

Article

Anaerobic Conversion of Microalgal Biomass to Biomethane in the Presence of Carbon-Based Materials: Additive Type, Concentration, and Impact on Microbial Communities

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Abstract

Today, waste is viewed as a renewable energy source and a potential supplier of new bioproducts. Microalgae are essential in wastewater treatment, and the anaerobic digestion (AD) of the harvested biomass leads to the production of biomethane. However, the process of producing methane from algae requires energy-intensive pretreatment steps. This study investigates the potential of carbon nanotubes (CNTs) and granular activated carbon (GAC) to enhance methane production through AD of untreated *Chlorella sorokiniana* and *Tetrademus obliquus* biomass. Indicators of process stability included organic acid, total ammonia nitrogen concentrations, methane production data, and microbial community structure and dynamics. The results suggest that adding CNTs (2.0 and 4.0 g L⁻¹) and GAC (4.0 g L⁻¹) following the acclimatization stage is an effective strategy to enhance the utilization of produced organic acids and promote long-term methane production from the tested biomass. Additionally, the significance of the bacterial genus *Ruminofilibacter* in biomass hydrolysis has been emphasized. Among methanogens, there was a decrease in the relative abundance of *Methanosarcina*, while the relative abundances of *Methanothrix* and *Methanoculleus* increased by day 12 in CNTs/GAC-added experiments, during which substantial differences in methane production were observed. This proposed approach has the potential to promote environmentally sound waste management and enhance the production of renewable energy.

Keywords: anaerobic digestion; green algae; methane; carbon nanotubes; granular activated carbon; microbial community structure; 16S rRNA gene; *mcrA* gene

1. Introduction

Anaerobic digestion (AD) is an effective technology for processing various substrates, including organic wastes, while simultaneously producing renewable biogas through a series of biochemical reactions carried out by bacteria and archaea. This technology plays a crucial role in addressing various resource and environmental challenges. AD of various biomass sources is gaining popularity. This trend is driven by the production of energy-rich biomethane and digestate, which can be used as a high-quality fertilizer. Biogas production and its subsequent application are sustainable and environmentally friendly approaches. They play a significant role in the energy sector and have high potential to mitigate climate change caused by fossil fuel consumption [1–3].

The entire AD process depends on the synergistic microbial activity across four key stages, including hydrolysis, acidogenesis, acetogenesis, and methanogenesis, to sequen-



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tially break down organic matter to form biogas. The bacterial community transforms different organic compounds into organic acids, alcohols, molecular hydrogen, and carbon dioxide. Meanwhile, methanogenic archaea generate methane through acetoclastic, hydrogenotrophic, and methylotrophic pathways. Balanced interactions among different anaerobic microbes in this biodegradation chain are crucial for the continuous conversion of the intermediate products formed and, consequently, for the efficient production of biogas. Methanogenesis is undoubtedly considered the system's bottleneck, being the most sensitive stage. Thus, volatile fatty acids (VFAs), when efficiency is low or the process is destabilized, accumulate and sharply decrease the pH in anaerobic digesters, disrupting the delicate balance between the bacterial and methanogenic stages [4–6]. Therefore, VFAs, total ammonia nitrogen (TAN; sum of ammonium nitrogen (NH_4^+ -N) and free ammonia nitrogen (FAN; NH_3 -N)), as well as FAN itself, serve as critical indicators that influence process stability and efficiency. Despite promising prospects, the efficiency of AD is often reduced by technical challenges, including the complexity of substrates, low biogas yields, long acclimatization periods for microorganisms, and accumulation of high concentrations of VFAs, TAN, FAN, and sulfides, leading to system instability. Numerous studies have focused on identifying the relationships between methane yield and substrate composition, particularly regarding lignocellulosic biomass, along with the factors that affect its transformation [7–11]. A narrow but important series of studies has focused on identifying the conditions necessary for acclimatization and development of acidogenic/methanogenic communities under various nutrition regimens and direct participants in methanogenesis, in particular acetoclastic methanogens, to enhance methane production [12,13]. Thus, a detailed understanding of the development of microbial communities in anaerobic digesters and conditions for their well-coordinated and stable activity is essential for the fundamental and practical development and improvement of the entire AD process.

To overcome these limitations, different approaches have been investigated, including anaerobic process optimization and microbiota enrichment. Among recent strategies, the incorporation of carbonaceous materials, including granular activated carbon (GAC), biochar, and carbon nanotubes (CNTs), has received considerable attention. These materials can improve the stability and efficiency of AD by mediating conductive particle-mediated interspecies electron transfer (CIET), changing the structure of anaerobic microbial communities and providing superficies for microbial colonization [14]. The surfaces of carbon additives, including biochar and activated carbon, enhance the anaerobic process stability by removing different toxic compounds, including ammonia. This also aids in balancing pH, which is particularly crucial during both mono- and high-load AD. The high conductivity, geometric properties, and size-dependent characteristics of CNTs positively influence methanogenesis in anaerobic digesters that process various substrates. Finally, carbon-based material additives promote increased energy recovery efficiency and process sustainability, offering a promising avenue for cost-effective and scalable waste-to-energy solutions [2,3,6–8]. Further comprehensive research in the field of CIET is needed to understand the physiology and ecology of this biochemical process, as well as to subsequently apply this new knowledge in practice.

Among the various species of microalgae, the phylum Chlorophyta (green algae) includes a wide variety of species that are used and potentially useful in biotechnology as efficient producers of biomass and specific valuable chemical compounds. A number of microalgal metabolic products are of great practical interest for agriculture, energy, nutraceutical, pharmaceutical, and cosmetic industries [13,15]. Among the valuable algal compounds, lipids, proteins, polysaccharides, carotenoids, and vitamins are noteworthy [16]. Furthermore, the incorporation of various microalgal species into microbial communities for the bioremediation of environmental objects is being actively studied [17,18]. However, despite

some work in this field of research, only a few microalgae species are nowadays used for the production of food products, valuable chemicals, and wastewater treatment [19,20].

A narrow range of studies is devoted to the anaerobic conversion of microalgal biomass and its residues to produce biogas [21–23]. Microalgae typically have high protein content (hence the low C/N ratio) [24], which can increase ammonia levels during long-term AD. Therefore, the inoculum and substrate loading parameters play an important role in methane productivity [25,26]. AD of microalgal biomass often suffers from low methane yield due to difficult-to-degrade cell walls [22], which must be destroyed by different pretreatment methods to increase methane yield [27]. However, additional such procedures increase the cost of the biotechnological process itself. Despite the known properties of carbonaceous materials and the necessity to optimize methane production from microalgal biomass, AD of microalgal-bacterial harvested biomass after wastewater treatment in the presence of carbon-based material additives remains largely unexplored. Additionally, the impact of these additives under conditions of high substrate concentration has not been thoroughly investigated. The specific microorganisms involved and their ecological functions also remain unexamined.

This study investigates the potential application of two types of carbon-based additives—multiwalled carbon nanotubes and granular activated carbon—to improve the efficiency of mesophilic AD of untreated microalgal biomass (*Chlorella sorokiniana* and *Tetradesmus obliquus*). Additionally, after the microorganisms adapted to the substrate and additives, the composition of the anaerobic microbial communities involved in this process was examined.

2. Materials and Methods

A schematic diagram summarizing the experimental design of the present study is shown in Figure 1.

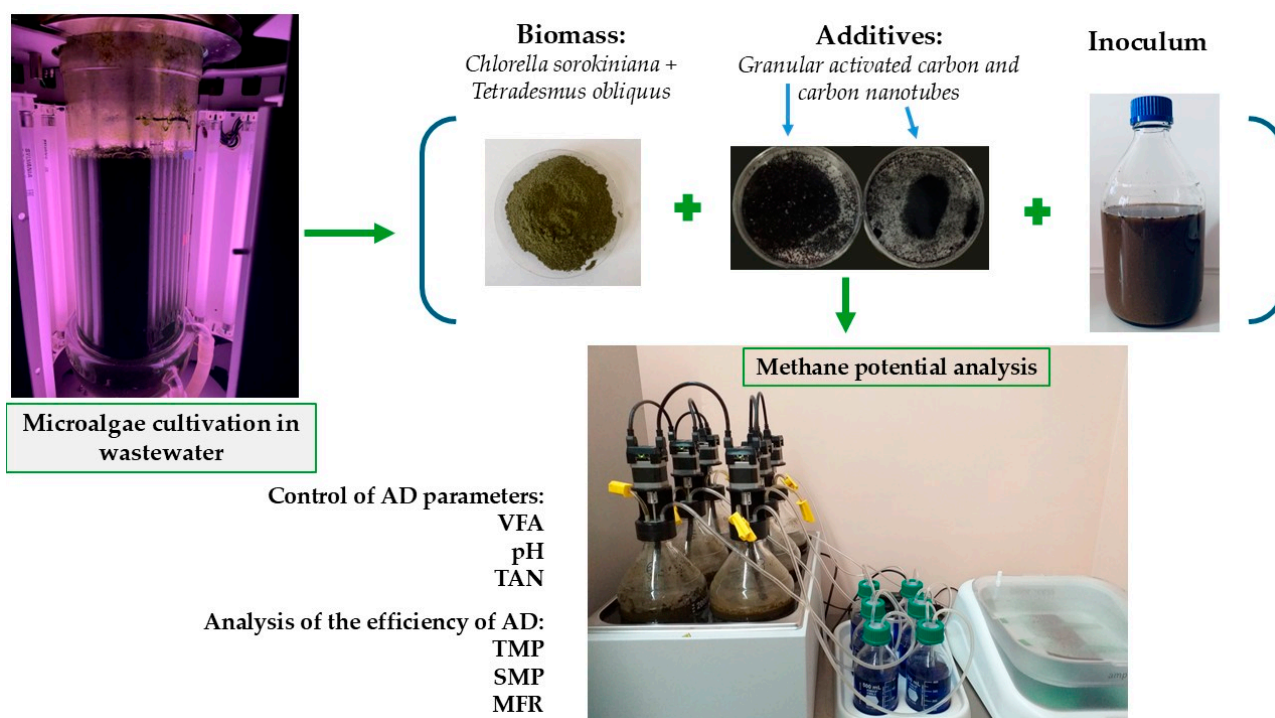


Figure 1. A schematic diagram summarizing the experimental design of the present study.

