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Anaerobic Digestion of Chicken Manure Assisted by Carbon Nanotubes: Promotion of Volatile Fatty Acids Consumption and Methane Production

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Abstract: In this study, the effect of different concentrations of carbon nanotubes (Taunit-M; 0.5–6.5 g L^{-1}) on the efficiency of anaerobic digestion of chicken manure is investigated. The highest positive effect on the specific production of methane is obtained when 5.0 g L^{-1} of carbon nanotubes are added to the anaerobic reactors. In addition, carbon nanotubes at these concentrations stimulate the biodegradation of volatile fatty acids, mainly acetate, butyrate, and finally propionate. The maximum production rate of methane increases by 15–16% in the presence of carbon nanotubes (5.0–6.5 g L^{-1}). Also, addition of carbon nanotubes at certain concentrations increases total methane production. Finally, the addition of carbon nanotubes to the anaerobic reactors is found to the favor consumption of volatile fatty acids and improve the methane production kinetics and productivity during the anaerobic digestion of chicken manure.

Keywords: anaerobic digestion; chicken wastes; biochemical methane potential; methane; volatile fatty acids; carbon nanotubes Taunit-M

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1. Introduction

Intensive development of farms is necessary to provide the growing population with livestock products. Therefore, we are currently faced with a tremendous amount of biowaste that requires proper recycling and the development of new sustainable technologies for waste disposal. Anaerobic digestion of animal wastes effectively avoids the direct discharge of fresh manure into soil and water systems [1]. In addition, nutrients from digestate can be used to produce algal biomass for various biotechnologies [2,3].

Chicken manure contains significant amounts of biodegradable components and can cause unpleasant environmental problems if not properly processed. Improper application of manure with high nitrogen and phosphorus content to fields can lead to contamination of the soil and groundwater and the eutrophication of surface water resources. Various technologies are used to process poultry manure. One of the options is anaerobic digestion since, during this process, different organic substances in raw materials are decomposed to form energy-rich biomethane [4–6].

Anaerobic digestion is a complex microbial process that includes several stages: hydrolysis, acidogenesis, acetogenesis (performed by bacteria), and finally methanogenesis (performed by methanogenic archaea), and many factors affect process efficiency [6,7]. One strategy for increasing the productivity of biogas reactors is the addition of different organic or inorganic materials. Organic additives include green biomass, microbial cultures, and different carbon-based supplements. Various mineral elements (macroelements and microelements) and minerals (e.g., magnetite, zeolites) are also used as inorganic additives [8–12].

Carbon nanotubes are becoming increasingly popular in various fields of human activity. Researchers have shown that the addition of different nanoparticles can improve biogas production [13]. Carbon-based nanostructures have small dimensions, high mechanical and thermal strength, and high electrical and thermal conductivity [14]. The use of carbon nanomaterials for anaerobic digestion of organic substrates can positively affect the efficiency of the process, which is also explained by the property of conductivity [15–17]. In addition to electrical conductivity, carbon nanotubes have adsorption properties, actively binding to different inorganic compounds. This is because the extent of the adsorption process increases with an increase in the specific surface area of the particles. Besides, the structures of carbon nanotubes allow strong interactions with organic compounds [18].

It was recently shown that the addition of conductive materials stimulates direct interspecies electron transfer (DIET) in microbial communities, which is essential for syntrophic cultures of microorganisms in anaerobic systems [19,20]. The introduction of a conductive material as a channel can replace microbial pili or *c*-type cytochromes for electron transport. It is possible to increase the efficiency of DIET by adding granular activated carbon, carbon cloth, graphite, biochar, and magnetite (Fe_3O_4) nanoparticles [21–23]. It was previously found that different carbon nanotubes stimulate the anaerobic digestion process at mesophilic temperatures through the DIET mechanism among bacteria and methanogenic archaea [24,25]. However, it should also be noted that carbon nanotubes can improve anaerobic digestion performances due to non-DIET mechanisms, as reported by Salvador et al. [17].

In recent years, the number of relevant and promising studies for practice on the effect of carbon materials and other conductive materials on anaerobic microorganisms has increased. However, in most studies, the direct effect of conductive materials exclusively on pure cultures of microorganisms was noted, while the effect under the conditions of formed microbial communities as well as the interaction between bacteria and archaea in the presence of these materials are still insufficiently studied. Therefore, in the present study, carbon-based nanostructures are chosen as representative conductive materials to explore their role in real anaerobic microbial communities involved in the anaerobic digestion of complex substrates.

