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Improvement of Methane Production from Sugar Beet Wastes Using TiO_2 and Fe_3O_4 Nanoparticles and Chitosan Micropowder Additives

Hossein Beiki[1] · Misagh Keramati[1]

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Abstract
An experimental study was performed to measure biogas production from sugar beet waste, which is, in fact, the chopped parts of the sugar beet not going through the sugar extraction process, at different additive concentrations. Medium molecular weight chitosan in microsize and TiO_2 and Fe_3O_4 nanoparticles were added to ten experimental reactors to investigate their effect on the anaerobic digestion process. Three different concentrations of 0.01, 0.04, and 0.12% *w*/*w* were used for each additive. Biogas production and methane content were compared with a control sample containing no additive. Adding chitosan in powder form did not help the process nor improved methanogenic activities. The results showed no effect on anaerobic digestion by the addition of TiO_2 nanoparticles in the mentioned concentrations, whereas adding Fe_3O_4 nanoparticles led to a slight increase in methane production and in volatile solid and total solid reduction. The maximum enhancement in methane and biogas production in the sample containing 0.04% Fe_3O_4, as compared with the control sample, reached 19.77% and 15.09%, respectively.

Keywords Biogas · TiO_2 and Fe_3O_4 nanoparticles · Chitosan powder · Additives · Sugar beet wastes

Highlights

- Unprocessed chopped parts of sugar beet have been used as substrate
- Chitosan powder in microsize and TiO_2 and Fe_3O_4 nanoparticles were used as additives
- Chitosan powder did not help the process nor improved methanogenic activities
- No effect on anaerobic digestion by addition of TiO_2 NPs
- Adding 0.04% Fe_3O_4 has led to a 19.77% increase in methane production

✉ Hossein Beiki
hbeiki@qiet.ac.ir

[1] Department of Chemical Engineering, Quchan University of Technology, Quchan 9477167335, Iran

Introduction

As mineral oil resources are being used up, the need to find sustainable renewable energy sources and alternative fuels has become a necessity [1–3]. Conversion of biomass to biogas is a potential solution [4–7]. This will lead to reduced greenhouse gas emission [8]. Biogas is a flexible energy source that can be obtained by anaerobic digestion (AD) of energy crops, animal manure, and organic wastes [8–10]. Sugar beet waste (SBW) is a by-product in a sugar beet factory and is the chopped parts of sugar beets that do not go through the process of sugar extraction and that is conventionally dumped in the landfill or used as cattle feed [11]. As an example, there are 28 sugar beet factories in Iran consuming 5.5 million tons of sugar beets annually [12]. This amount of processed sugar beets results in a large amount of SBW that makes it an attractive substrate for AD.

Additives are substances that can be added to working materials of processes in low concentrations. They can promote the process performance. Some of these additives are in microscale and some other are in nanoscale. In the abovementioned sizes, the additives' large surface-to-volume ratio leads to an increase in their activity. Metals, metal oxides, and polymers are materials that can be used as additives in order to enhance biogas production and AD's performance. Although heavy metals such as chromium, nickel, copper, and zinc could reduce biogas and methane production [13], some chemical additives have been studied for the purpose of biogas enhancement under different operating conditions [14]. Iron salts could enhance the rate of biogas production as reported by references (15, 16). Iron powder was also reported to increase methane yield [14, 17]. There have been few studies done on using TiO_2 and Fe_3O_4 nanoparticles (NPs) and medium molecular weight chitosan (MMWC) as additives in the case of biogas production enhancement [18–22]. Casals et al. [22] investigated using 100 ppm of 7 nm Fe_3O_4 NP addition to a digester system, and they reported a 180% increase in biogas production as compared with a control. Suanon et al. [23] also reported an improvement in biogas production with the addition of two different iron NPs, namely, zerovalent iron and Fe_3O_4, in a mesophilic condition (37 ± 1 °C). They observed that using NPs at an optimum concentration (0.5 wt%) could improve biogas production up to 46% relative to the control. Similarly, the effect of trace metal (nickel and cobalt) and metal oxide (Fe_3O_4 and MgO) NPs on biogas and biohydrogen production from microalgae was investigated, showing that the highest biogas and biohydrogen production, as compared with the control, was achieved by Fe_3O_4 (28%) and Ni (51.42%), respectively [24]. Mu et al. [25], Gonzalez-Estrella et al. [26], and Chen et al. [27] used different concentrations of TiO_2 NPs with sizes of less than 25 nm, 25 nm, and 185 nm, respectively. They stated no effect on biogas production in their works. García et al. [28] inspected the effect of using 7.5 nm TiO_2 NPs on biogas enhancement. On the other hand, they reported about 10% increase in biogas production at 1120 mg/L concentration in both thermophilic (55 °C) and mesophilic conditions (37 °C). Yadav et al. [29] studied the effect of TiO_2 NPs on biomass activity of an upflow anaerobic sludge bed (UASB) system. They noticed a slight reduction in biogas production as compared with the control.