2.1. Chemicals

Taunit-M multiwalled carbon nanotubes (CNTs), quasi-one-dimensional nanoscale thread-like graphite structures in the form of a black powder, were received from Zavkom (Tambov, Russia; available online: <https://zavkom.com/en/industries/other-industries/>; accessed on 3 October 2026). They had a hollow cylindrical structure over 2 μm long, with an external diameter of 10–30 nm and an internal diameter of 5–15 nm, and a specific surface area $\geq 270 \text{ m}^2 \text{ g}^{-1}$. Coconut-based granular activated carbon Silcarbon K835 (GAC; 0.5–2.36 mm particle size) was purchased from Silcarbon Aktivkohle Gmb (Kirchhundem, Germany). Before use, GAC was washed using distilled water and dried in an oven at 105 °C overnight. GAC was then cooled in a desiccator and kept in airtight plastic bottles at room temperature. NaOH, H₂SO₄, thymolphthalein, sodium acetate, sodium propionate, n-butyric acid, isobutyric acid, and Nessler's reagent were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Inoculum for AD

Cow dung was obtained from the local dairy farm (near Kazan, Republic of Tatarstan, Russia). Cow dung was diluted using tap water in the ratio 1:1 (*w/w*) and then anaerobically digested at 38 °C for 6 weeks to obtain active inoculum. After AD, the inoculum was kept in closed plastic bottles, under shade at room temperature, for subsequent use. The inoculum for the start of experiments had a total solids (TS) content of $3.24 \pm 0.04\%$ and a volatile solids (VS) content of $2.39 \pm 0.03\%$.

2.3. Substrate for AD

Untreated dry microalgal–bacterial biomass grown in wastewater was used as a substrate for biomethane production. After harvest, the main share of biomass consisted of *Chlorella sorokiniana* and *Tetrademus obliquus* (hereinafter referred to as algal biomass). The species *C. sorokiniana* and *T. obliquus* were grown in different wastewaters in photobioreactors [17,18,24]. The resulting algal biomass, washed twice with distilled water, was dried, vacuum-packed, and stored at −20 °C. *C. sorokiniana* biomass had a TS content of $95.15 \pm 0.16\%$ and a VS content of $90.81 \pm 0.14\%$, whereas *T. obliquus* biomass was characterized by a TS content of $94.04 \pm 0.21\%$ and a VS content of $90.46 \pm 0.17\%$.

2.4. Anaerobic Digestion Test Protocol and Set-Up

Batch AD tests were carried out. Automatic Methane Potential Test Systems (AMPTS II Light, Bioprocess Control, Lund, Sweden) were used as equipment for methane potential analysis. The digested cow dung (inoculum), untreated dry algal biomass, and tap water were mixed in 2 L glass bottles, with a 1.6 L working volume. The first batch assays amended with CNTs were named CNT_2 and CNT_4, where the digits denote the concentration of CNTs in the assays, i.e., 2.0 g L^{−1} and 4.0 g L^{−1}, respectively. The batch assays amended with GAC were named GAC_2 and GAC_4, where the digits denote the concentration of GAC in the assays, i.e., 2.0 g L^{−1} and 4.0 g L^{−1}, respectively. All the assays were triplicated, and inoculum glass bottles (blank reactors) were also prepared with no addition of algal biomass to estimate the background methane production. Control reactors (inoculum + substrate, named as C1) were also kept to estimate the methane production in the absence of CNTs and GAC. The inoculum-to-substrate ratio (*C. sorokiniana* + *T. obliquus*) was 29.8/(14.9 + 14.9) g of VS for the first round. Anaerobic digestion was performed at a TS concentration of 4.5%. All anaerobic reactors were purged with N₂ for 1 min to obtain the anaerobic environment. The glass bottles were kept in a water bath under the mesophilic regimen (38 °C) at 60 rpm for 2 min with a 1 min rest interval. The duration of this round was 62 days.

Before starting the second batch, the anaerobic reactors were opened, and a new batch of algal biomass was added (without adding any additional CNTs or GAC). The risks associated with opening anaerobic reactors were reduced by quickly introducing a new batch of biomass. The second batch assays amended with CNTs were named as CNT_2_2 and CNT_2_4, where the last digits denote the concentration of CNTs in the assays, i.e., 2.0 g L⁻¹ and 4.0 g L⁻¹, accordingly. The second batch assays amended with GAC were named as GAC_2_2 and GAC_2_4, where the last digits denote the concentration of GAC in the assays, i.e., 2.0 g L⁻¹ and 4.0 g L⁻¹, accordingly. All the assays were triplicated, and blank reactors were also used. Control reactors (named C2) were also used to estimate the methane production in the absence of CNTs and GAC. Biomass of *C. sorokiniana* and *T. obliquus* was added at a concentration of 18.8 and 21.5 g of VS, respectively. TS concentration for the second round was 6.1%. All anaerobic reactors were further purged with N₂ for 1 min to obtain the anaerobic environment. In each round, the system overestimated the volume of gas initially used for flushing the system. All reactors were incubated under the mesophilic regimen (38 °C); mixing at 60 rpm for 2 min with a 1 min rest interval was set. The duration of this round was 47 days.

The final TS content in each bottle was maintained at the level determined by the characterization of the inoculum and substrate; tap water was added as necessary. For all rounds, the produced biogas was initially passed through 400 mL of 3 M NaOH solution with thymolphthalein as a pH indicator. After retaining acid gas fractions, CO₂ and H₂S, by chemical interaction with NaOH, the CH₄ yield was calculated using a gas volume measuring device. The generated files by AMPTS contained the volume of gas already normalized (1.0 standard atmospheric pressure, 0 °C and zero moisture content) and with the elimination of the headspace overestimation.

2.5. Analytical Methods

Biogas released from the reactors was periodically sampled, and the percentage of biomethane was periodically analyzed by gas chromatography using a gas chromatograph Clarus 580 (Perkin Elmer, Shelton, CT, USA). Samples (digestates) were periodically taken from the reactors for various analyses, including determination of VFAs, pH, and TAN as process stability indicators, as well as microbial community structure analyses. TS and VS contents were estimated at the beginning and the end of the experimental period as parameters to monitor the efficiency of AD. TS and VS contents were determined according to the standard methods using a drying oven SHS-10-02 SPU (Smolensk, Russia) and a muffle electric furnace EKPS-10 (Smolensk, Russia), respectively. pH was measured using a Starter 300 pH meter (OHAUS Corporation, Shanghai, China) and an ESC-10601 electrode (Measuring Equipment, Moscow, Russia). TAN levels (a critical indicator of protein-rich biomass conversion) were estimated using the Nessler's reagent photometric method. Nessler's reagent was added to the supernatant of the sample and allowed to react for 5 min to develop the characteristic yellow color. The absorbance of the mixture was measured at 425 nm using a Lambda 35 spectrophotometer (Perkin Elmer, Singapore). FAN was then calculated from the obtained TAN values when necessary [28]. Concentrations of individual organic acids (acetic, propionic, n-butyric, and isobutyric) as vital metabolites of acidogenesis and acetogenesis stages were analyzed by an UltiMate 3000 HPLC system (Thermo Fisher Scientific, Hamburg, Germany) equipped with an autosampler, a pump, a variable wavelength detector, a temperature-controlled column compartment, a precolumn, and a RezexTM ROA-Organic Acid H+ (8%) column (Phenomenex, Torrance, CA, USA). Organic acids detection occurred at 210 nm. Separation was performed at 40 °C after filtration of samples through membrane filters. The mobile phase consisted of 0.005 N

H₂SO₄ at a flow rate of 0.5 mL min⁻¹. All the samples were analyzed in triplicate and reported as the average value with standard deviations.