The aim of this study is to increase the rate of the anaerobic process and its efficiency during the anaerobic digestion of chicken manure. Industrial multi-walled carbon nanotubes called "Taunit", which are quasi-one-dimensional, filamentous polycrystalline graphite cylindrical formations with internal channels, are used in this study.

2. Materials and Methods

2.1. Experimental Setup

Chicken manure with total solids (TS) of 60.5 $\pm$ 0.8% and volatile solids (VS) of 49.7 $\pm$ 0.6% (for the first batch tests) and chicken manure with TS of 59.5 $\pm$ 0.4% and VS of 40.5 $\pm$ 0.5% (for the second batch tests) were collected from a local poultry farm (Kazan, Republic of Tatarstan, Russia). In the experiment, hydrophilic ("soluble" in water (up to 0.2%)) carbon nanotubes (CNTs) in the form of powder were used as additives. Multi-walled carbon nanotubes of the "Taunit" series, which are quasi-one-dimensional, nanoscale, filamentous polycrystalline graphite cylindrical formations with internal channels in the form of a black powder, namely, its modification "Taunit-M", were used. They had a hollow cylindrical structure, at least 2 μm long, with an external diameter of 10–30 nm and an internal diameter of 5–15 nm. "Taunit" was produced by chemical vapor deposition; its purity is above 95% (Available online: http://eng.nanotc.ru/; accessed on 10 November 2022).

2.2. Biochemical Methane Potential Experiments

The biochemical methane potential (BMP) of chicken manure was estimated by using Automatic Methane Potential Test Systems (AMPTS II Light, Bioprocess Control, Lund, Sweden). During the experiments, all batch anaerobic reactors were started using an inoculum (digested cattle manure) and chicken manure as a substrate. Inoculum to substrate ratios (ISR) were 37.8/45.3 g of VS (TS concentration of 6.4%) and 33.7/47.2 g of VS (TS concentration of 7.0%) for the first and second batch tests, respectively. The 2 L bottles with a working volume of 1.6 L were then incubated at 38 °C for 32 days. CNTs at concentrations of 0.5–6.5 g L^{-1} were selected and separately added to experimental reactors. Control reactors were operated without the addition of CNTs. Blank reactors contained only the inoculum (to compensate for the biomethane level produced by the inoculum itself). The biogas was first passed through a solution of 3 M NaOH to remove CO_2 and H_2S, and the CH_4 yield was estimated by using a gas flow meter system. The AMPTS II Light instruments agitated the digestion medium at 60 rpm for 1 min with a 3 min rest interval. Each batch test was carried out in duplicate.

2.3. Analytical Methods and Statistical Analysis

Methane values were obtained automatically from the AMPTS II instruments and normalized to 1.0 standard atmospheric pressure, 0 °C, and zero moisture content. Specific methane production (SMP; mL $g^{-1}{}_{VS}$), methane flow rate (MFR; mL day^{-1}), and maximum methane production rate (mL $g^{-1}{}_{VS}$ day^{-1}) were further calculated. Digestates were periodically taken from all batch reactors for various analyses, including volatile organic acids (VOA) and total ammonia nitrogen (TAN) concentrations. These analyses were performed as detailed by Ziganshina et al. [26]. In addition, individual organic acids (acetic, propionic, and n-butyric) were analyzed by an UltiMate 3000 HPLC system (Thermo Fisher Scientific, Hamburg, Germany) equipped with a Rezex™ ROA-Organic Acid H^+ (8%) column (Phenomenex, Torrance, CA, USA). All these analyses were measured in triplicate, and the mean values are presented together with standard deviations.

The Tukey method and 95% confidence were used to compare differences (Minitab software version 20.2.0, State College, PA, USA).

3. Results and Discussion

The efficiency of anaerobic digestion of chicken manure in mesophilic anaerobic batch reactors was estimated by volatile solids degradation with the production of methane.

3.1. Process Stability and Methane Production (First Batch of Chicken Manure)

During the first batch of experiments, the anaerobic biogas reactors functioned under mesophilic conditions for 32 days. To increase the efficiency of anaerobic digestion of chicken manure, CNTs were introduced into anaerobic reactors at three different concentrations: 0.5 g L^{-1}, 2.0 g L^{-1}, and 5.0 g L^{-1}.

Chicken manure was used as a substrate and added equally to all reactors except blank reactors (to compensate for the CH_4 level produced by the inoculum itself). The purpose of the anaerobic digestion test was to determine the effect of adding nanostructured carbon materials on the production of CH_4 from chicken manure. Figure 1 shows the specific methane production and methane flow rate from chicken manure-added treatments in the absence and presence of CNTs.