Chitosan is a natural degradable polymer which does not have adverse effects on the human body. Chitosan can help anaerobic bacteria to aggregate and flocculate [18, 19]. There have been few efforts in the literature regarding the use of chitosan in AD [18–21]. Lertsittichai et al. [21] investigated anaerobic sludge performance enhancement from tropical fruit processing industrial wastewater by adding chitosan in small amounts (two injections with 2 mg chitosan/g suspended solid at each injection) in UASB. They have noticed a 35% increase in biogas production in the reactor with chitosan addition as compared with the control sample. Nuntakumjorn et al. [19] used chitosan in different forms (solution, bead, and powder) and compared sludge granulation and UASB performance with the control sample. They stated that the reactor with chitosan in solution form generated up to 25% more biogas in comparison with the sample without chitosan addition. It can be inferred that chitosan addition aids sludge granulation. There has been no significant improvement with the addition of chitosan powder [19]. Khemkhao et al. [20] studied the effect of chitosan addition on palm oil mill effluent (POME) during a transition from the mesophilic to the thermophilic condition in a UASB system. They declared that biogas production was enhanced by adding chitosan from insignificant to 18%. A summary of efforts done to enhance biogas production using additives is shown in Table 1.

As shown in Table 1, results obtained in this field are either few or contradictory. As a result, more investigations are needed in this area to clarify the effects of additives on biogas production.

The aim of this experimental work is to investigate possible effects of chitosan powder and nanoparticle addition in different concentrations on biogas production from SBW in batch reactors. Specific biogas production and methane content were compared with the control sample.

Substrate and Inoculum

For the purpose of this study, sugar beet was stored at 4 °C according to [30, 31]. Storing sugar beet does not affect its ability to generate biogas [32]. We used 4-month-stored sugar beets as the sole substrate in all experiments. The sugar beet was washed and chopped (as done in the sugar factory). Then, the waste parts in the root were chopped into semicubic pieces smaller than 0.5 cm in size. Anaerobic sludge was collected from wastewater treatment plant (WWTP) from Quchan Industrial Town. The physiochemical characteristics of both the sugar beet and the sludge are shown in Table 2.

Experimental Setup and Procedures

The schematic diagram of the experimental setup is shown in Fig. 1. The experimental setup consisted of a thermostatic heater (1), water circulator (2), a batch reactor (3), liquid sampling valve (4), gas collector (5), and water bath (6).

Plastic bottles were used as reactors with total volume of 500 mL. A hole was punched in the reactor body, and then the area was sealed with aquarium glue so that liquid sampling could be plausible and the system would not be exposed to the environment. Every four reactors were put in a circulating water bath equipped with a thermostatic heater as can be seen in Fig. 1. Temperature has a substantial effect on AD's performance [8, 33]. While the thermophilic condition provides benefits like more biogas production and substrate degradation, its reactor stability makes the mesophilic condition preferable [8, 33]. Thus, the temperature was set at 37 ± 1 °C.

