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Effect of metal oxide based TiO₂ nanoparticles on anaerobic digestion process of lignocellulosic substrate

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Abstract

Lignocellulosic materials are recalcitrant to bioconversion, due to their rigid physiochemical structure. In this work, the effects of Fe_2O_3 - TiO_2 and NiO - TiO_2 nanoparticles (NPs) and FeCl_3 and NiCl_2 salts, on the anaerobic digestion (AD) of wheat straw have been investigated. For this purpose, metal oxide- TiO_2 NPs were synthesized and fully characterized. Results showed that addition of 0.252 mg of NiO - TiO_2 /g total solids (TS) to batch assays resulted in increase of soluble chemical oxygen demand (COD) and 67% increase in volatile fatty acids (VFAs) concentration compared to control tests during the first 4 days of experiments. These results indicate that hydrolysis and acidogenesis rates have been enhanced due to addition of NPs. No significant effects have been observed in soluble COD and VFAs concentrations compared to control experiments by adding salts to the batch assays. Addition of NPs or salts led to an increase in methane production rate during the first 4 days of experiments.

Keywords

Nanoparticles; Anaerobic digestion; Lignocellulose; TiO_2 ; Trace elements

1 Introduction

In recent years, interest in anaerobic digestion (AD) of organic waste such as cow manure, poultry litter, food waste, and agriculture residues has increased. The AD process can contribute to treatment of organic wastes, and at the same time produce a renewable fuel (biogas) and thereby contribute to reduction emission of greenhouse gases [1]. Four major steps are involved in biological conversion of organic matters to

biogas. Different groups of microorganisms perform the four steps, i.e., hydrolysis, acidogenesis (primary fermentation), acetogenesis (secondary fermentation), and methanogenesis during AD of organic matters [2]. Among various types of feedstocks, lignocellulosic materials, i.e. agriculture residue, can be potentially one of the important substrates in AD process, due to their availability, large abundance and high carbohydrate content [3]. Generally, The hydrolysis of lignocellulosic biomass to fermentable substances is considered the rate limiting step in overall process [4]. So in order to increase the overall biogas production rate, accelerating hydrolysis step is necessary. Anaerobic thermophilic hydrolytic bacterium such as *Clostridium thermocellum* produces extracellular enzymes and perform the initial break down of cellulose polymers [5]. In order to facilitate the hydrolysis of lignocellulosic biomass many strategies have been implemented, such as co-digestion with other substrates, chemical or physical pre-treatments and/or combination of them [6], and addition of biological (i.e. enzymes) and non-biological additives (i.e. metal salts of trace elements, metallic and non-metallic nanostructures) to the AD system [7,8]. Most of the present AD processes dealing with lignocellulosic feedstocks rely on chemical/physical pre-treatments and high dosage of enzymes, which can lead to high operational costs of the plant[3]. Liu et al. [9] stated that the addition of external inorganic compounds, i.e. magnetite and zeolite, increased methane production *via* influencing the microorganisms and enzymatic transformation of the AD processes.

The activity and growth of AD microorganisms highly depend on necessary nutrients and presence of micronutrients such as the trace elements iron (Fe), nickel (Ni), molybdenum (Mo), cobalt (Co), and selenium (Se) which are constituent of enzymes of microorganisms and are essential for numerous reactions [10]. It has been shown that

the biogas production from agriculture residues can be limited by the shortage of essential trace elements. However, high concentrations of trace elements can result in adverse effects on biological degradation processes in anaerobic reactors [11].

Lignocellulosic biomass contains low concentrations of trace metals and thereby can potentially lead to low performance of overall AD process. Kim et al. [12] reported that Fe, Co, and Ni stimulate hydrolysis at both mesophilic and thermophilic temperatures. Generally different methanogens can be activated by specific trace metals, although all methanogens require Co, Ni and Fe [13]. Fe is an inorganic cofactor for some proteins (i.e. cytochromes) that are relating to energy metabolism, hence it is essential for the growth of almost all microorganisms involved in AD process [14]. Ma et al. [15] showed that various types of iron oxide (Fe_2O_3 , $\text{FeO}(\text{OH})$, Fe_3O_4) increase hydrolytic efficiency and methane production from lignocellulosic biomass via generating Fe^{2+} ion that served as nutrient supply for the microorganisms. Moreover, Zhang et al. [16] reported that iron oxides promoted microbial hydrolysis-acidification of complex organic matter, resulting in more soluble organic matter for methanogenesis. Addition of 30 mg/g VS of FeCl_3 during AD of corn straw was also reported to result in an increase of 14% in methane production [17]. Ni is an essential element of F_{430} , a low molecular weight coenzyme, present in all methanogens which facilitate methane production. Furthermore, Ni is also essential for the growth of acetoclastic organisms [18]. It was reported that the biogas production from manure could be enhanced by addition of NiCl_2 [11]. Oppositely, high level of trace elements can have adverse effect on anaerobic digestion process. For instance, it has been reported that 1 mg/L of Ni was inhibiting AD of sewage sludge [19]. Several researches, recommended concentration of Fe and Ni elements in anaerobic digesters was reported in the range of 1-10 mg/L and