2.6. DNA Extraction, 16S rRNA, and *mcrA* Gene Sequencing

Samples for the analysis of the structure of bacterial and archaeal communities in several tests were taken at two different times of operation of the anaerobic reactors from the second batch. Equal amounts of samples from three identical reactors were mixed, centrifuged at 12,000 × *g* for 10 min, and total DNA was extracted from 0.5 g of sediment using a FastDNA spin kit for soil (MP Biomedicals, Solon, OH, USA). DNA concentrations were estimated using a Qubit 2.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA). The primer set, Bakt_341F (5'-CCT ACG GGN GGC WGC AG-3') and Bakt_805R (5'-GAC TAC HVG GGT ATC TAA TCC-3'), was used to amplify the bacterial 16S rRNA gene fragments, while the primers mlas (5'-GGT GGT GTM GGD TTC ACM CAR TA-3') and *mcrA*-rev (5'-CGT TCA TBG CGT AGT TVG GRT AGT-3') were used to amplify the *mcrA* gene of methanogenic archaea. Amplicon sequencing was carried out using a FASTASeq 300 (GeneMind, Shenzhen, China) with a read length of 300 bp. The obtained sequence data were then processed with QIIME2 according to recommended protocols [29]. Archaeal 16S rRNA gene sequences were filtered from samples amplified with the primer set Bakt_341F and Bakt_805R. Operational taxonomic units (OTUs) representing less than 0.01% of the total reads were removed from further analysis. Alpha diversity was assessed at the OTU level. For the taxonomic assignment of the bacterial OTUs, the MiDAS 5 database was used [30], while for the taxonomic assignment of methanogenic OTUs, the NCBI nucleotide database was applied.

2.7. Statistical Analysis

Data for normal distribution were estimated by using the Kolmogorov–Smirnov test. Obtained data were reported as mean ± one standard deviation. Data were statistically compared using the Tukey method and 95% confidence (Minitab software version 22.0.0.0, State College, PA, USA).

3. Results

3.1. Process Stability and Methane Production

The efficiency of AD of algal biomass in anaerobic reactors was evaluated based on the breakdown of volatile solids and the production of methane. Additionally, the concentrations of individual VFAs, TAN, FAN, and pH values were measured. Batch AD experiments, conducted over two rounds, took place at mesophilic temperatures for 62 days in the first round and 47 days in the second round to study the effects of CNTs and GAC on AD of algal biomass. Five different conditions were monitored: control reactors, reactors with two different CNT concentrations, and reactors with two different GAC concentrations.

Anaerobic reactors produced methane immediately after the addition of algal biomass (*C. sorokiniana* and *T. obliquus*), indicating both a good initial substrate and a well-prepared inoculum. Figures 2a and 2b show the total amount of methane produced (TMP) and methane flow rate (MFR) during the first round of AD, respectively. As can be seen, methane was efficiently produced across all treatments; however, the addition of GAC enhanced methane production during the initial 16 days. In contrast, the addition of CNTs decreased this process. Throughout all experiments, approximately four major peaks in methane production were observed. The two largest peaks occurred on days 6/7 and 9. The final peaks of methane formation resulted from the microbial decomposition of less biodegradable compounds. The final levels of TMP (on day 62) for reactors C1, CNT_2, CNT_4,

GAC_2, and GAC_4 were 8430 ± 81 mL, 8215 ± 53 mL, 8155 ± 42 mL, 8343 ± 68 mL, and 8304 ± 47 mL, respectively. The final values of SMP (on day 62) for reactors C1, CNT_2, CNT_4, GAC_2, and GAC_4 reached 283 ± 2.7 mL g⁻¹_{VS}, 275 ± 1.8 mL g⁻¹_{VS}, 273 ± 1.4 mL g⁻¹_{VS}, 280 ± 2.3 mL g⁻¹_{VS}, and 278 ± 1.6 mL g⁻¹_{VS}, respectively.

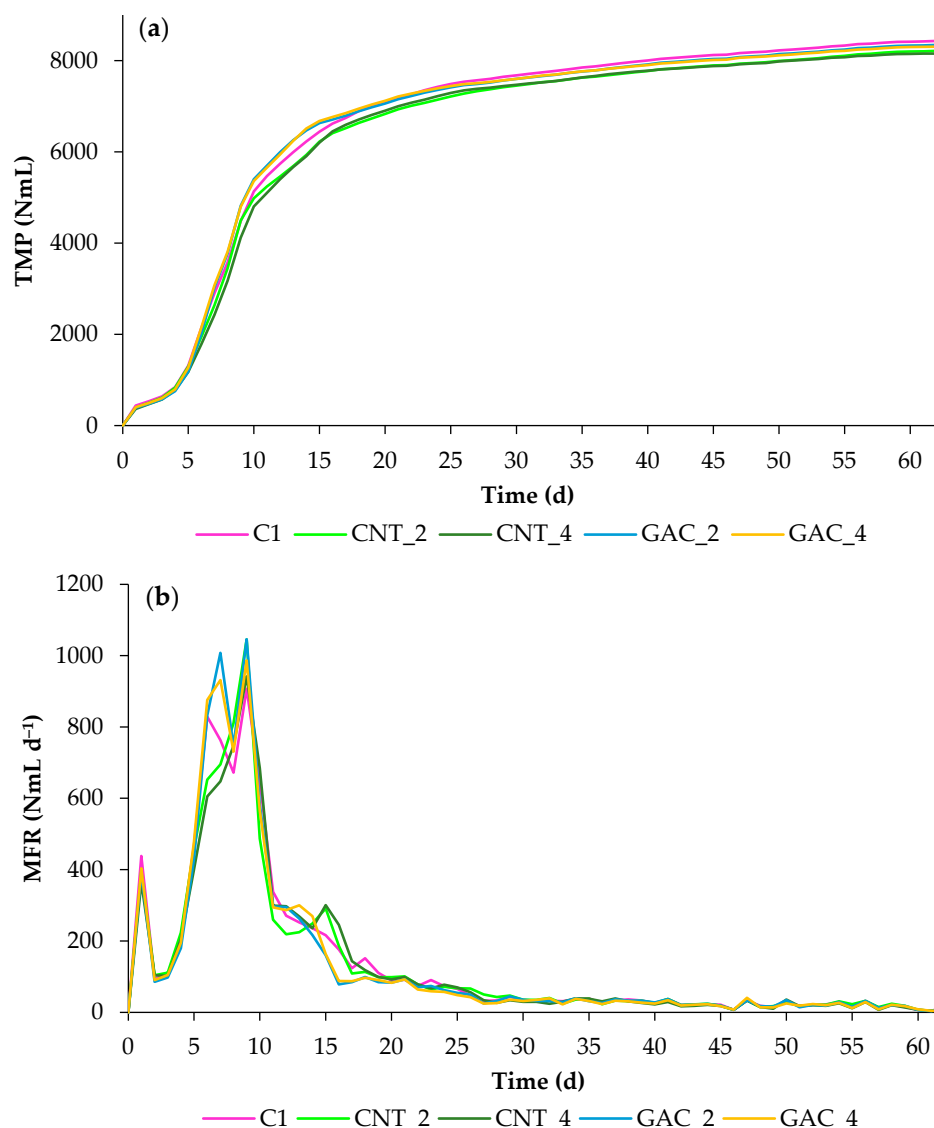


Figure 2. Influence of CNTs and GAC on TMP (a) and MFR (b) during the first round of AD of algal biomass. Different concentrations of CNTs and GAC were added to anaerobic reactors (labeled as C1 (0 g L⁻¹), CNT_2 (2 g L⁻¹), CNT_4 (4 g L⁻¹), GAC_2 (2 g L⁻¹), and GAC_4 (4 g L⁻¹)).

One important indicator for assessing the stability of the anaerobic process is the concentration of VFAs, which accrue in reactors if their subsequent transformation to biogas is suppressed. Figure 3a shows the formation and utilization of acetic, propionic, n-butyric, and isobutyric acids. Initially, all reactors exhibited approximately the same total levels of these VFAs, with acetic acid being the predominant type. Organic acid concentrations decreased during the anaerobic conversion of algal biomass. Throughout the AD process, almost all detected organic acids in all reactors were effectively directed to the final stage of methanogenesis. During the first round of AD of algal biomass, pH values decreased from the initial 7.46–7.62 (day 0) to 6.81–6.91 (day 2), before rising again to 7.49–7.55 (day 62) in different experiments. AD of biomass led to the accumulation of TAN across all anaerobic systems (Figure 3b). Initially, TAN concentrations increased

but subsequently decreased to varying levels. Lower levels were noted in experiments involving CNTs ($0.94\text{--}1.01\text{ g L}^{-1}$ on day 62). In terms of FAN data, concentrations reached between 40 and 46 mg L^{-1} in different reactors on day 62.