Three hundred milliliters of sludge was added together with SBW pieces. As our preliminary experiments showed, the substrate to inoculum (S/I) ratio of 0.5:1 is suitable for biogas production without the necessity to adjust the pH [7]. So, we used this ratio for our

Table 1 A summary of previous works on additives' effects on biogas production

Investigator	Substrate	Additive (size)	Mass concentration	Effect (additive concentration)
Lertsittichai et al. [21]	Tropical fruit processing industrial wastewater	Chitosan solution	Two shots of 2 mg/g SS	1.35 enhancement in biogas production (BP)
Nuntakumjorn et al. [19]	Synthetic wastewater	Chitosan solution	2 mg/g SS	Up to 1.25 enhancement in BP
		Chitosan bead (1.67 mm)		8% increase in BP
		Chitosan powder (6.8 μm)		Insignificant
Khemkhao et al. [20]	POME wastewater	Chitosan solution	2 mg/g VSS	Up to 18% increase in BP
Agani et al. [14]	Fecal sludge	Iron powder (0.2 mm)	0.5–3 g Fe per 400-g wet sludge	2.06 (1 g Fe per 400-g wet sludge) enhancement in methane production
Suanon et al. [17]	Sewage sludge	nZVI (160 nm)	0.1%	25.2% increase in methane yield
		Iron powder (0.2 mm)	1.6%	40.8% increase in methane yield
Casals et al. [22]	Cellulose	Fe_3O_4 (7 nm)	100 ppm	180% increase in BP
Suanon et al. [23]	Dewatered excess sludge	nZVI (50 nm)	0.5% and 1%	1.46 enhancement in methane production (0.5%)
		Fe_3O_4 (20 nm)		1.26 enhancement in methane production (0.5%)
Zaidi et al. [24]	Green microalgae *Enteromorpha*	Ni (< 100 nm)	1 and 10 mg/L	26% enhancement in cumulative biogas production (1 mg/L)
		Co (< 100 nm)		9% enhancement in cumulative biogas production (1 mg/L)
		Fe_3O_4 (< 100 nm)		28% enhancement in cumulative biogas production (10 mg/L)
		MgO (< 100 nm)		8% enhancement in cumulative biogas production (10 mg/L)
Mu et al. [25]	Waste activated sludge	TiO_2 (< 25 nm)	6, 30, and 150 mg/g TSS	No effect
Gonzalez-Estrella et al. [26]	Anaerobic granular sludge	nZVI (46–60 nm)	1500 mg/L	No effect
		Fe_2O_3 (40 nm)		No effect
		TiO_2 (~25 nm)		No effect
Chen et al. [27]	Waste activated sludge	TiO_2 (185 nm)	6 to 150 mg/g TSS	No effect
García et al. [28]	Wastewater treatment sludge	TiO_2 (7.5 nm)	1120 mg/L	10% increase in BP
Yadav et al. [29]	Dewatered sludge	TiO_2 (< 100 nm)	100 mg/L	A slight reduction in BP

BP biogas production; *NZVI* nano zerovalent iron; *SS* suspended solid; *TSS* total suspended solid; *VSS*; volatile suspended solid

Table 2 Physiochemical characteristics of substrate and inoculum

	TS (mg/L)	DM (%)	VS (mg/L)	VS (%)
SBW	7876	43.46	7561	95.68
Anaerobic sludge	24,500	–	14,300	58.37

DM dry material; *SBW* sugar beet wastes; *TS* total solid; *VS* volatile solid

experiments. Continuous mixing of the reactors was not possible due to the small size of the reactors. The reactors were stirred 100 times a day manually though [34]. In order to replace the air, all reactors were initially flushed and bubbled with an inert gas (argon) for 2 min.