0.005-0.5 mg/L, respectively [20–23]. Kloss et al. [24] reported that Fe dose of 10-200 mg/L increased the cumulative CH₄ production. Zhang et al. [25] mentioned that the process related to methane production is inhibited when Ni concentrations exceed 1.2 mg/L. Altas [26] and Wu et al. [27] reported that 0.5-16 mg/L of Ni increased the cumulative CH₄ production. Demirel and Scherer [28] stated that the optimum or stimulatory concentrations of Ni for batch cultures of methanogens range between 0.012 and 5 mg/L.

Nanoparticles (NPs), with at least one dimension less than 100 nm, have widely been applied in engineering and environmental processes [29]. NPs have excellent thermal, mechanical, structural, and morphological properties which enable them to be used in different environmental applications [30]. The challenges of sustainability, energy, climate change are attracting researchers to explore the nanoscale science as a new source of improvements for the energy and environment section since the nanoscale materials show extraordinary properties that are not shown by bulk materials [31]. NPs, due to their small size, can pass across biological barriers and influence uptake, absorption, distribution and metabolism of compounds [32]. The positive effect exerted by the NPs was explained by their high surface area, high reactivity, high specificity, self-assembly and dispersibility [33]. It has been proven that supplementation of trace elements in form of NPs improved AD performance [34]. On the other hand, the effluent of the AD process, i.e. digestate, can be used as compost and soil conditioner after dewatering [35]. In some cases, the digestate may become inappropriate and toxic to be applied as a bio-solid due to the accumulation of NPs. Yang et al. [36] showed that silver ions released to soil can lead to a significant reduction in denitrification activity which is unfavourable. The effects and fate of NPs in effluent of AD processes have not

yet been established completely, therefore digested containing NPs should be investigated in relation to phytotoxicity and bacterial toxicity should be assessed before reusability of bio-solid. One of the advantageous properties of NPs is the slow dissolving rate of trace elements which facilitates the bioavailability of metal ions in the AD medium and enhances enzymes activity [1]. In general, adsorption of NPs on enzymes will cause conformational transitions, accompanied by the inhibition or enhancement of the enzymatic activity [37]. Wang et al. [29] reported that in case of waste activated sludge, 10 mg/g TS of nano-zero valent iron (NZVI) and 100 mg/g TS of Fe_2O_3 NPs increased the population of microorganisms (Bacteria and Archaea) and activities of key enzymes, while 500 mg/g TS of Ag and MgO NPs inhibited them. Abdelsalam et al. [11] reported that during AD of raw manure, using 2 mg/L of Ni NPs gave the highest specific methane production in comparison to other tested concentrations (0.5, 1, and 2 mg/L), their salts (CoCl_2 and NiCl_2) and the control. The stimulating effects of NPs may be attributed to their uptake inside the microorganisms, integrating them with metabolic intermediates and key enzyme activities involved in the AD process [38]. However, the relatively high density of NPs of trace elements (Fe and Ni) may cause an uneven dispersion in the AD medium, which could limit the bioavailability of the trace element and weaken the interaction between iron oxide minerals and functional microorganisms [39]. Coating the trace metal oxides, i.e. iron oxide and nickel oxide, on a carrier may be a potential strategy to solve this problem [40]. Distributing-or coating of metal oxide NPs over the inert support materials (i.e. TiO_2 , Al_2O_3 , SiO_2), increases the fraction of surface- exposed atoms of NPs and consequently increases the reactivity of these metals due to desire dispersion of metal oxides on surface of support [41]. Despite this interest, a few researchers have studied

the effect of supported NPs and to the best of the authors knowledge no one used TiO_2 , as carrier/support of NPs in AD processes. TiO_2 is a kind of inexpensive and non-cytotoxic mineral with a large surface area and crystalline structure that can be used as support of trace element NPs. Gonzalez-Estrella [42] reported that among four metal oxide NPs (TiO_2 , Al_2O_3 , SiO_2 and ZnO) only ZnO show inhibitory effect on methane generation. This is mainly due to the release of toxic divalent Zn ions that influence the microorganisms. In another research Garcia et al. [43] observed no inhibitory effect on AD of cellulose substrate due to addition of bare TiO_2 .