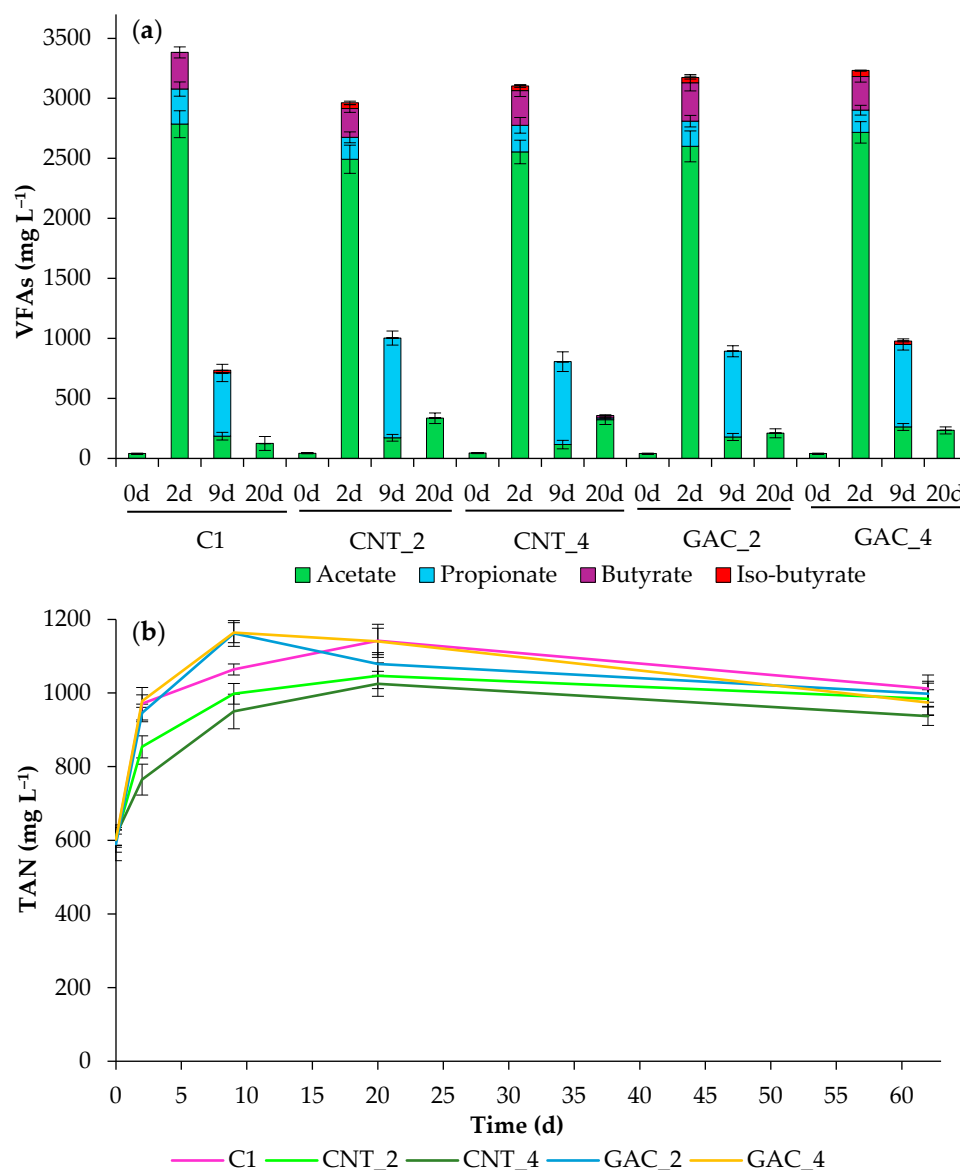


Figure 3. Influence of CNTs and GAC on VFA (a) and TAN (b) concentrations during the first round of AD of algal biomass. Different concentrations of CNTs and GAC were added to anaerobic reactors (labeled as C1 (0 g L^{-1}), CNT_2 (2 g L^{-1}), CNT_4 (4 g L^{-1}), GAC_2 (2 g L^{-1}), and GAC_4 (4 g L^{-1})). Error bars represent one standard deviation ($n = 3$).

During the second round, certain dosages of CNTs and GAC led to a substantial improvement in methane flow rates, as evidenced by a shift in the peaks of maximum methane yield. Also, compared to the control, the maximum flow rate of methane increased by 11.5% with CNTs (4.0 g L^{-1}) and by 6.5% with GAC (4.0 g L^{-1}). Thus, the TMP, MFR, accumulation, and utilization of VFAs and TAN resulting from AD of algal biomass, both in the absence and presence of different concentrations of CNTs and GAC, are illustrated in Figures 4 and 5. Unlike previous experiments, these trials yielded the most favorable outcomes when using carbon nanotubes at both tested concentrations, as well as activated carbon at the highest concentration studied. Thus, four main peaks in methane production were observed in all experiments. Increasing the amount of biomass added to the reactors

has led to a higher methane yield. The average maximum peaks of methane production from C2, CNT_2_2, CNT_2_4, GAC_2_2, and GAC_2_4 were 981/1144 mL, 1110/1147 mL, 1275/1216 mL, 1023/1152 mL, and 1219/1078 mL, respectively, and the corresponding time periods were 6/8, 5/7, 4/6, 7/8, and 5/7 days, respectively. The final levels of TMP (on day 47) for reactors C2, CNT_2_2, CNT_2_4, GAC_2_2, and GAC_2_4 were $11,291 \pm 60$ mL, $11,372 \pm 41$ mL, $11,499 \pm 55$ mL, $11,102 \pm 63$ mL, and $11,220 \pm 38$ mL, respectively. Additionally, the final values of SMP (on day 47) from reactors C2, CNT_2_2, CNT_2_4, GAC_2_2, and GAC_2_4 achieved 281 ± 1.5 mL g⁻¹_{VS}, 283 ± 1.0 mL g⁻¹_{VS}, 286 ± 1.4 mL g⁻¹_{VS}, 276 ± 1.6 mL g⁻¹_{VS}, and 279 ± 0.9 mL g⁻¹_{VS}, respectively.

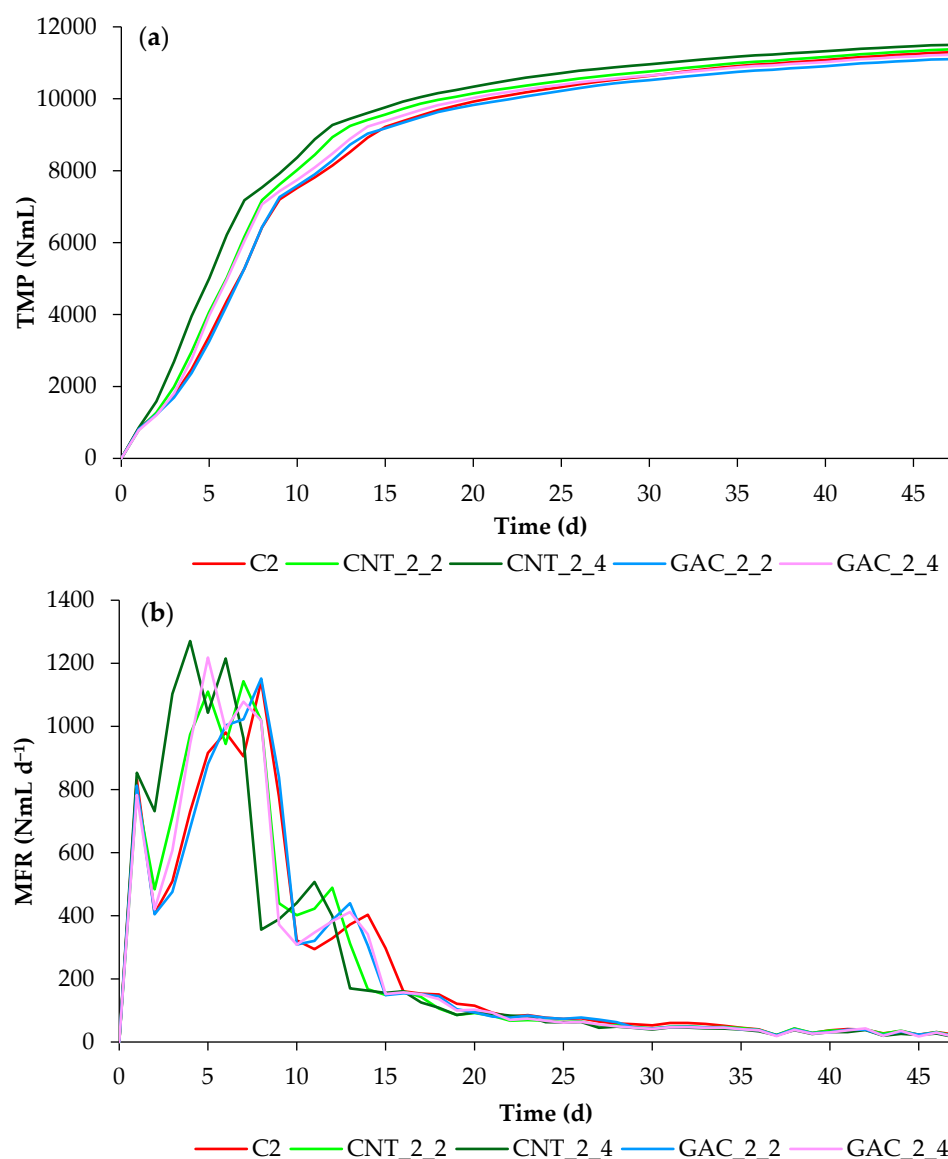


Figure 4. Influence of CNTs and GAC on TMP (a) and MFR (b) during the second round of AD of algal biomass. Different concentrations of CNTs and GAC were added to anaerobic reactors (labeled as C2 (0 g L⁻¹), CNT_2_2 (2 g L⁻¹), CNT_2_4 (4 g L⁻¹), GAC_2_2 (2 g L⁻¹), and GAC_2_4 (4 g L⁻¹)).