Suspension Preparation

After weighing the required amount of additives, the NPs and MMWC were gradually added to the sludge in small portions, in order to avoid granulation.

The NPs included suspensions and were mechanically stirred and also sonicated for 22 min after each step of adding NPs. To create a homogenous and stable suspension, after each step of NP addition, the suspension was stirred for 22 min by using a mechanical mixer. The suspensions were sonicated then for 22 min via an ultrasonic vibrator (400 W and 24 kHz) which was made by Top Sonics Co. (Iran). This process was repeated 11 times to make sure the dispersion was thorough [35, 36].

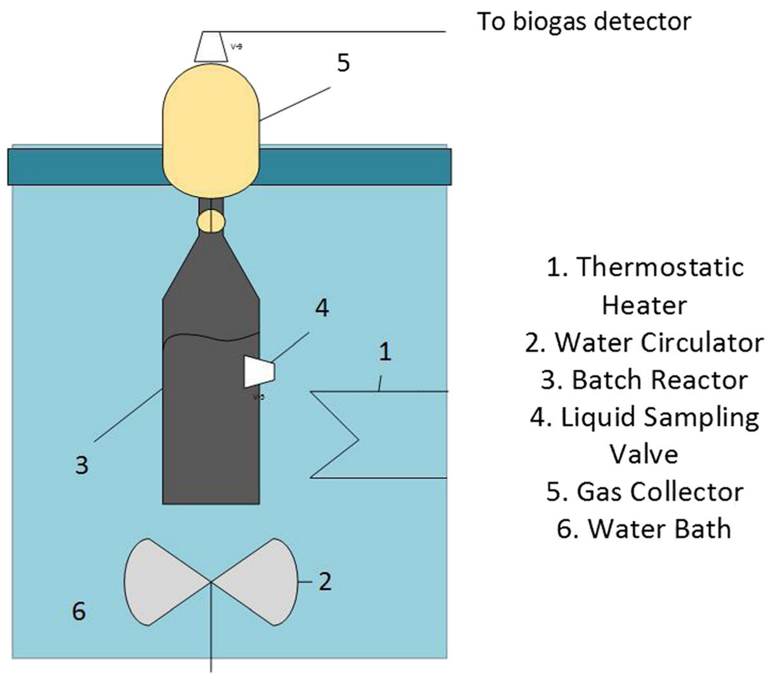

Fig. 1 Schematic design of the setup

The purchased MMWC was sieved with a 200-mesh size screen. So, the maximum particle size would be 74 μm. The chitosan-included samples were just stirred without being sonicated. After completing MMWC addition, the suspension was stirred for 8 h.

The characteristics of NPs and chitosan powder used in the experiments are shown in Table 3.

Particle size distribution inside two different types of nanofluids was investigated using the dynamic light scattering (DLS) technique (Cordouan Vasco3, France). The average diameters of dispersed nanoparticles inside the based fluid are listed in Table 4. To find out how stability of nanoparticles in the based liquid, the zeta potential of the mentioned nanofluids was measured (CAD Zeta Compact, France). The suspension with a higher negative or positive value of zeta potential (less than − 30 mV or higher than + 30 mV) has a higher stability due to a large electrostatic repulsive force between nanoparticles [2]. According to Fig. 2, the mean values of the zeta potential of nanofluids are less than − 30 mV, indicating that the mentioned nanofluids have good stability.

Ten samples were initiated with the substrate and inoculum as mentioned beforehand. Table 5 shows different batches (B1–B9) with different mass (weight) percent. The control sample was a sample loaded with the same S/I ratio and without any additives.

The total solid (TS) and volatile solid (VS) were calculated in accordance with the standard methods [37]. Liquid samples were collected from sample valves using a syringe on a daily basis from all the reactors. Gas samples were taken periodically. The collected gas was tested using portable gas detector Smart Charger Type PGDC2 (portable gas detector, UK).