Higher degradability and thus, methane production was achieved using a fibre-rich feedstock in thermophilic compared to mesophilic conditions [44]. Furthermore, the thermophilic AD process can lead to improved process performance at increased loading rates and also, produces a more organically-stable, pathogen-free digestate than mesophilic [45]. The mentioned characteristics settle thermophilic AD interesting for real-life applications and hence, the present study was conducted at 55 °C. In the present work, 5.0 wt% Fe_2O_3 -coated TiO_2 and 5.0 wt% NiO -coated TiO_2 NPs were synthesized by ultrasonic-assisted co-precipitation method. Batch experiments (BMPs) were conducted under thermophilic conditions, with the purpose of evaluating the effect of the Fe_2O_3 - TiO_2 and NiO - TiO_2 NPs on the AD of wheat straw. For comparison, the experiments with no additive, i.e. control, and with metal salts, i.e. FeCl_3 and NiCl_2 , as the bulk trace elements were also conducted. Chemical oxygen demand (COD), volatile fatty acids (VFAs), and cumulative biogas and methane production, were measured along the AD process to detailed investigation of the effect of trace elements in form of NPs and corresponding salts, on AD of lignocellulosic feedstock.

2. Materials and methods

2.1. Substrate and inoculum

Wheat straw was obtained from an agriculture farm in Zealand, Denmark. The wheat straw was cut down to 1-2 cm length by a cutting mill (Retsch SM 2000) and used as mono substrate in this study. As inoculum the digestate of a well performing lab-scale reactor fed with source separated municipal waste was used. The digestate was sieved in order to remove remaining organic particles. The digestate was degassed before use, by incubating at thermophilic conditions (54 ± 1 °C) for 10 days to reduce the background biogas production. The physiochemical analysis of wheat straw and inoculum were performed in triplicate and presented in Table 1.

Table 1. Physicochemical characterization of wheat straw and inoculum

Characteristics	Wheat Straw	Inoculum
pH	-	8.08
Total COD (g/kgTS)	1060.92 ± 43.26	24.78 ± 1.19
TS (g/kg)	928.39 ± 0.01	26.70 ± 0.20
VS (g/kg)	867.22 ± 3.85	17.54 ± 0.22
total Kjeldahl nitrogen (TKN) (g/kg)	4.8 ± 0.1	2.57 ± 0.10
ammonium nitrogen (NH ₄ N) (g/kg)	0.8 ± 0.1	1.72 ± 0.05
Fe (mg/g TS)	0.038 ± 0.002	0.10 ± 0.00
Ni (mg/g TS)	0.0009 ± 0.0001	0.00245 ± 0.00012
cellulose (%TS)	42.02 ± 0.72	—

hemicellulose (%TS)	30.75 ± 0.55	—
klason Lignin (%TS)	26.73 ± 2.35	—

2.2. Preparation of metal oxide- TiO_2 nanoparticles

For synthesizing TiO_2 -based metal oxides, 5.0 wt% NiO/TiO_2 and 5.0 wt% $\text{Fe}_2\text{O}_3/\text{TiO}_2$, a sono-chemical method was applied [46]. Diluted ammonia solution was added to 0.5M TiCl_4 solution under stirring and ultrasonication for 120 min at 80 °C. After cooling down to room temperature, the slurry was centrifuged and washed several times with distilled water. TiO_2 powder was collected and dried overnight at 100 °C. In order to coat the surface of TiO_2 with 5 wt% metal oxide (NiO or Fe_2O_3), TiO_2 powder was introduced to pre-calculated amount (twice the stoichiometric amount) of Na_2CO_3 aqueous solution. Then the pre-calculated amount of metal salt, i.e. NiCl_2 in the case of NiO and FeCl_3 in case of Fe_2O_3 , was dissolved in distilled water and metal containing solution was gradually added to TiO_2 slurry under stirring and ultrasonication for 120 minutes. The metal oxide- TiO_2 NPs were centrifuged and washed several times with distilled water, following by overnight drying at 100 °C. Finally, the NPs were calcined at 250 °C at a rate of 5 °C/min for 3 h. $\text{Fe}_2\text{O}_3\text{-TiO}_2$ and NiO-TiO_2 NPs are denoted as FT and NT, respectively.