Thus, CH_4 production was relatively stable, which makes the substrate suitable for biomethane production. The period from the loading of the anaerobic reactors to the onset of methane formation was short. The active production of methane lasted for 32 days. After one day of incubation, similar methane yields were obtained for all treatments, and the average data ranged from 142 mL to 152 mL. But already, after two days of anaerobic digestion, reactors supplied with CNTs produced more methane compared to control treatments (Figure 1).

The addition of CNTs at a concentration of 5.0 g L^{-1} significantly increased the specific methane production throughout the experiment. The final SMP levels (at day 32) for the reactors R_0, R_0.5, R_2.0, and R_5.0 were 153 ± 3.1 mL $g^{-1}{}_{VS}$, 153 ± 2.5 mL $g^{-1}{}_{VS}$, 161 ± 2.2 mL $g^{-1}{}_{VS}$, and 167 ± 2.8 mL $g^{-1}{}_{VS}$, respectively (Figure 1a). The highest average values in MFR were obtained on days 2 and 4 for the reactors supplied with 5.0 g L^{-1} of CNTs (591 mL and 605 mL, respectively), while for the reactors operated without CNTs these peaks fell on days 4 and 8 (397 mL and 523 mL, respectively) (Figure 1b). All other concentrations did not have such a large impact, but the productivity of R_2.0 was slightly higher than the productivity of R_0 and R_0.5. The obtained data indicate that the addition of CNTs (mainly 5.0 g L^{-1}) improved the performance of these reactors. The maximum production rate of methane increased by 16% in the presence of CNTs (5.0 g L^{-1}).

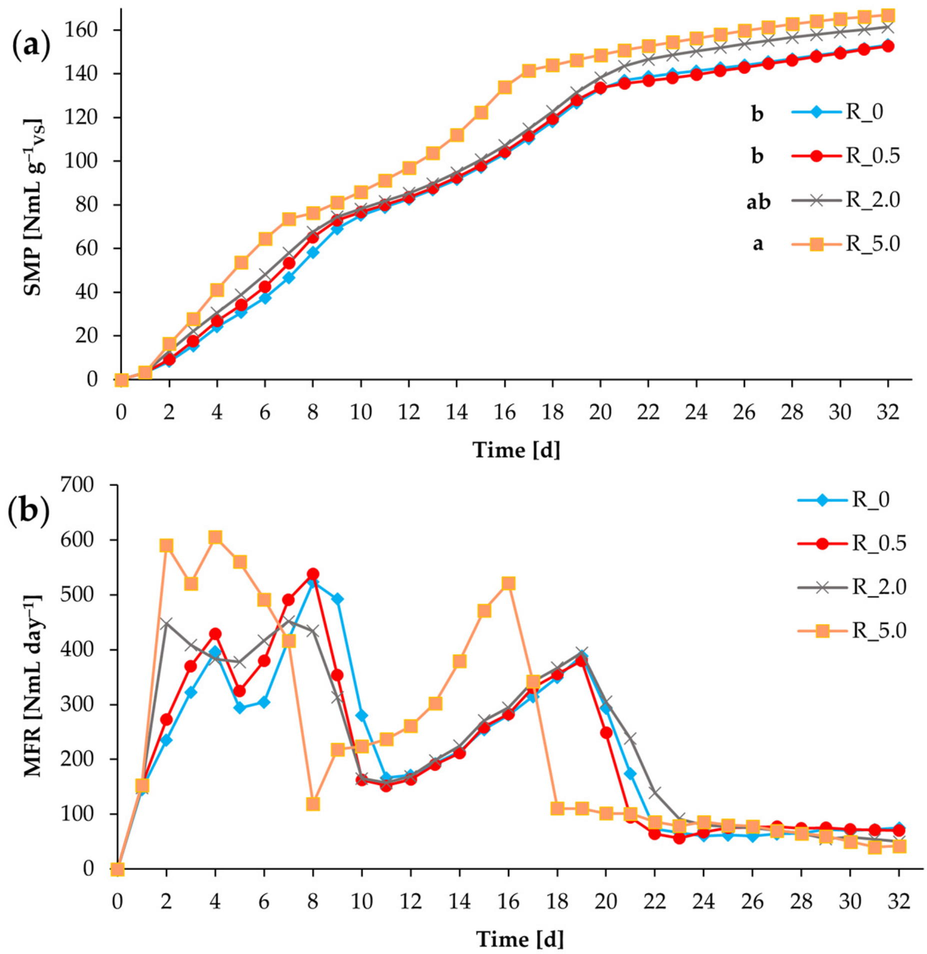

Figure 1. Impact of CNT addition on SMP (**a**) and MFR (**b**) during the anaerobic digestion of chicken manure (first batch of chicken manure). Different concentrations of CNTs (0.5 g L^{-1}, 2.0 g L^{-1}, and 5.0 g L^{-1}) were added to the reactors (R_0.5, R_2.0, and R_5.0, respectively). R_0 operated without the addition of CNTs. Values at day 32 (SMP) were statistically compared; [a,b] arithmetic means (averages) that do not share a letter are statistically significantly different from each other according to the Tukey method and 95% confidence.