Results and Discussion

In order to examine the reliability of the results, the experiments were done in triplicate. The standard deviation (STDEV) was calculated afterward. The maximum STDEV for pH and volume of gas measurements were 3.16% and 10.02%, respectively. As can be seen, the experiments are repeatable within an acceptable error range.

As shown in Fig. 3, all the reactors went through the same route having a pH drop in the first days of operation due to formation of volatile fatty acids (VFAs). The produced VFA was used to generate biogas afterwards and the pH increased consequently. Specific biogas production, which is a ratio of cumulative volume of biogas production to grams of VS, from SBW is shown in Fig. 4. As can be seen in Figs. 3 and 4, almost all the reactors were stabilized after 7 days of operation. On the other hand, biogas generation started roughly on the same day (Fig. 4). As can be seen, using additives has not shown any effect on system stability nor accelerated biogas production.

Table 3 Characteristics of the employed additives

Properties	TiO_2	Fe_3O_4	MMWC
Morphology	Spherical	Spherical	Powder
Color	White	Dark brown	White
Average particle size	10–15 nm	20–30 nm	≤ 74 μm
Density (g/cm^3)	3.84	4.8–5.1	–
Molecular weight (Da)	–	–	310,000
Producer	Tecnan, Spain	US Research Nanomaterials, USA	Sigma-Aldrich, USA

Table 4 The results of DLS measurements for nanoparticles dispersed in the based liquid

Nanofluids	Nanoparticles' average diameter (nm)	Polydispersity index (PDI)
Fe_3O_4 nanofluids	39.21	0.22
TiO_2 nanofluids	18.64	0.17

As can be seen in Fig. 4, using NPs and MMWC in 0.01% *w/w* concentration did not improve biogas production. In fact, an adverse effect can also be seen in the case of TiO_2 NPs. As the concentrations rise to 0.04% *w/w* (or for greater concentrations of nanoparticles), this adverse effect perishes in the case of TiO_2 and changes into an increase in the case of Fe_3O_4 NPs. Figure 4 b also represents this incremental effect for the sample containing Fe_3O_4 NPs. This could be due to the use of electron-donating NPs and acetate production which increases the activity of methanogenic archaea. Employing TiO_2 NPs did not help the methanogen process to improve in any cases, which is in agreement with previous studies [25–27]. Chitosan powder was reported to have an insignificant effect on biogas production enhancement [19]. These experiments share the same results. Lack of effective interactions between the particles and microorganisms can be one of the dominant reasons. Therefore, the suitable nanoparticle mass concentration in this study obtained to be 0.04% of Fe_3O_4 NPs shows 15.09% enhancement in specific biogas production relative to the control sample as shown in Fig. 4.

Specific biogas production enhancement ratio (SBPER), which is the ratio of specific biogas production from the additive-included sample to that from the additive-free sample, from SBW for various additives, is shown in Fig. 5. Previous results for using TiO_2 NPs for biogas production have been in contrast. Some reported a 10% increase [28], some mentioned no effect [25–27], and some stated a slight decrease [29] in biogas production. In this study, using TiO_2 NPs has not shown a noticeable difference in biogas production from SBW according to Fig. 5a. A microbial community is an important aspect in sludge fermentation. The abundance of bacteria and methanogenic archaea is linked with fermentation of the sludge as well [27]. It can be concluded that adding TiO_2 NPs did not influence microbial activity or their abundance to our benefit in order to generate more biogas. The results vary in the error range and are in agreement with previous efforts [25–27].