2.3. Characterization of synthesized nanoparticles

X-ray powder diffraction (XRD) patterns of the synthesized metal oxide- TiO_2 NPs were recorded using a Philips Analytical (X'pert Pro MPD) X-ray powder diffractometer with $\text{CuK}\alpha$ radiation. The average crystallite size of the NPs were calculated using the Scherrer's equation [47].

Brunauer-Emmett-Teller (BET) specific surface area of NPs was measured by N_2

physical adsorption, using micrometrics Gemini surface area analyzer. Assuming spherical particle, the approximate average particle size of NPs was calculated using the following equation:

$$d_{BET} = \frac{6000}{\rho \times S_{BET}} \quad (1)$$

where S_{BET} is the BET surface area (m^2/g) and ρ is the true density (g/cm^3) of a nanoparticle [46].

The morphology and size distribution of the synthesized NPs were observed by using transmission electron microscopy (FEI Titan 80-300ST TEM). Selected area electron diffraction (SAED) and energy dispersive X-ray spectroscopy (EDX) in the TEM were used for crystallographic and elemental analysis of NPs. For TEM, nanoparticle samples were diluted with water, dropped onto holey carbon supporting Cu grids and dried out in air.

2.4. BMP tests

Biochemical methane potential tests (BMPs) were performed in triplicate under thermophilic conditions ($55^\circ C$). Glass bottles with working and total volumes of 164 and 547 mL respectively, were used for BMP experiments. Based on the volatile solid content of substrate and inoculum (Table 1), a constant amount of inoculum (i.e., 68.38 g) was mixed with a suspension of 0.47 g wheat straw in 95.25 g water so that the substrate to inoculum ratio (S/I) and initial organic load of substrate for all BMPs set to 2.0 gVS/gVS and 2.5 gVS/L, respectively. The trace metal salts, iron chloride ($FeCl_3$) and nickel chloride ($NiCl_2$), were selected for addition at three levels of concentrations based on TS of substrate according to the dosages used in literature [11,33,48]. These dosages are in the range of recommended concentrations for digesters mentioned in

introduction. Based on levels presented in Table 2, level 1 of FeCl_3 and NiCl_2 contain 0.1 g/g TS of Fe and 0.009 g/g TS of Ni element, respectively. Trace elements in form of NPs, $\text{Fe}_2\text{O}_3\text{-TiO}_2$ and NiO-TiO_2 , were used as additives in three different dosages. In order to prevent aggregation of NPs and increase their suspension stability, the specific amount of NPs was added to 10 mL of distilled water and ultra-sonicated for 5 minutes prior to adding them to batch assays. The dosages of $\text{Fe}_2\text{O}_3\text{-TiO}_2$ and NiO-TiO_2 NPs were calculated according to the amounts of Fe and Ni elements in the FeCl_3 and NiCl_2 additives, respectively (Table 2). As an example, for the level 3 of nickel containing additives, the amount of Ni element in both NiCl_2 and NiO-TiO_2 was equal to 0.9 mg/gTS. In order to regulate pH, 0.1 g of sodium bicarbonate was added to each bottle. Triplicate of batch reactors containing inoculum, water, and substrate were prepared and incubated in order to determine the biomethane production of mixture in the absence of NPs and salts. In order to provide anaerobic conditions, all bottles were sparged with N_2 gas flow for 5 minutes and immediately sealed with butyl rubber stopper and aluminum crimps. The bottles were incubated for 18 days and were shaken manually twice a day during the whole incubation period, in order to ensure homogeneous conditions. Methane concentration was measured twice per week from the headspace of the glass bottles, until the end of process. 5 mL of the liquid phase was taken from one of the three replicates of each BMP bottles, weekly, for soluble COD and total VFAs analysis.