Initially, VFA concentrations increased and finally decreased during the AD of algal biomass, with the best results observed in reactors with CNTs (4.0 g L⁻¹) and GAC (4.0 g L⁻¹). This indicates more efficient degradation of short-chain fatty acids under formed conditions. The addition of CNTs and GAC to reactors promoted earlier consumption of acetate and butyrate. In contrast, propionate biodegradation began approximately 9 days after the start of the experiments, coinciding with the appearance of the last methane

peak. This indicates that propionate-degrading microorganisms became active following a significant decrease in acetate concentration and the nearly complete utilization of butyrate, and that the addition of the carbon-based additives stimulated microbial activity. Finally, these materials facilitated the consumption of the resulting VFAs. During the second round of AD of algal biomass, pH values decreased from the initial 7.49–7.55 (day 0) to 6.95–7.13 (day 3), before rising again to 7.43–7.50 (day 47) in different experiments. Unlike the first experiments, where a smooth increase and then decrease in TAN levels was observed, in these experiments TAN levels fluctuated. TAN and FAN concentrations in different reactors reached 1.1–1.25 g L⁻¹ and 39–52 mg L⁻¹, respectively, on day 47.

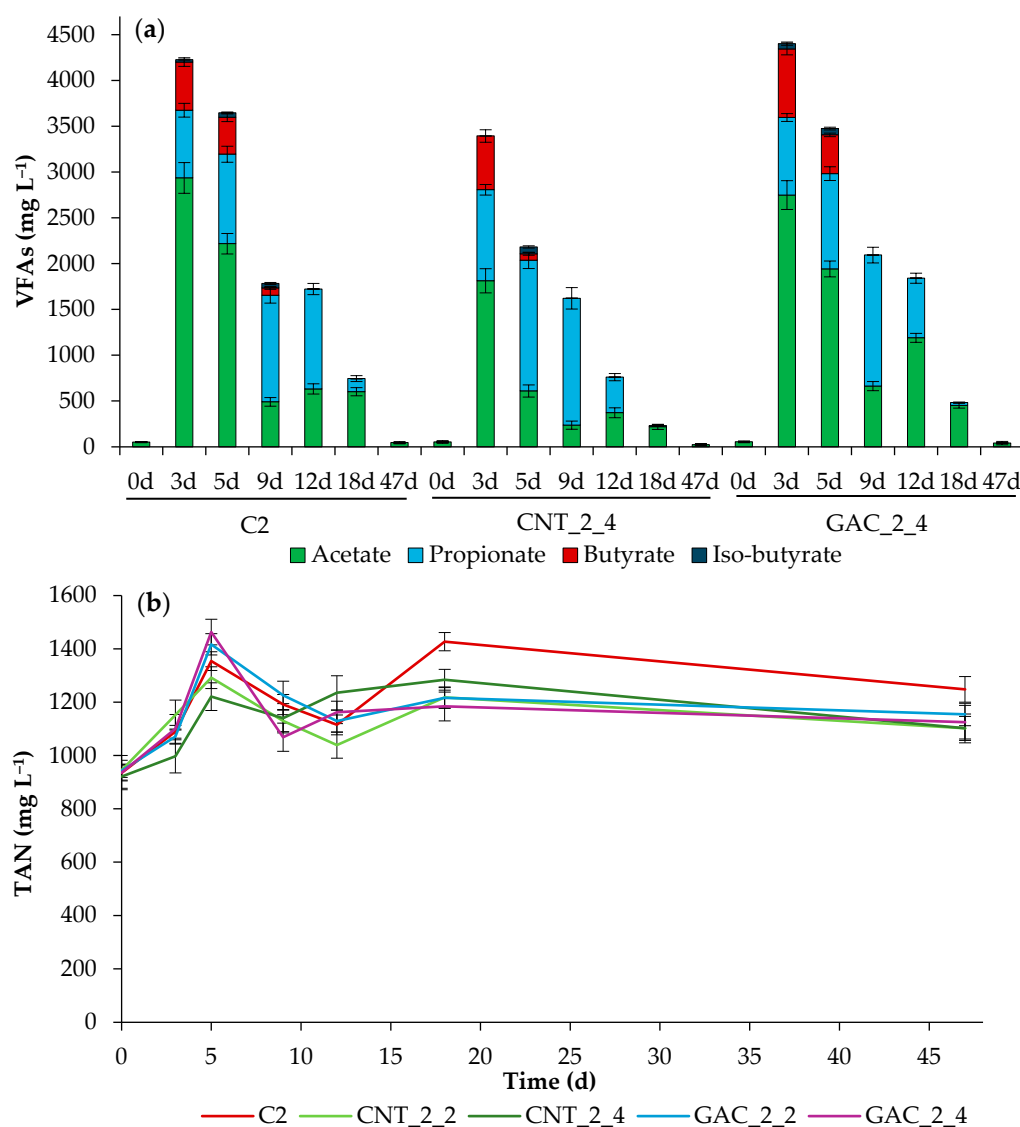


Figure 5. Influence of CNTs and GAC on VFA (a) and TAN (b) concentrations during the second round of AD of algal biomass. Different concentrations of CNTs and GAC were added to reactors (labeled as C2 (0 g L⁻¹), CNT_2_2 (2 g L⁻¹), CNT_2_4 (4 g L⁻¹), GAC_2_2 (2 g L⁻¹), and GAC_2_4 (4 g L⁻¹)). VFA concentrations were measured only in reactors C2, CNT_2_4, and GAC_2_4. Error bars represent one standard deviation ($n = 3$).

The analysis of data regarding VFAs accumulation and utilization, along with the primary kinetic parameters of methanogenesis (see Table 1), revealed a positive effect of the addition of the test agents during the anaerobic conversion of untreated algal biomass after the acclimatization stage. Consequently, a statistically significant increase (at $\alpha = 0.05$) in all analyzed metrics of methane production was observed in the experiment that included

carbon nanotubes at a concentration of 4.0 g L⁻¹ (round 2). A positive effect of GAC/CNTs on maximum MFR in the first round of AD was also observed.

Table 1. Key metrics of methane production obtained during AD of untreated algal biomass ($n = 3$). Means that do not share a letter are statistically significantly different according to the Tukey method and 95% confidence level.

Treatment	Methane Production Metrics		
	Maximum Methane Flow Rate (NmL d ⁻¹)/Day	Specific Methane Production Rate (NmL g ⁻¹ vs)	Total Methane Production (NmL)
First round (acclimatization stage)			
C1	908 ± 18 ^{c,d} /9	283 ± 2.7 ^a	8430 ± 81 ^a
CNT_2	1046 ± 17 ^a /9	275 ± 1.8 ^{b,c}	8215 ± 53 ^{b,c,d}
CNT_4	942 ± 25 ^{b,c} /9	273 ± 1.4 ^c	8155 ± 42 ^d
GAC_2	1048 ± 21 ^a /9	280 ± 2.3 ^{a,b}	8343 ± 68 ^{a,b,c}
GAC_4	987 ± 16 ^b /9	278 ± 1.6 ^{a,b,c}	8304 ± 47 ^{a,b,c,d}
Second round			
C2	1144 ± 18 ^d /8	281 ± 1.5 ^{b,c}	11,291 ± 60 ^b
CNT_2_2	1147 ± 19 ^d /7	283 ± 1.0 ^{a,b}	11,372 ± 41 ^{a,b}
CNT_2_4	1275 ± 22 ^a /4	286 ± 1.4 ^a	11,499 ± 55 ^a
GAC_2_2	1152 ± 21 ^{c,d} /8	276 ± 1.6 ^d	11,102 ± 63 ^d
GAC_2_4	1219 ± 17 ^b /5	279 ± 0.9 ^{c,d}	11,220 ± 38 ^{c,d}

3.2. Microbial Community Structure and Dynamics

After processing six samples from the second round of AD, more than 210 thousand high-quality bacterial 16S rRNA gene sequences were obtained. Alpha diversity indices calculated at the OTU level are presented in Table 2.

Table 2. Alpha diversity of bacterial communities (analyzed on days 5 and 12).

Indices	OTUs	Chao1	Shannon	Simpson
C2 (day 5)	418	475	6.87	0.98
C2 (day 12)	512	565	6.35	0.96
CNT_2_4 (day 5)	445	502	6.54	0.97
CNT_2_4 (day 12)	341	387	6.61	0.98
GAC_2_4 (day 5)	526	586	6.68	0.97
GAC_2_4 (day 12)	513	578	6.74	0.97

The number of bacterial OTUs in samples obtained after the second round of AD of algal biomass ranged from 341 to 526 (relative abundance > 0.01%). The relative abundance of specific bacterial groups at two sampling points was analyzed at different taxonomic levels, such as phylum, class, order, family, and genus. A total of 21 bacterial phyla, 51 classes, 94 orders, and 228 genera were identified in the six samples.