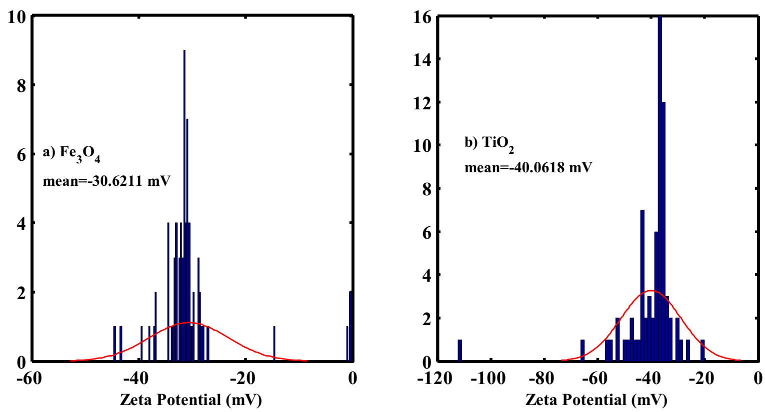

Fig. 2 Zeta potential diagram for nanofluids. **a** Fe_3O_4. **b** TiO_2

Table 5 Specifications of the reactors

Sample	Additive	Concentration (wt%)
B1	TiO_2	0.01
B2	TiO_2	0.04
B3	TiO_2	0.12
B4	Fe_3O_4	0.01
B5	Fe_3O_4	0.04
B6	Fe_3O_4	0.12
B7	MMWC	0.01
B8	MMWC	0.04
B9	MMWC	0.12
Control sample	–	0

Figure 5 b shows the specific biogas production from SBW with different Fe_3O_4 NP concentrations. Fe_3O_4 NPs have previously been reported to increase biogas production [17, 23]. Suanon et al. [23] stated that the volume of methane gas was dependent on the dose of Fe_3O_4 NPs added to the digester. They observed an adverse effect on biogas generation at higher Fe_3O_4 NP concentrations. According to Fig. 5b, adding 20–30 nm Fe_3O_4 NPs at 0.01 concentration did not help anaerobic digestion. By adding 0.04% and 0.12% Fe_3O_4 NPs, about 29% and 39% augmentation was obtained, after 4 days of operation. But for operation days greater than 4, there was a minor fluctuation in biogas production that can be addressed to deviation in measurement.

Figure 5c shows the effect of chitosan additive on biogas production. Chitosan has shown good effects on enhancing sludge granulation and shortening the startup period of UASB systems [20, 21]. But considering the STDEV, no significant change was observed in the reactors with the chitosan additive (Fig. 5c). The results are in agreement with Nuntakumjorn et al. [19]. As they concluded, although there were enough contacts between the chitosan

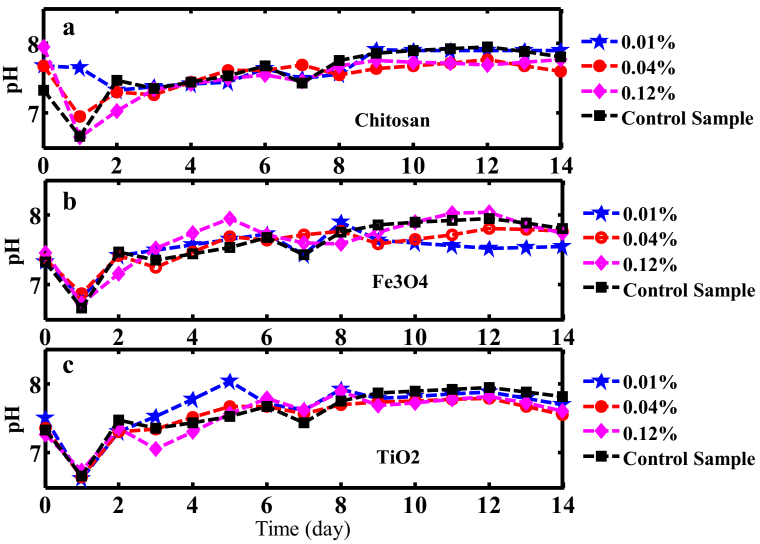

Fig. 3 Change of pH during anaerobic digestion of SBW with the addition of different additives. **a** Chitosan powder. **b** Fe_3O_4 nanoparticles. **c** TiO_2 nanoparticles