Table 2. Nanoparticles and trace element salts concentrations supplemented to the biomethane potential (BMP) experiments

Additive	Concentration,		
	mg of additive/gTS substrate (mg of Ni or Fe element/L)		
	Level 1	Level 2	Level 3
FeCl ₃	0.29 (0.269)	2.9 (2.69)	29 (26.9)
NiCl ₂	0.02 (0.024)	0.2 (0.24)	2 (2.4)
Fe ₂ O ₃ -TiO ₂ (FT)	2.85 (0.269)	28.5 (2.69)	285 (26.9)
NiO-TiO ₂ (NT)	0.252 (0.024)	2.52 (0.24)	25.2 (2.4)

2.5. Analytical methods

Total solids (TS) and volatile solids (VS), and chemical oxygen demand (COD) were determined according to the Standard Methods [49]. Structural carbohydrates and lignin content of wheat straw were determined according to the procedure reported by Sluiter et al [50]. Volatile fatty acid concentrations (VFAs) were determined using a gas-chromatograph (GC-TRACE 1300) equipped with flame ionization detector (FID) and Agilent J & W capillary Column (30 m x 0.530 mm i.d., film thickness 1.50 µm) with helium as carrier gas [51]. The methane concentration in the headspace of the batch reactors was measured using a gas chromatograph (Shimadzu GC-8A) equipped with a flame ionization detector (FID). The volume of produced methane was corrected at STP condition, and BMPs were determined per unit of gVS in the substrate added to each batch reactor. All analytical methods were performed in triplicate and average values were reported.

2.6. Statistical analysis

Statistical analysis was applied to all data. Each experiment was conducted three

times and the average values and standard deviations were calculated. The analysis indicated that the results were similar and there was no significant difference between replicates for each set of experiments. The maximum standard deviations including all data from GC, VFAs, and COD results were 4.33 mL CH₄/gVS, 0.0218 g/L, and 0.084 g/L, respectively. In order to compare quantitative variables among different experiments, one-way ANOVA was applied, following Tukey post hoc test ($p < 0.05$).

3. Results and Discussion

3.1. Characterization of nanoparticles

The crystallographic forms of synthesized NPs were characterized by XRD and SAED in the TEM (Fig. 1). XRD patterns of the Fe₂O₃-TiO₂ and NiO-TiO₂ NPs, calcined at 250 °C, are shown in Fig. 1a. The XRD patterns of both NPs have well-developed peaks, which correspond to the d-spacings of anatase (JCPDS file No. 21-1272) or rutile (JCPDS file No. 21-1276). Anatase and rutile phases are marked by A and R, respectively. No significant peaks for Fe₂O₃ or NiO were detected in the Fe₂O₃-TiO₂ and NiO-TiO₂ NPs. This is probably due to the low content of metal oxide and/or highly dispersion of metal oxides on the surface of TiO₂ [52]. Similar observations have been reported in previous works [53]. According to the XRD spectra, the average crystallite sizes (d_{XRD}) of the NPs, using Scherrer's equation, are presented in Table 3. SAED patterns of Fe₂O₃-TiO₂ and NiO-TiO₂ NPs are shown in Figs. 1b and 1c, respectively. Based on d-spacing values measured from the SAED patterns [54], the distinct diffraction rings were indexed with either rutile or anatase phase [55]. The d-spacing values measured with SAED are in excellent agreement with those identified with XRD analysis shown in Fig 1a. No apparent diffraction spots for Fe₂O₃ or NiO

were observed; suggesting that Fe or Ni is present as an amorphous phase and/or too small crystallites.

Fig. 1 – X-ray diffraction patterns of $\text{Fe}_2\text{O}_3\text{-TiO}_2$ and NiO-TiO_2 NPs (a), SAED patterns of $\text{Fe}_2\text{O}_3\text{-TiO}_2$ (b), SAED patterns of NiO-TiO_2 nanoparticles (c).

The BET surface areas of pristine (bare) and metal oxide-coated TiO_2 NPs are presented in Table 3. NPs had surface areas in the range of 120-160 m^2/g . Coating the TiO_2 surface with 5wt% of Fe_2O_3 or NiO leads to decrease in pore volume and to smaller surface area due to pore blockage with metal oxides [56].

Table 3. Textural and structural features of synthesized nanoparticles

Nanoparticle	BET surface area (m ² /g)	Average particle size measured by TEM (nm)	Crystallite size measured by XRD (nm)
TiO ₂	161	8.8	9.1
Fe ₂ O ₃ -TiO ₂	126	11.1	10.2
NiO-TiO ₂	144	9.8	9.2

The particle size distributions of Fe₂O₃-TiO₂ and NiO-TiO₂ NPs were obtained by measuring 100 particles from TEM images. Figs. 2a and 2b shows that the particle sizes are somewhat in accordance with the particle size calculated by BET and XRD analysis (Table 3).

Fig. 2 – TEM micrographs and corresponding particles size distribution (inset) of Fe₂O₃-TiO₂ (a) and NiO-TiO₂ (b) nanoparticles.