The bacterial community profile (at the class level) in reactors C2, CNT_2_4, and GAC_2_4 is presented in Figure 6. The most frequently observed classes in the analyzed anaerobic reactors were Bacteroidia and Clostridia. In contrast to C2, where the relative abundance of Bacteroidia increased, in CNT_2_4 and GAC_2_4, the relative abundance of Bacteroidia decreased over the analyzed period. Furthermore, the relative abundance of representatives of Clostridia decreased, while the relative abundance of members of Anaerolineae increased over the same analyzed period in all systems. Other classes that accounted for more than 0.5% of the 16S rRNA gene sequences in at least one of the samples included RBG-16-55-12 (Actinobacteriota), Caldatribacteriia, Campylobacteria, Bacilli, Limnochordia,

Syntrophomonadia, Dojkabacteria, Microgenomatia, Phycisphaerae, Alphaproteobacteria, Gammaproteobacteria, MVP-15 (Spirochaetota), Spirochaetia, Synergistia, Thermotogae, and Lentisphaeria (Figure 6).

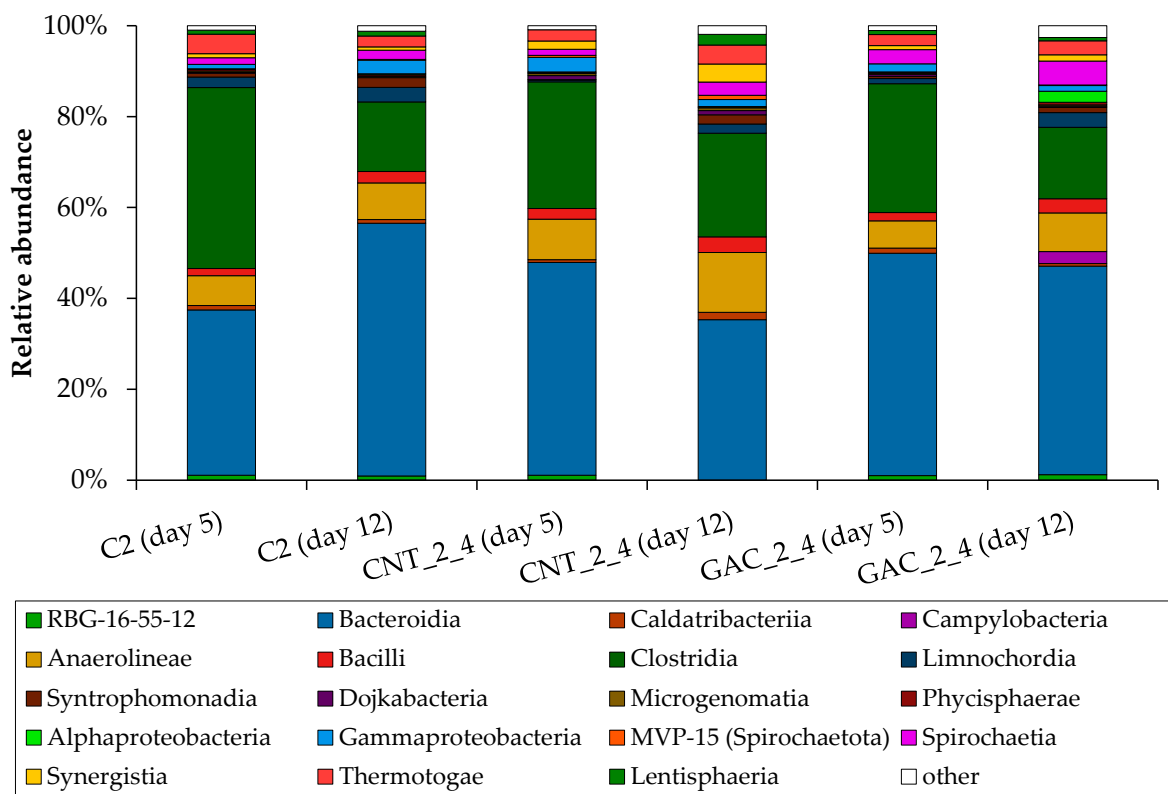


Figure 6. Taxonomic composition of bacterial communities in reactors C2, CNT_2_4, and GAC_2_4 (sampled on days 5 and 12). Bacterial community composition according to amplicon sequencing of the bacterial 16S rRNA gene is shown at the class level. Classes with relative abundances below 0.5% are summarized as “others”.

Figure 7 illustrates the bacterial community profile at the genus level in reactors C2, CNT_2_4, and GAC_2_4. Regardless of the experiment, the bacterial community was mainly represented by the genus *Ruminofilibacter*. In contrast to C2, where the relative abundance of *Ruminofilibacter* increased, in CNT_2_4 and GAC_2_4, the relative abundance of *Ruminofilibacter* decreased by day 12. Interestingly, in all reactors, the relative abundance of the genera *Pelolinea*, *DMER64* (Rikenellaceae), *Syntrophomonas*, and *Treponema* increased, with *DMER64* and *Treponema* being detected at notably higher levels in the additive-containing treatments. In addition, the percentages of *Clostridium_sensu_stricto_8*, *Cellulosilyticum*, and unclassified OTUs of Lachnospiraceae decreased during the operation of all anaerobic reactors. Compared with other reactors, in the best operating mode, CNT_2_4, unclassified *Bacteroidetes_vadinHA17*, *Petrimonas*, *Proteiniphilum*, *Lentimicrobium*, *Ca_Caldatribacterium*, UCG-004 (Erysipelatoclostridiaceae), *Clostridium_sensu_stricto_1*, and *Paeniclostridium* increased by day 12.

The structure and dynamics of methanogenic communities in anaerobic reactors without carbon-based supplements (C2) and with carbon-based supplements (CNT_2_4 and GAC_2_4) were further assessed using amplicon sequencing of the *mcrA* gene. After processing six samples, more than 150 thousand high-quality sequences were obtained. The reactor-specific profile of methanogenic communities (at the genus level) in various reactors is presented in Figure 8.

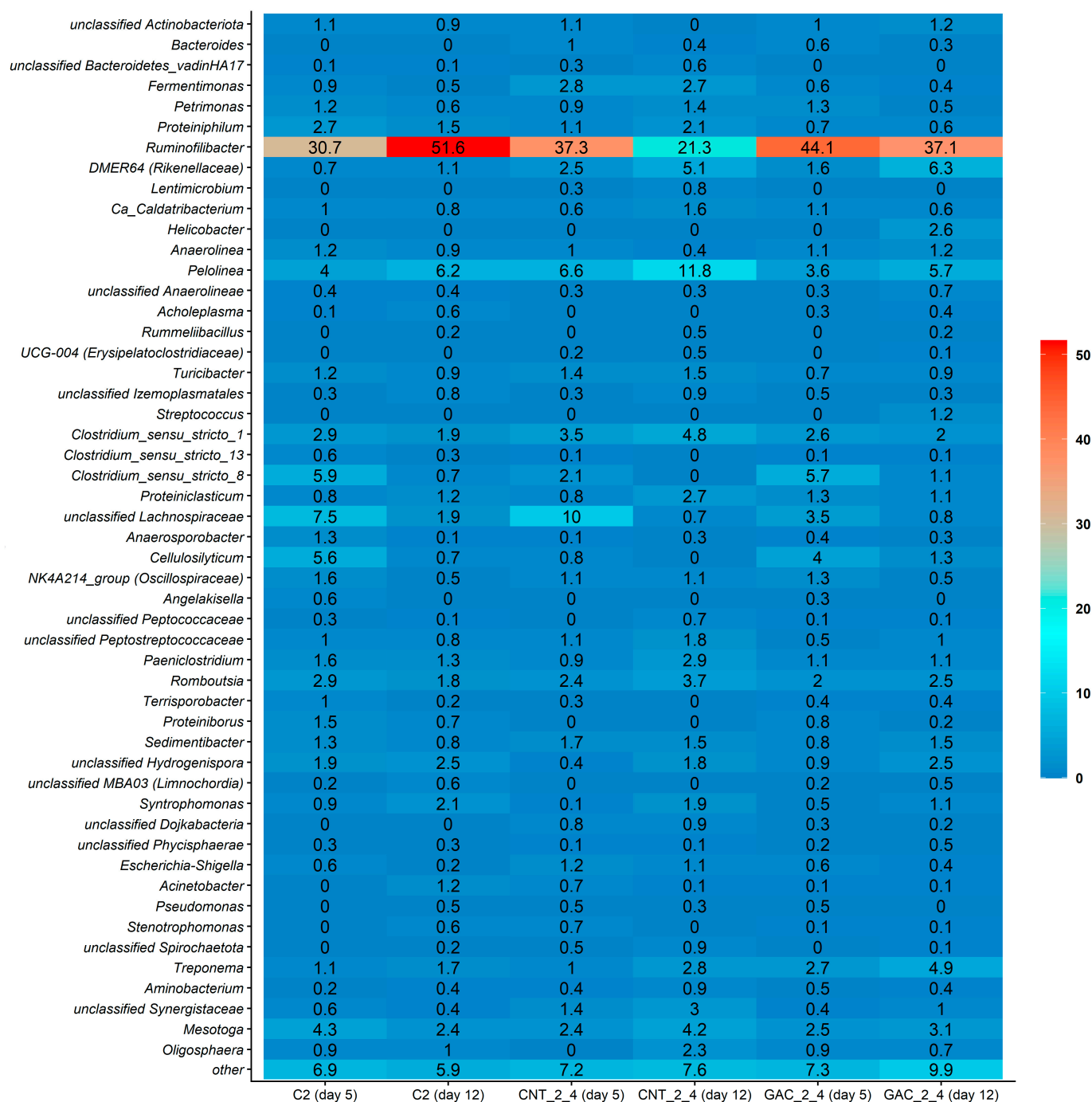


Figure 7. Heatmap illustrating the relative abundances (%) of bacterial taxa in reactors C2, CNT_2_4, and GAC_2_4 (sampled on days 5 and 12). Bacterial community composition according to amplicon sequencing of the bacterial 16S rRNA gene is shown at the genus level. Only taxa comprising at least 0.5% relative abundance in at least one sample are presented.