Concentrations of the produced organic acids were high due to the large amount of organic matter in chicken manure (Figure 2a). If the VOA levels in all experiments with the introduction of CNTs regularly decreased during the entire anaerobic digestion process, then in the experiments performed in the absence of CNTs, a lower level of their utilization was observed. Thus, CNTs at a concentration of 5.0 g L^{-1} stimulated the rapid utilization of VOA. It should also be noted that some of the organic acids were derived from the inoculum. TAN levels did not vary substantially in all treatments and were in the range of 1.6–1.8 g L^{-1}, though a lower level of TAN was observed in R_5.0 (Figure 2b).

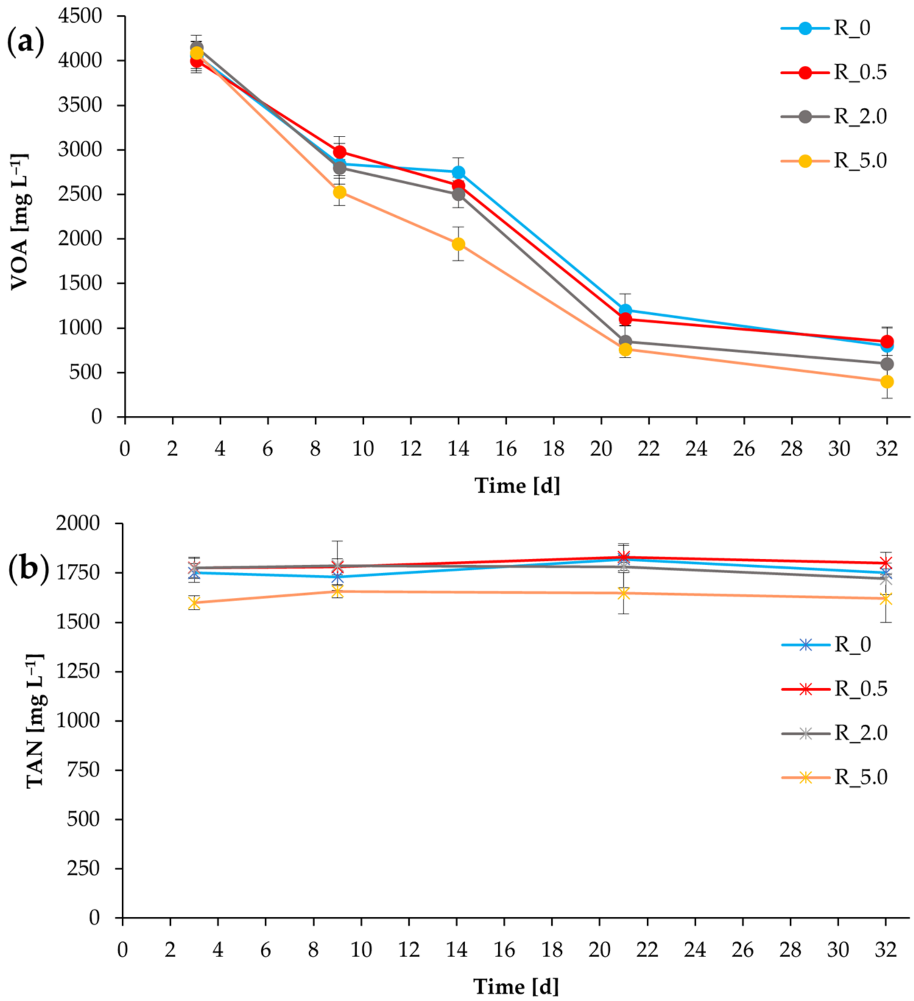

Figure 2. Impact of CNT addition on VOA concentrations (**a**) and TAN concentrations (**b**) during the anaerobic digestion of chicken manure. Different concentrations of CNTs (0.5 g L^{-1}, 2.0 g L^{-1}, and 5.0 g L^{-1}) were added to the reactors (R_0.5, R_2.0, and R_5.0, respectively). R_0 operated without the addition of CNTs.