TEM-EDX spectra of Fe₂O₃-TiO₂ and NiO-TiO₂ NPs shown in Fig. 3 confirms the presence of Ti, O, Ni, and Fe in the NPs. It should be noted that the signals of Cu and C

are from the used TEM grids. Also Na is due to impurity in the synthesis step. The compositions measured in different regions (~200 nm) are almost constant, which may imply that Fe or Ni distributes homogeneously on the surfaces of TiO₂ NPs. The small peak for Fe in Fig. 3b probably originates from the microscope pole piece.

Fig. 3 – EDX spectra of Fe₂O₃-TiO₂ (a), and NiO-TiO₂ (b) nanoparticles.

3.2. Effect on COD and VFAs concentration

COD and VFAs are important performance indicators for the AD process. Soluble COD and total VFAs concentrations measured during the batch assay testing different dosages of additives are presented in Table 2. Fe was tested at higher concentrations

compared to Ni is in accordance to previous reported values showing potential effect [57]. In addition, threshold concentration for toxicity of Ni is much lower than for Fe [14]. Effect of Fe and Ni trace elements in form of salts (FeCl_3 and NiCl_2) and NPs ($\text{Fe}_2\text{O}_3\text{-TiO}_2$ and NiO-TiO_2 compound) on soluble COD and total VFAs concentrations is illustrated in Fig.4 and Fig. 5, respectively. The COD and VFAs values including the VFA components in the Stacked Graphs were the average values of triplicate measurements.

Fig. 4 – Effect of different concentrations of trace elements (in form of TiO_2 -based NPs and metal salts) on soluble COD variations.

Fig. 5 – Effect of different concentrations of trace elements (in form of TiO_2 -based NPs and metal salts) on VFAs variations (L1: level 1, L2: level 2, L3: level 3).

It was observed that in the beginning of the experiment (day 4) the concentrations of soluble COD and total VFAs were higher in case of using Fe_2O_3 - TiO_2 and NiO - TiO_2 NPs compared to the ones exhibited by the control and salt (i.e. FeCl_3 and NiCl_2) experiments. This indicates that the hydrolysis rate and subsequently acidification degree of substrate was profoundly enhanced by adding NPs. Soluble COD and total VFAs concentration in case of using level 1 of NiO - TiO_2 NPs at day 4 was 13% and 67% higher than that of control at day 4, respectively. Furthermore, Fig. 5 indicates the predominant VFA content during the experiment was acetic acid, followed by propionic acid and trace amount of other VFAs, i.e. butyric and valeric acids. Butyric and longer chain acids were converted before day 4. Specifically, the maximum acetate concentration (0.195 ± 0.0181 g/L) was found at day 4 in case of using level 1 of NiO -

TiO₂ NPs which was 95% higher than that in the control bottle. It is also clear that using NPs caused the accumulation of VFAs content and the hydrolysis of substrate may not have been the rate-limiting step in the AD process of wheat straw. Decomposition and conversion of cellulose and hemicellulose leads to increase in soluble COD and subsequently VFAs concentrations [58]. The mechanism behind the enhancement of soluble COD and VFAs in the presence of metal oxide-TiO₂ NPs, probably could be related to the fact that in early days of the process, metal oxide-TiO₂ composites accelerate conversion of the accessible macromolecules (cellulose and hemicellulose) into monomers (soluble sugars). Probably, TiO₂-based NPs can serve a high surface area to enhance enzymatic activity of hydrolytic enzymes. This is in agreement with the results obtained by Lu et al. [39] confirming that presence of iron oxide-zeolite NPs leads to a higher total VFAs in AD process of cow manure and rice straw. NPs interact with proteinous compounds (i.e enzymes), membranes, cells, DNA, and organelles [59]. The interaction between non-functionalized NPs, i.e. metal-oxide coated TiO₂, and biological systems (i.e. enzymes) is based on physical adsorption, related to dipole-dipole, hydrophobic, van der Waals, or hydrogen forces [37]. The accumulation of enzymes on the surface of non-toxic NPs enhances enzymatic activity due to higher enzyme density [60]. Furthermore, the effect of TiO₂ NPs on the electron transfer reaction of the activity process of enzyme causes an increase in enzyme activity [61]. Boisset et al. [62] reported that the specific activity of the multi hydrolase enzyme complexes is higher than that of free enzyme systems. Furthermore, Verma and Stellacci (2010) showed that NP surface properties (surface area, shape, and size) affect NP- microorganism interactions and boost bioavailability of lignocellulosic biomass [39]. As it is presented in Table 3, TiO₂-based NPs provide a large BET surface area for

boosted enzymatic activities. There is no significant difference between the COD and VFAs levels of all BMPs in day 11 and also in day 18. So the difference is in the early days and after that the final levels of COD and VFAs are in the same range for all the experiments. It means almost all of the accessible substrate to enzymes, i.e. cellulose and hemicellulose, is degraded during first 4-5 days of experiment. It is notable that the pH values for all the BMPs during 18 days of experiments were in the range of 6.5-8.