During the experimental period, the most frequently observed archaeal genera in reactors without and with carbon-based supplements were *Methanosarcina*, *Methanotherix*, *Methanoculleus*, an unclassified OTU of the family Methanosarcinaceae, and *Methanobacterium*. Compared to control experiments, in carbon-additive-containing treatments, the relative abundance of *Methanosarcina* decreased, whereas the relative abundance of *Methanotherix* and *Methanoculleus* increased, which was probably due to more active methane generation in the presence of CNTs or GAC (Figure 8).

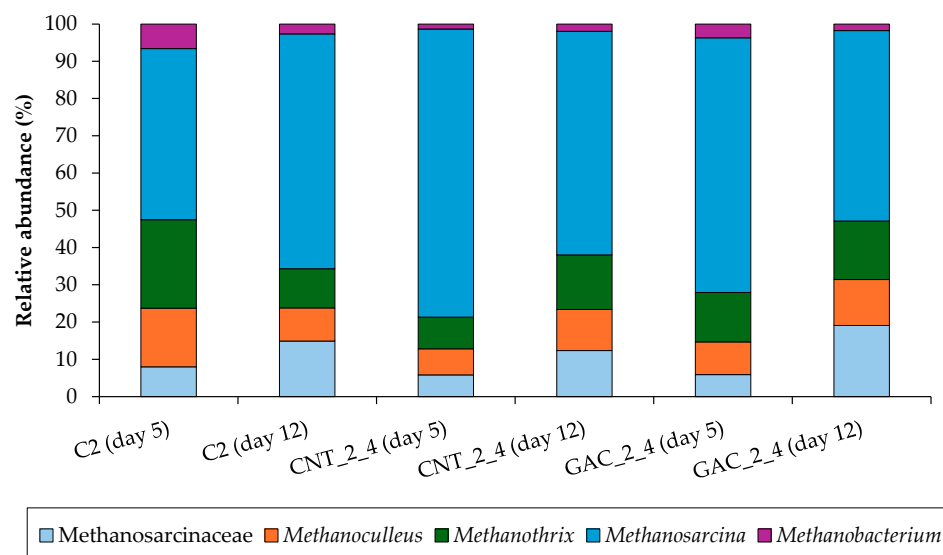


Figure 8. Taxonomic composition of methanogenic communities in reactors C2, CNT_2_4, and GAC_2_4 (sampled on days 5 and 12). Methanogenic community composition according to amplicon sequencing of the *mcrA* gene is shown at the genus level.

4. Discussion

In this study, to investigate the effects of different carbon-based additives, specifically carbon nanotubes and granular activated carbon, and their different concentrations on AD efficiency of algal biomass (*C. sorokiniana* and *T. obliquus*), mesophilic anaerobic batch experiments were carried out. The structure and dynamics of the anaerobic microorganisms involved in this process were additionally investigated. The findings of this study will enhance the potential for using carbon-based additives to increase the efficiency of microalgal biomass valorization.

Several early studies have focused on the AD of microalgal biomass, including the residual biomass remaining after the extraction of valuable products, for biogas production [21–23]. Additionally, microalgae cultivated in the presence of high concentrations of available nitrogen tend to have elevated protein content [24], which suggests that the AD of this biomass can lead to an increased TAN level, as well as FAN, over a long-term AD period. This study confirmed that TAN concentration increased during a two-round AD process involving algal biomass, with anaerobically digested cow manure used as an inoculum. This inoculum was initially characterized by a low TAN content. Additionally, research has demonstrated that the use of carbon-based additives can effectively decrease TAN levels in anaerobic reactors, providing an alternative to expensive methods aimed at decreasing toxic ammonia concentrations. Moreover, AD of microalgal biomass is often hampered by their tough-to-decompose cell walls, ultimately resulting in low methane yields from potentially rich biomass [22]. In our study, in the presence of certain additive concentrations, accelerated gas production from the substrate was observed, resulting in high methane yields (273–286 mL g^{−1}_{VS}) that are comparable to and may even exceed previously reported values [22,31]. This method could serve as an alternative to expensive algal biomass pretreatment methods for enhancing overall methane yields. The data obtained from this study suggest that using carbon-based additives can be considered as a way to improve the efficiency of the entire biotechnological process, particularly in the effective biomass hydrolysis, which is followed by biomethane production. Fermentation of biomass or biowaste obtained from microalgae processing offers an attractive solution for optimizing the anaerobic process. This approach fosters increased microbial activity, which can lead to higher methane yields. Therefore, developing tailored biomass processing

technologies necessitates a detailed understanding of the microbial processes involved in the AD of non-traditional biomass.

Several previous studies have demonstrated that the use of granular activated carbon, biochar, graphene oxide, and carbon nanotubes can enhance the efficiency of methane production from various other substrates, and therefore, they can be effectively applied in AD processes of various biomass [7,8,32–36]. However, it is worth noting that the kinetics of methane generation from the reactors can also depend on the type of inoculum, the substrate itself, and cultivation parameters; consequently, the formation of the metabolites themselves (gas, organic acids, and TAN) may vary from case to case. Nevertheless, the trends demonstrating the positive effect of these materials will persist. Some literature indicates that these additives improve AD by promoting interspecies electron transfer. DIET (direct interspecies electron transfer via electrically conductive cell surface structures) and CIET are the main modes of interspecies electron exchange in a number of anaerobic biogas reactors. Other reports indicate that these materials can enhance the stability and efficiency of AD by altering the structure of anaerobic microbial communities, maintaining a more negative redox potential in anaerobic microcosms, creating surfaces for microbial colonization, and providing protection for microorganisms from inhibitory factors. Finally, carbon-based additives contribute to improved energy recovery efficiency and process sustainability, offering a promising avenue for cost-effective and scalable waste-to-energy solutions [2,3,6–8]. Therefore, further comprehensive studies of microorganisms are needed to understand the physiology and ecology of this biochemical process, as well as to subsequently apply this new knowledge in practice. However, for a more detailed understanding of this anaerobic process, it is necessary to evaluate its efficiency using other substrates and inocula.

A more detailed study of the composition of bacterial communities in various anaerobic reactors, determined by the method of amplicon sequencing of bacterial 16S rRNA gene, revealed that the main participants among the hydrolytic bacterial communities were bacteria of the genus *Ruminofilibacter* (Bacteroidia). Unlike C2, where the relative abundance of *Ruminofilibacter* increased, in CNT_2_4 and GAC_2_4, the relative abundance of *Ruminofilibacter* decreased by day 12. This may suggest a more active role of *Ruminofilibacter* in the anaerobic transformation of microalgal biomass in biogas reactors containing carbon-based additives, as well as their more rapid replacement by other bacteria, such as organic acid-oxidizing bacteria. It is worth noting that the structure of bacterial communities formed on the 12th day of the experiments was assessed during the period of intensive destruction of propionate in reactors containing CNTs and GAC and only its partial destruction in the control reactors. GAC has recently been shown to promote propionate conversion, may reduce oxidative stress, and enhance bacterial membrane stability [37]. Bacterial species belonging to the genus *Ruminofilibacter* are generally poorly described in the literature. The only known representative of this genus, *Ruminofilibacter xylanolyticum*, is recognized for its xylanolytic activity and has previously been detected in some other biogas reactors that utilize plant biomass [38–40]. Across all reactors, the relative abundance of the genera *Pelolinea* (Anaerolineae), *DMER64* (Bacteroidia), *Syntrophomonas* (Syntrophomonadia), and *Treponema* (Spirochaetia) increased, with *DMER64* and *Treponema* being found in notably higher percentages in the CNTs- and GAC-containing systems. Similar to *R. xylanolyticum*, the sole representative of the genus *Ruminofilibacter*, the genus *Pelolinea* also includes one known species, *Pelolinea submarina*, previously isolated from marine subsurface sediments [41]. This strictly anaerobic species, when grown on sugars, produces acetate, lactate, ethanol, and hydrogen, which can be finally funneled into the methanogenesis stage [42]. The members of the genus *DMER64* have been proposed as potential syntrophic bacteria and electroactive agents that could be involved in magnetite-mediated interspecies electron transfer with some methanogenic

archaea [43]. Members of the genus *Syntrophomonas* are known as syntrophic fatty acid oxidizers in a variety of anaerobic environments. For example, *Syntrophomonas wolfei* can convert butyric acid to acetic acid and molecular hydrogen in syntrophic association with hydrogenotrophic methanogenic archaea [44]. *Syntrophomonas sapovorans* ferments different saturated fatty acids to propionic acid, acetic acid, and molecular hydrogen in coculture with hydrogenotrophic methanogenic archaea [45]. *Syntrophomonas* species have also been proposed as electron-donating partners in the DIET mechanism [46,47]. However, it has also been previously shown that acetate production from butyrate by *S. wolfei* was faster in the presence of carbon nanotubes, but no evidence of DIET was found between *S. wolfei* and *Methanospirillum hungatei*. The reported role of Spirochaetaceae and *Treponema* in lignocellulose degradation is also consistent with the strong correlation of several *Treponema* OTUs with cellulase and xylanase activity in other anaerobic systems [40]. *Mesotoga* has been reported to efficiently ferment carbohydrates and proteinaceous substrates. Acetic acid was the primary product formed during xylose fermentation, along with significantly smaller amounts of n-butyric, isobutyric, and isovaleric acids [48].