The efficiency and stability of the anaerobic digestion process are entirely dependent on the coordinated and syntrophic activity of the involved microorganisms [26–28]. Cattle manure was used as an inoculum, which is well suited for running anaerobic digesters as it contains a diverse microbial community that can quickly adapt to changing operating conditions [29]. Notably, the rumen microbiota includes methanogens, which use acetate, H_2, and CO_2 to produce methane and decrease acetate and hydrogen accumulation during the anaerobic process [30].

The average methane yield during the anaerobic digestion of chicken manure is 70–140 m^3 t^{-1}, while the average methane yield from cattle slurry and cattle dung is comparatively lower: 11–19 m^3 t^{-1} and 33–36 m^3 t^{-1}, respectively [31]. Chicken manure is the mixture of feces and urine excreted, which contains varying amounts of undigested feeding stuff, desquamated intestinal epithelium, residues of secretion, microorganisms from the intestinal flora, metabolites excreted with the urine, as well as other components, e.g., feathers, egg leftovers, bedding material, grid material, and soil [32]. So, chicken manure has a diverse material composition, and regular testing of raw materials is recommended before anaerobic digestion on large scales.

3.2. Process Stability and Methane Production (Second Batch of Chicken Manure)

During the second batch of experiments, five different conditions were monitored: control reactors and reactors supplemented with different concentrations of CNTs (2.0 g L^{-1}, 3.5 g L^{-1}, 5.0 g L^{-1}, and 6.5 g L^{-1}). The operation of the anaerobic reactors lasted for 32 days, and during this period, six samples were taken from them to analyze the composition of the digestate. Figure 3 illustrates the SMP and MFR, whereas Figures 4 and 5 demonstrate the concentrations of the individual main volatile fatty acids (such as acetic acid, propionic acid, and butyric acid) and the total ammonia nitrogen level during the whole anaerobic digestion process.