3.3. Effect on methane production

Generally, trace elements are crucial cofactors in many enzymatic reactions involved in biochemistry of methane formation [23]. Fig. 6 illustrates the cumulative production of methane, in the absence and presence of different levels of $\text{Fe}_2\text{O}_3\text{-TiO}_2$ and NiO-TiO_2 NPs and their corresponding salts (FeCl_3 and NiCl_2). Average values of methane production and error bars were calculated based on triplicate measurements.

Fig. 6– Effect of different concentrations of $\text{Fe}_2\text{O}_3\text{-TiO}_2$ and NiO-TiO_2 nanoparticles, and Fe and Ni salts on the cumulative biomethane production from the AD of

wheat straw.

Cumulative methane production of control sample during time was consistent with pervious researches on anaerobic mono-digestion of wheat straw [64,65]. As it can be seen, the addition of NPs and their salts with various concentrations did not result in a significant enhancement of final methane production, and the amount of produced gas at the end of the process is in the same range for all of the additives and levels. This is in agreement with the results obtained by [6] that declaring that trace elements (i.e. Ni, Co, Se) did not result in significant enhancement of the AD of wheat straw. The difference is in first 4 days of experiment, in which adding both salt and NPs has increased the methane production in comparison with control experiments. This can be attributed to the fact that either NPs or corresponding salts can served as cofactors in enzymes which are involved in process of methane formation [58]. Based on results presented in Table 4, cumulative methane production at day 4 was 0.6, 6.1 and 11.4% more than control experiment when the BMPs conducted respectively in the presence of 2.9 mg/gTS (level 1), 28.5 mg/gTS (level 2), and 285 mg/gTS (level 3) of $\text{Fe}_2\text{O}_3\text{-TiO}_2$ NPs. As it has been presented in Table 2, level 1, level 2, and level 3 of $\text{Fe}_2\text{O}_3\text{-TiO}_2$ NPs contained 0.3, 2.7, and 26.9 mg/L of Fe in solid state. Recommended concentrations of Fe in anaerobic digesters have been reported to be in the range of 1-10 mg/L [20]. Using the third level of $\text{Fe}_2\text{O}_3\text{-TiO}_2$ NPs, i.e. 285 mg/gTS, had the most profound effect on methane production at first 4 days. It seems that, the accessible Fe from $\text{Fe}_2\text{O}_3\text{-TiO}_2$ NPs, which is exposed to reaction media, doesn't exceed the recommended range and there is no adverse effect of $\text{Fe}_2\text{O}_3\text{-TiO}_2$ nanoparticle in the present dosing range. NPs act as the carrier and can be first dispersed and then Fe progressively dissolved to supply Fe for

the microorganisms [1].

Table 4. Percentage of increase in cumulative biomethane production in presence of additives in comparison to control experiments

Type of additive	Dosage	Increase in cumulative methane production in comparison with control experiment (%)			
		Day 4	Day 7	Day 14	Day 18
FeCl ₃	Level 1	5.9	12.6	3.3	3.4
	Level 2	18.7	2.0	1.5	0.9
	Level 3	15.1	5.6	0.4	0.4
Fe ₂ O ₃ -TiO ₂ (FT)	Level 1	0.6	2.8	0.2	0.50
	Level 2	6.2	7.3	4.6	4.7
	Level 3	11.4	9.4	4.0	4.2
NiCl ₂	Level 1	15.1	8.7	2.9	4.2
	Level 2	29.0	9.5	8.3	5.8
	Level 3	15.4	8.5	0.1	1.9
NiO-TiO ₂ (NT)	Level 1	4.4	2.6	0.2	0.6
	Level 2	21.1	9.2	0.1	0.6
	Level 3	5.9	3.2	0.3	0.8