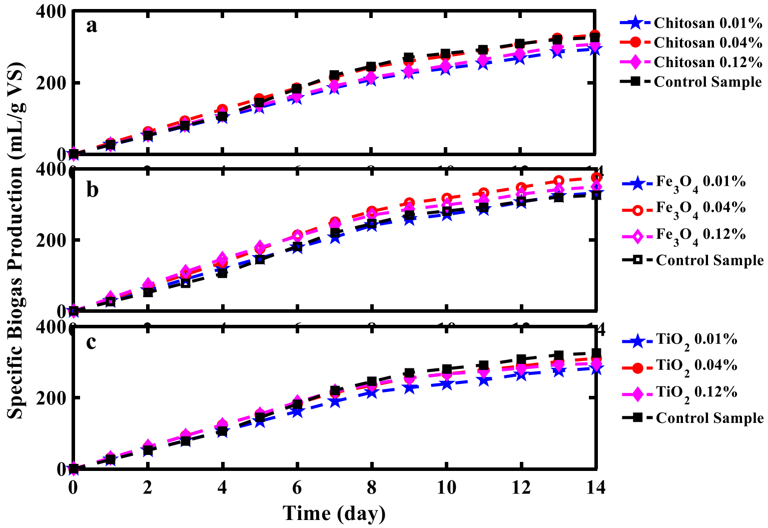

Fig. 4 Specific biogas production from SBW with the addition of different additives. **a** Chitosan powder. **b** Fe_3O_4 nanoparticles. **c** TiO_2 nanoparticles

powder and the substrate, they could not appreciably reduce the electrostatic interaction between negatively charged bacteria.

It is believed that the type and size of additives can have important effects on biological degradation of compounds. For smaller particles, the surface-to-volume ratio is larger. Therefore, this would create suitable sites for microorganism colonies, whereby the hydrolysis rate and reactions increase. On the other hand, considering that metal and metal oxide particles act as an electron donor, they can increase biogas yield. It can be concluded that electronegative particles are more effective in the anaerobic digestion process. Hence, metal oxide nanoparticles are preferred than the chitosan powder. Also, chaotic movement of particles causes micromixing and microstirring inside the samples. This can stimulate bacterial activity and promote biogas production. These interactions (specific area, electron donor effect, and chaotic motion) between the chitosan powder and microorganisms are much less than those between the NPs and microorganisms.

Methane is a valuable product that resulted from AD. Methane content can be used to assess the performance of AD. Table 6 shows the average and maximum methane content and the TS and VS reduction for AD of SBW in the presence of the mentioned additives. As can be seen, the highest methane content was obtained from the B5 reactor which was loaded with 0.04 wt% Fe_3O_4. But the methane production did not show a noticeable enhancement after all. Thus, the additives did not improve the biogas generation. On the other hand, no sign of an inhibitory effect was observed. The TS and VS reduction of the control sample were 37.16% and 59.49%, respectively. Fe_3O_4 or TiO_2 NPs or MMWC addition did not show a significant effect (except at 0.04% Fe_3O_4) on reduction of TS or VS of SBW as reported in Table 6.

Conclusion

Biogas and methane production from SBW were measured using a simple and inexpensive method in lab-scale anaerobic digesters. Fe_3O_4 and TiO_2 NPs were added to reactors in 0.01%,