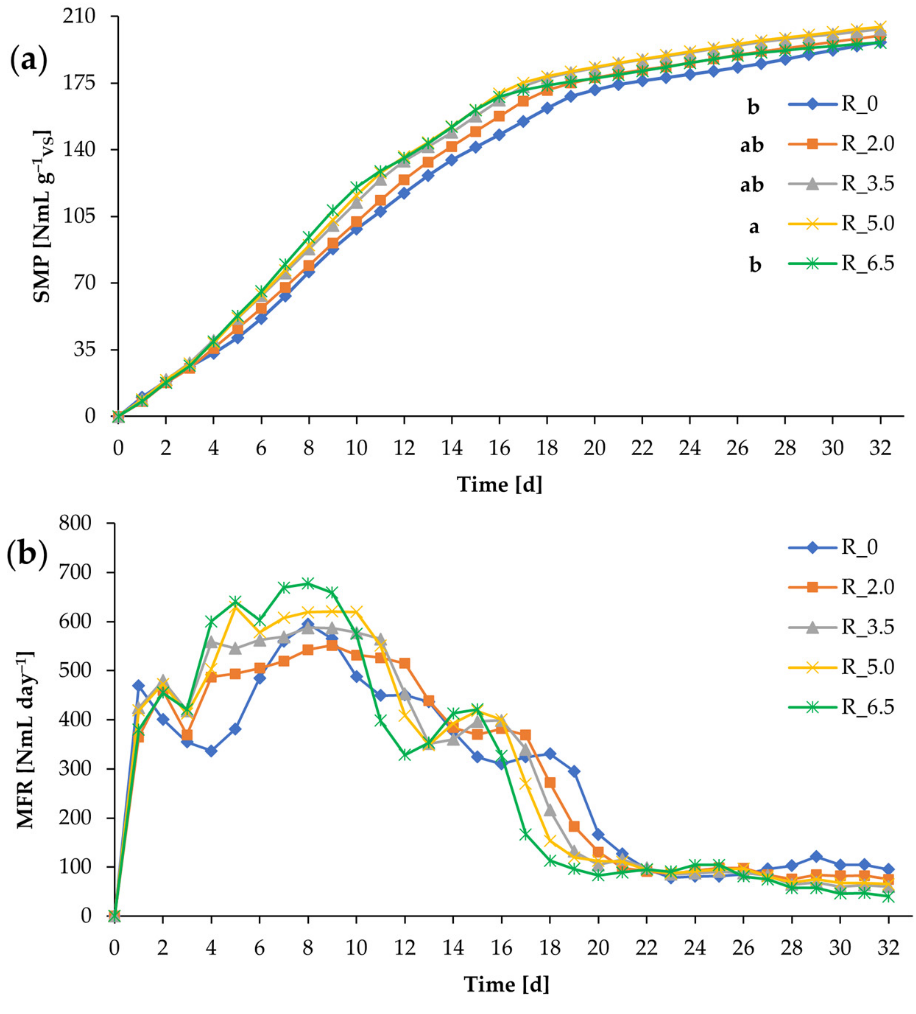

Figure 3. Impact of CNT addition on SMP (**a**) and MFR (**b**) during the anaerobic digestion of chicken manure (second batch of chicken manure). Different concentrations of CNTs (2.0 g L^{-1}, 3.5 g L^{-1}, 5.0 g L^{-1}, and 6.5 g L^{-1}) were added to the reactors (R_2.0, R_3.5, R_5.0, and R_6.5, respectively). R_0 operated without addition of CNTs. Values at day 32 (SMP) were statistically compared; [a,b] arithmetic means (averages) that do not share a letter are statistically significantly different from each other according to the Tukey method and 95% confidence.

As can be observed, methane was produced efficiently in all cases. The second batch of chicken manure generated a higher level of methane compared with the first batch of chicken manure.