Positive effect was observed at first 4 days in the presence of 0.29 mg/gTS, 2.9 mg/gTS, and 29 mg/gTS of FeCl₃. The best performance was observed in case of using level 2 (2.9 mg/gTS) of FeCl₃. Applying higher dosage of FeCl₃, i.e. 29 mg/gTS (26.9 mg Fe/L), was not as effective as level 2. This is due to the fact that, addition of metal

ions with dosages higher than recommended range (1-10 mg Fe/L) leads to less positive effect on AD. At very high concentrations, adverse effect on AD process could be dominant and that can inhibit the biogas production [14]. Similar trend was observed in case of adding NiO-TiO₂ NPs and NiCl₂ salt. Using 2.5 mg/gTS of NiO-TiO₂ nanoparticle and 0.2 mg/gTS of NiCl₂ resulted in increase of 21.1% and 29.0% of methane production at day 4 in comparison with control experiment (Table 4). As it can be seen from Fig. 4 and Fig. 5, the NiO-TiO₂ NPs effectively increased the hydrolysis of substrate. The concentration of soluble COD and total VFAs at day 4 in case of using NiO-TiO₂ is more than that of NiCl₂; while as it can be seen in Table 4 the methane production in case of using NiCl₂ is higher than that of NiO-TiO₂ during first 4 days of experiments. This means that, NiCl₂ probably could stimulate the methanogenesis more effective than NiO-TiO₂ NPs when Ni content is equal for both forms, i.e. NPs and salt. This is due to the fact that the bioavailability of Ni for microorganisms in form of NPs is less than its bioavailability in form of salt. The content of Ni metal ions (1 ± 0 µg/g VS of substrate) found in the wheat straw was lower than that of Fe (41 ± 2.3 µg/g VS of substrate). Using level 3 of both NiO-Fe₂O₃ NPs and NiCl₂ salts (Ni contents is equal to 2.4 mg /L) had lower positive effect in comparison to level 2. This is probably due to the toxic effect of Ni as trace element. This is in agreement with the results obtained by Schattauer et al. [23] indicating that Ni element concentration in range of 0.005-0.5 mg/L is recommended for anaerobic digesters. Exceeding this range could have inhibitory effect on AD process. Bozym et al. [66] reported that the toxic threshold for nickel in fermented wastes is equal to 10 mg/L. The toxic effect of heavy trace elements (i.e. Fe, Ni, Co, Mo, Cu) is due to the chemical binding of these metals to the enzymes and microorganisms, leading to the disruption of enzyme structure and activities [67].

At relatively high concentrations, heavy metals can form unspecific compounds, altering the optimum biochemistry and performance of the processes [68]. These results indicate that the best concentrations of $\text{Fe}_2\text{O}_3\text{-TiO}_2$ and $\text{NiO-Fe}_2\text{O}_3$ NPs and FeCl_3 and NiCl_2 salts are 285, 2.52, 29, and 0.2 mg/gTS ($p < 0.05$), respectively. The large difference between amounts of Fe containing additives (i.e. $\text{Fe}_2\text{O}_3\text{-TiO}_2$ NPs and FeCl_3 salt) and Ni containing additives (i.e. NiO-TiO_2 NPs and NiCl_2 salt) is due to the fact that microorganisms require much more Fe concentration than Ni [23].

4. Conclusions

Highly surface area $\text{Fe}_2\text{O}_3\text{-TiO}_2$ and NiO-TiO_2 nanoparticles were synthesized and characterized to be evaluated in the AD of wheat straw. The effects of Fe and Ni in the form of TiO_2 -based NPs and their salts, i.e. FeCl_3 and NiCl_2 , were investigated for three different dosages. The results showed an enhancement in production of soluble COD up to 13% and total VFAs up to 67% with the addition of NPs during first 4 days of experiments. Coating high surface area TiO_2 NPs with trace metals, gives promising prospects to promote AD process of feedstocks with harsh conversion. As future works, coating of several trace elements in form of NPs on surface of recyclable cm-scale pellet of TiO_2 would be of great interest as well as the application of these multi-component structures at mesophilic temperature condition that could be used on the industrial scale production.

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Highlights

- TiO_2 nanoparticles (NPs) coated with Fe_2O_3 or NiO were synthesized and characterized
- The effects of trace metals were tested on anaerobic digestion of wheat straw
- Up to 13% increase in soluble COD is observed due to increase of hydrolysis rate
- Total VFAs production rate increased up to 67% using NPs
- NPs and salts boosted methane production rate up to 21.1% and 29%, respectively