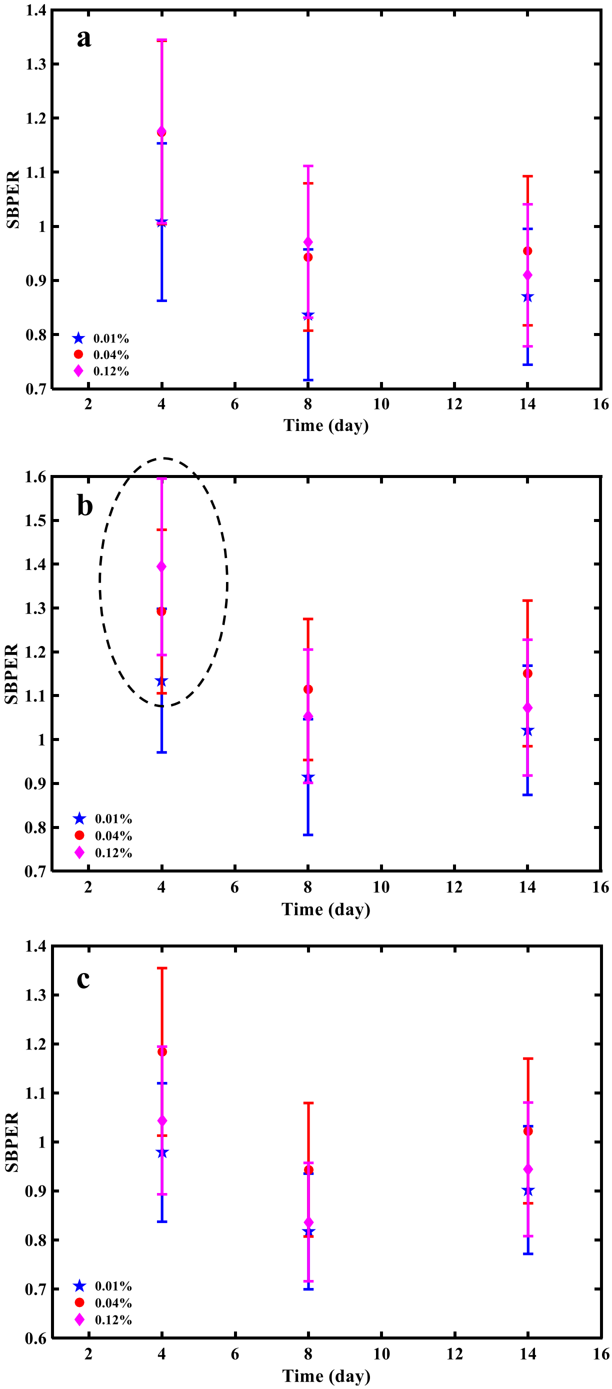

Fig. 5 Additives' effect on biogas production enhancement. **a** TiO_2. **b** Fe_3O_4. **c** Chitosan powder

Table 6 Average and maximum methane content and TS and VS reduction

Sample	Average methane content (mol%)	Maximum methane content (mol%)	TS reduction (%)	VS reduction (%)
B1	44.85	58.13	40.30	57.26
B2	47.11	65.31	34.96	57.70
B3	42.59	60.31	28.05	52.36
B4	39.61	54.54	37.47	56.37
B5	55.28	74.45	48.78	66.16
B6	45.24	64.38	34.96	65.72
B7	52.5	67.5	30.87	48.35
B8	48.13	61.88	38.73	55.92
B9	53.92	67.19	38.10	60.38
Control sample	47.43	62.16	37.16	59.49

0.04%, and 0.12% *w*/*w* concentrations in order to investigate their effect on AD of SBW. MMWC was also added in the same concentrations in microsize (less than 74 μm). Compared to the control, none of the additives showed inhibitory effects on anaerobic digestion. NP addition did not enhance biogas production; it did not influence TS or VS reduction at all concentrations either, except for Fe_3O_4 at 0.04 wt%, which led to an increase in both methane production (19.77%) and TS or VS reduction. Also, by adding 0.04% Fe_3O_4 NPs, about 15.09% augmentation was obtained. This could be due to the use of electron-donating Fe_3O_4 NPs and acetate production, which increases the activity of methanogenic archaea. On the other hand, adding MMWC showed no effect. It could be concluded that MMWC did not improve methanogen bacteria's abundance and their flocculation. This could be due to the powder form and lack of suitable interactions between the MMWC and the substrate.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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