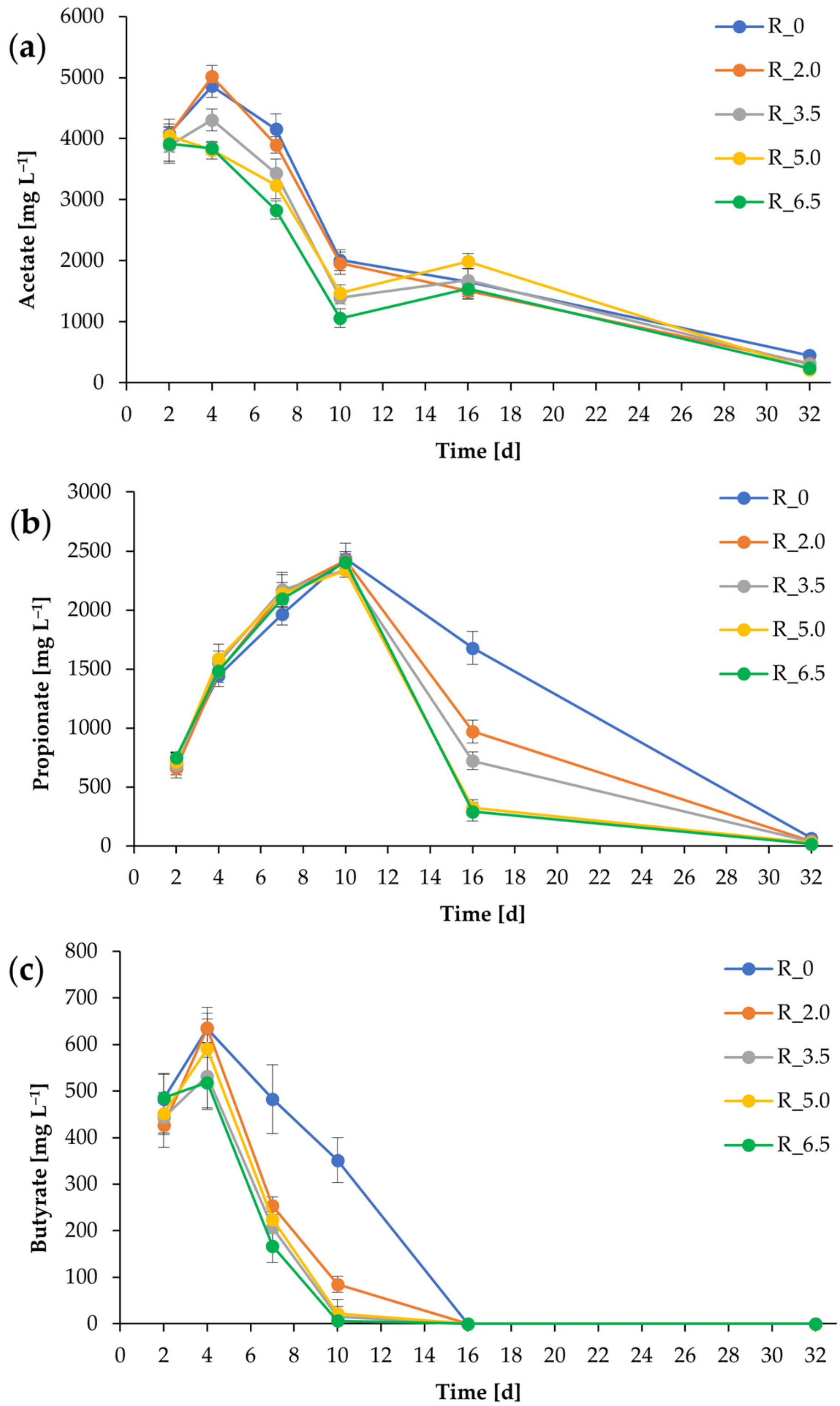

Figure 4. Impact of CNT addition on acetate (**a**), propionate (**b**), and butyrate (**c**) during the anaerobic digestion of chicken manure. Different concentrations of CNTs (2.0 g L^{-1}, 3.5 g L^{-1}, 5.0 g L^{-1}, and 6.5 g L^{-1}) were added to the reactors (R_2.0, R_3.5, R_5.0, and R_6.5, respectively). R_0 operated without the addition of CNTs.

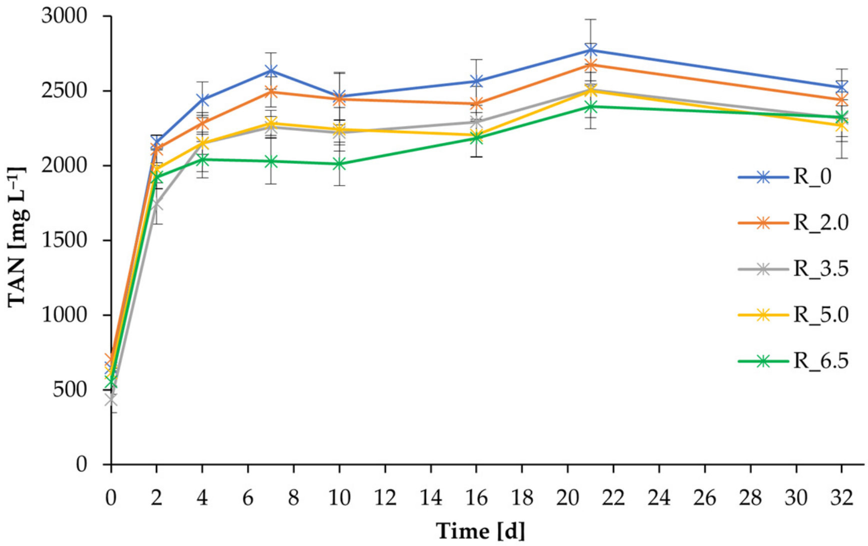

Figure 5. Impact of CNT addition on TAN accumulation during the anaerobic digestion of chicken manure. Different concentrations of CNTs (2.0 g L^{-1}, 3.5 g L^{-1}, 5.0 g L^{-1}, and 6.5 g L^{-1}) were added to the reactors (R_2.0, R_3.5, R_5.0, and R_6.5, respectively). R_0 operated without the addition of CNTs.

The reactors started to produce methane immediately after the addition of the substrate. The highest average peaks in MFR were received on days 5 and 8 for reactors supplied with 6.5 g L^{-1} of CNTs (640 mL and 677 mL, respectively), but only on day 8 for reactors operated in the absence of CNTs (595 mL). The introduction of CNTs into the anaerobic reactors in the range of 2.0–6.5 g L^{-1} improved the rate of methane formation throughout the experiment, while the greatest positive effect was observed when CNTs were used in amounts of 5.0–6.5 g L^{-1}.

The other two concentrations tested were also positive but less effective. The received data indicate that the introduction of CNTs into the reactors improved their performance. The maximum production rate of methane increased by 15% in the presence of carbon nanotubes (6.5 g L^{-1}). The final SMP levels (at day 32) for the reactors R_0, R_2.0, R_3.5, R_5.0, and R_6.5 were 196 $\pm$ 1.2 mL $g^{-1}{}_{VS}$, 200 $\pm$ 1.5 mL $g^{-1}{}_{VS}$, 203 $\pm$ 2.1 mL $g^{-1}{}_{VS}$, 205 $\pm$ 1.8 mL $g^{-1}{}_{VS}$, and 196 $\pm$ 2.4 mL $g^{-1}{}_{VS}$, respectively (Figure 3).

