase of bacterial activities. Furthermore, Fe_3O_4 magnetic nanoparticles ensure the distribution of iron ions in slurry, with corrosion of the nanoparticles maintaining a sustained supply of iron ions in the reactor. In fact, not only the hydrolysis of soluble protein but the transformation activity of electron donors of the redox-driven proton translocation in methanogenic Archaea expressed by coenzyme F_{420} was significantly controlled by higher concentrations of ZnO NPs [31]. The coenzyme F_{420} activity was ZnO NPs dosage dependent, which was respectively 99.3%, 89.8% and 66.2% of the control at ZnO NPs of 1, 30 and 150 mg/g TS. In case of Co and Fe NPs additives, our results agree with Zandvoort et al. [32] who reported that the optimal concentration of cobalt is approximately 7 μmol L^{-1} (0.91 mg/L based on total cobalt addition). Additionally, our results confirmed that the highest biogas and methane production were achieved when the substrates were treated with 1 mg/L Co NPs which is in line with Qiang et al. [33] who concluded that the addition of 10 mg/L Fe, 1 mg/L Co and 1 mg/L Ni to the thermophilic digester every 45 days successfully enhanced methane production. Furthermore, our results indicated that the addition of Co NPs to slurry reduced the lag phase and the time to reach the peak of biogas production which agrees with Krongthamchat et al. [29] who treated digester sludge with 0.1 mg/

Table 2
The averages of biogas and methane yields affected by the addition of different Ni NPs during different time intervals of HRT.

HRT (day)	Additives									
	Control		1 mg/L Co NPs		2 mg/L Ni NPs		20 mg/L Fe NPs		20 mg/L Fe_3O_4 NPs	
	Biogas	CH_4	Biogas	CH_4	Biogas	CH_4	Biogas	CH_4	Biogas	CH_4
(1–5)	159.3	2.2	596	94.6	658	173.3	580.7	128.9	633.3	206.9
(6–10)	296	21.3	846	182	893.3	359.2	739.3	263.4	848.7	368.2
(11–15)	658	202.9	1096.7	287.8	1178	577.5	941.3	414.9	1053.3	559
(16–20)	829.3	380.3	1270	458.8	1305.3	826.8	1068.7	609.8	1269.3	793.4
(21–25)	826.7	453.1	1311.3	649.6	1376	1026.7	1100.7	731	1374.7	1022.1
(26–30)	876	520.5	1336	704.6	1390	1027.2	1126	812	1386.7	1048.6
(31–35)	865.3	523.5	1347.3	736.7	1378	905.9	1172	749.4	1378.7	900.7
(36–40)	825.3	505.9	1333.3	693.6	1348	760.1	1152.7	649.8	1287.3	729.3
Average	**667.0**	**326.2**	**1142.1**	**476.0**	**1190.8**	**707.1**	**985.2**	**544.9**	**1154.0**	**703.5**

$LSD_{Biogas} = 14.38$, $LSD_{CH4} = 11.72$, $p < 0.05$.

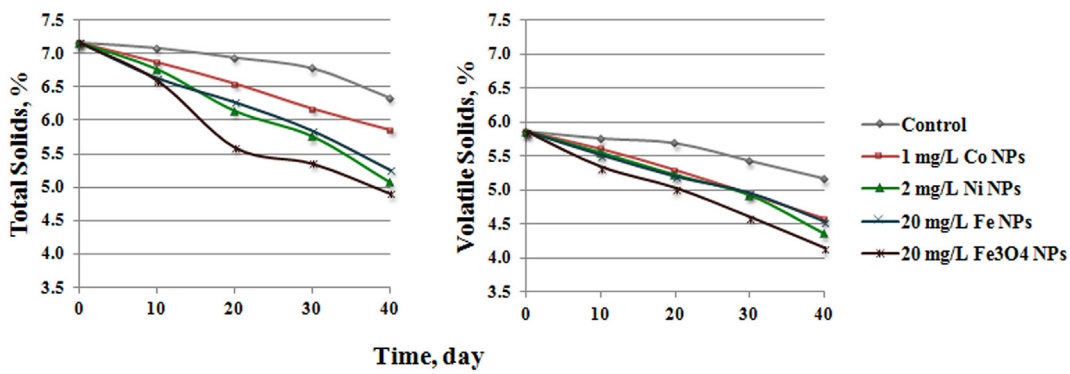

Fig. 6. Total and volatile solids affected by the addition of different NPs determined every 10 days during HRT.

L $CoCl_2$ to produce biogas. Furthermore, an enhancement of methane production by 100% was observed when the substrates were treated with 1 mg/L Co NPs compared with the control. This is in accordance with Banks et al. [34] and Feng et al. [6] who reported that cobalt is an important metal for methanogenesis. The addition of Fe NPs additive reduced the lag phase, increased biogas and methane production which accords with Krongthamchat et al. [29] who stated that the addition of iron, as a trace metal, had the capacity to reduce the lag phase of mixed culture. Fe additive could increase the methane production as a result of the enhanced generation of acetate in the presence of Fe additives which provides a suitable substrate for methanogenesis. Furthermore, Fe could directly serve as an electron donor for reducing CO_2 into CH_4 through autotrophic methanogenesis causing the improvement of methane production based on the following reactions presented by Feng et al. [1]:

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

$$CO_2 + 4H_2 = CH_4 + 2H_2O \quad (2)$$

Based on our understanding, the abovementioned process will deprive the substrate from the hydrogen ions (H^+) which will increase the pH of the substrate. Similarly, capturing CO_2 will prevent the formation of carbonic acid (H_2CO_3) within the substrate which will increase the pH. Therefore, controlling the pH of the substrate is an important key issue. Furthermore, the enhancement of biogas and methane with the addition of 20 mg/L Fe NPs in agreement with Feng et al. [1] who reported that the methane production increased with the increase of the ZVI dosage to 20 g/L. The function of ZVI was dependent on the surface reaction of ZVI, i.e. $Fe^0 + 2H^+ = Fe^{2+} + H_2$. However, the scrap iron has a small surface area, and therefore increasing the dosage provided a larger surface area to release more Fe^{2+} which explains why Fe NPs have more reactivity due to the large surface area [1]. Furthermore, the released Fe^{2+} at 20 g/L of dosage were only 80 mg/L after 20 days. Fe^{2+} reached 15.7 mg/L when the scrap iron was used in anaerobic system with a HRT of 2 days in the study of Liu et al. [30], which confirmed that the most effective concentration of Fe NPs is 20 mg/L. Our results indicated that the methane production with 20 mg/L Fe NPs additive increased by 67%, which is higher than the methane yielded by Feng et al. [1] which increased only by 43.5% at the dosage of 20 g/L compared with control. Furthermore, the methane percentage significantly increased to 68.9% when ZVI dosage further increased to 20 g/L, while our results indicated that the methane percentage reached more than 65% on days 21–32 of HRT and the peak reached 73.1% on day 28. This indicated that Fe NPs improved the methane production and enhanced the activity of methanogens, which agrees with Dinh et al. [35] who stated that ZVI enhanced the activity of methanogens.

5. Conclusions

According to the results of this study, it can be concluded that:

1. The addition of trace metals in form of nanoparticles reduced not only the lag phase but also the time to achieve the highest biogas and methane production (peak).
2. Nanoparticles have demonstrated biostimulating effects on the methanogenic activity during the start up of the anaerobic digestion of cattle slurry process through the hydraulic retention time till the end of the experiments.

3. Ni NPs produced the highest significant biogas and methane production compared to Co, Fe and Fe_3O_4 NPs and the control.

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