In the reactors R_5.0 and R_6.5, acetate accumulated for up to 2 days, and in the reactors R_0, R_2.0, and R_3.5, its maximum concentration was reached after 4 days (Figure 4). In the case of butyrate and propionate, their accumulation in all systems occurred until days 4 and 10, respectively. The addition of CNTs promoted earlier consumption of acetate and butyrate, and the highest concentration of CNTs stimulated more efficient consumption of these volatile fatty acids. As already noted, the biodegradation of propionate occurred after day 10 with an increase in the level of acetate in some reactors and the appearance of the last peak of methane generation. This indicates that propionate degrading microorganisms were activated after a decrease in the level of acetate and almost complete utilization of butyrate, and CNTs stimulated their activity. Finally, this suggests that the addition of CNTs promoted the uptake of the produced volatile fatty acids (Figure 4). It should be noted that some concentrations of organic acids were obtained from the inoculum.

In these experiments, TAN levels were higher than the values obtained in the first experiments and were in the range of 2.2–2.5 g L^{-1} (day 32; Figure 5). Interestingly, higher levels of CNTs resulted in a lower level of TAN. Carbon nanotubes have adsorbing properties, actively binding to other molecules [18,33]. Therefore, ammonia and/or other nitrogen-containing compounds (from which ammonium was formed) could bind to carbon nanotubes. Moreover, the higher microbial activity in the reactors with CNTs could stimulate the increased assimilation of ammonia by the cells. However, these assumptions require further research.

3.3. CNTs as Promising Agents in Anaerobic Digestion

Carbon nanotubes are among the most promising nanomaterials for diverse applications due to their attractive physicochemical properties, such as their large surface area, mechanical and thermal strength, and electrochemical activity [34].

Many scientific groups consider different carbonaceous materials promising agents for improving the performance of anaerobic digesters, including those operating under stressful conditions for microorganisms [35–38]. For example, the results of the work of Yan et al. [36] showed that the presence of carbon nanotubes in anaerobic systems can mitigate the inhibition of the process by ammonia. The authors noted a positive correlation between the concentration of carbon nanotubes and the polysaccharide, which creates the physical structure to protect the cell membrane of microorganisms. In another study, Shen et al. [38] evaluated the effect of carbon nanotubes on methane generation through syntrophic acetate oxidation (SAO) in extreme environments (at high acetate concentration and high temperature). The authors noted the acceleration of methane production via decreasing the lag phase and increasing maximum methane production rates with the addition of electroconductive carbon nanotubes. They reported the DIET pathway in SAO consortiums, which made it possible to accelerate methane production [38].

The experimental results from another work also showed an increase in methane production rate in reactors with carbon nanotube hollow-fiber media, and the authors attribute this to a shift in the microbiome and stimulation of syntrophic microbial metabolism associated with DIET [39]. The authors also attributed the improvement in anaerobic process performance to the lower concentration of organic acids in experimental systems, which made syntrophic metabolism thermodynamically exergonic. There are other works that showed that CNTs can establish DIET between syntrophic partners due to their conductive properties, which stimulate the anaerobic digestion process [24,36]. Li et al. [24] showed that the introduction of carbon nanotubes into the anaerobic process intensified the anaerobic wastewater treatment. The authors noted that carbon nanotubes at concentrations up to 1.0 g L^{-1} induced much faster substrate utilization and methane production rate by anaerobic microorganisms. In addition, carbon nanotubes as materials with high conductive properties increased the electrical conductivity of the sludge, which could promote the DIET between anaerobic fermentative bacteria and methanogens during the anaerobic digestion process.

It should be added that carbon nanotubes can improve anaerobic digestion performance due to non-DIET mechanisms. Thus, the study performed by Salvador et al. [17] confirmed that with an increase in the carbon nanotube dosage in systems, a more negative redox potential occurred, which is beneficial for methanogenesis. Carbon nanotubes can also have a cytotoxic effect on microorganisms [37,40], which emphasizes the importance of selecting an adequate dosage.

Finally, the scientific results have encouraged researchers in this field to note the effectiveness of introducing nanotubes into the processes of anaerobic conversion of various substrates. They additionally demonstrated the promising potential of their full-scale application.

4. Conclusions

The highest positive effect on the specific production of methane from chicken manure was obtained when 5.0 g L^{-1} of carbon nanotubes was added to the anaerobic reactors. In addition, carbon nanotubes enhanced the biodegradation of volatile fatty acids, mainly acetate, butyrate, and finally propionate. The maximum production rate of methane increased by 15–16% in the presence of carbon nanotubes (5.0–6.5 g L^{-1}). This indicates that carbon nanotubes, when used correctly, stimulate the production of methane during the anaerobic digestion of chicken manure. The results of this study encourage the use of carbon nanotubes in individual modifications in biogas plants to improve methane production efficiency.

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