The structure and dynamics of methanogenic communities in anaerobic digesters with and without CNTs/GAC addition were further studied using amplicon sequencing of the *mcrA* gene. Compared to control experiments, in the additives-containing treatments, the relative abundance of acetoclastic/methylotrophic/hydrogenotrophic *Methanosarcina* decreased, while the relative abundance of acetoclastic *Methanothrix* and hydrogenotrophic *Methanoculleus* increased by day 12, which was likely due to the addition of CNTs or GAC to these anaerobic systems. The use of acetate as a substrate for methanogenesis is a common feature of *Methanosarcina* and *Methanothrix*, with the latter having an extremely high affinity for acetate and metabolizing this metabolite at low levels. Methanogens belonging to the mixotrophic genus *Methanosarcina* are more effectively adapted and exhibit greater resistance to various environmental stresses, elevated concentrations of volatile fatty acids and ammonia, as well as pH fluctuations. In contrast, strict acetoclastic methanogens of the genus *Methanothrix* show less resilience [14,49]. This difference aligns with the more rapid attenuation of *Methanosarcina* after depletion of the pool of organic acids in the growth medium. The resilience of *Methanosarcina* species to various stress factors, their close syntrophic partnerships with bacterial partners, and their metabolic versatility—including the ability for extracellular electron uptake—are often regarded by researchers as key features that contribute to their significant and frequently dominant role in methanogenic communities [14,50]. The properties of *Methanosarcina* make it an especially valuable methanogen for research in biotechnological applications, including nanobiotechnology [51]. Methanogens of the genera *Methanothrix* and *Methanosarcina* employ different electron transfer strategies, both using conductive cell surface structures and using conductive particles to accept electrons [14,52–54]. The increased methane production rate observed in our CNTs/GAC-supplemented experiments may also be due to the latter mechanism. Further studies on the conversion of complex substrates using conductive materials will uncover electron transfer strategies that enable methanogenic communities to realize their full potential for sustainable methane management.

5. Conclusions

Taunit-M multiwalled carbon nanotubes and coconut-based granular activated carbon, when used appropriately, enhance methane production from microalgal biomass derived from the treatment of various wastewaters. These findings demonstrate that incorporating carbon nanotubes (2.0 and 4.0 g L⁻¹) and granular activated carbon (4.0 g L⁻¹) accelerates methane production by promoting the decomposition of organic matter and conversion of VFAs. Our experiments also highlight the importance of acclimating microorganisms

to substrate, carbon nanotubes, and granular activated carbon. The results obtained are anticipated to advance the development of dependable methane generation systems and promote the sustainable utilization of waste generated from microalgal biomass processing. Moreover, the proposed approach could enhance environmentally friendly waste management and renewable energy production, thereby contributing to the creation of resource-efficient and low-carbon societies.

Although the CNTs and GAC significantly affected the conversion rate of organic acids to biomethane in the second round, the influence of these additives on continuous stirred tank reactors utilizing algal biomass—both the initial biomass and the biomass following the extraction of target metabolites—requires further investigation for engineering applications. This study may suggest a method for enhancing the ability to resist the inhibition of organic acid accumulation that occurs in anaerobic systems with a sudden rise in organic load and ammonia level. Consequently, further research is necessary to explore other materials with conductive properties and to identify the specific microorganisms involved in the conductive particle-mediated interspecies electron transfer.

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Abbreviations

The following abbreviations are used in this manuscript:

AD	Anaerobic digestion
CIET	Conductive particle-mediated interspecies electron transfer
CNTs	Carbon nanotubes
DIET	Direct interspecies electron transfer
FAN	Free ammonia nitrogen
GAC	Granular activated carbon
MFR	Methane flow rate
SMP	Specific methane production
TAN	Total ammonia nitrogen
TMP	Total methane production
TS	Total solids
VFAs	Volatile fatty acids
VS	Volatile solids

References

1. Zhang, D.; Lu, P.; Zhang, M.; Wei, Y.; Liang, J.; Zhou, L. Elucidating interactive effects of sulfidated nanoscale zero-valent iron and ammonia on anaerobic digestion of food waste. *J. Biosci. Bioeng.* **2023**, *135*, 63–70. [[CrossRef](#)] [[PubMed](#)]
2. Parra-Orobio, B.A.; Soto-Paz, J.; Lara-Franco, S.J.; Castañeda-Restrepo, M.F.; Oviedo-Ocaña, E.R.; Wang, Z.; Sánchez, A. Nanomaterial-enhanced anaerobic digestion for sustainable bioenergy production: Opportunities, challenges and territorial issues. A systematic bibliometric review. *Biomass Bioenergy* **2025**, *203*, 108268. [[CrossRef](#)]

3. Ramesh, G.; Logan, M. Unlocking the potential of conductive materials for advancing the frontiers in anaerobic digestion. *Fuel* **2026**, *406*, 136755. [\[CrossRef\]](#)
4. Ziganshin, A.M.; Ziganshina, E.E.; Kleinsteuber, S.; Pröter, J.; Ilinskaya, O.N. Methanogenic community dynamics during anaerobic utilization of agricultural wastes. *Acta Nat.* **2012**, *4*, 91–97. [\[CrossRef\]](#)
5. Ziganshin, A.M.; Wintsche, B.; Seifert, J.; Carstensen, M.; Born, J.; Kleinsteuber, S. Spatial separation of metabolic stages in a tube anaerobic baffled reactor: Reactor performance and microbial community dynamics. *Appl. Microbiol. Biotechnol.* **2019**, *103*, 3915–3929. [\[CrossRef\]](#) [\[PubMed\]](#)
6. Azad, B.; Wu, L.; Duan, H.; Qian, J. Enhanced anaerobic digestion performance with carbon-based material additives towards sustainable energy production: A comprehensive review. *J. Clean. Prod.* **2025**, *525*, 146616. [\[CrossRef\]](#)
7. Ziganshina, E.E.; Belostotskiy, D.E.; Bulynina, S.S.; Ziganshin, A.M. Influence of granular activated carbon on anaerobic co-digestion of sugar beet pulp and distillers grains with solubles. *Processes* **2020**, *8*, 1226. [\[CrossRef\]](#)
8. Ziganshina, E.E.; Ziganshin, A.M. Magnetite nanoparticles and carbon nanotubes for improving the operation of mesophilic anaerobic digesters. *Microorganisms* **2023**, *11*, 938. [\[CrossRef\]](#) [\[PubMed\]](#)
9. Jiang, M.; Qiao, W.; Wang, Y.; Zou, T.; Lin, M.; Dong, R. Balancing acidogenesis and methanogenesis metabolism in thermophilic anaerobic digestion of food waste under a high loading rate. *Sci. Total Environ.* **2022**, *824*, 153867. [\[CrossRef\]](#) [\[PubMed\]](#)
10. Tongco, J.V.; Park, S.H.; Kim, S.I.; Hwang, S. Influence of fluctuating food waste concentrations on horizontal anaerobic reactor performance and biogas output. *Energies* **2025**, *18*, 5064. [\[CrossRef\]](#)
11. Riya, S.; Nitta, T.; Terada, A. Effect of the composition and hydrolyzability of lignocellulosic biomass on methane productivity in co-anaerobic digestion of lignocellulosic biomass and cow manure. *Waste Biomass Valorization* **2026**, *17*, 575–584. [\[CrossRef\]](#)
12. Chang, H.; Yin, Q.; He, K.; Vrieze, J.D.; Wu, G. Feeding regime selectively enriching acetoclastic methanogens to enhance energy production in anaerobic digestion systems. *Biochem. Eng. J.* **2025**, *220*, 109764. [\[CrossRef\]](#)
13. Amalapidman, V.; Ofori, P.A.; Abbey, L. Valorization of algal biomass to biofuel: A Review. *Biomass* **2025**, *5*, 26. [\[CrossRef\]](#)
14. Rotaru, A.; Gharib, G.; Jabaley, A.; Anestis, K.; Kotoky, R. Cell surface differences within the genus *Methanosarcina* shape interactions with the extracellular environment. *J. Bacteriol.* **2025**, *207*, e00112-25. [\[CrossRef\]](#) [\[PubMed\]](#)
15. Martínez-Ruiz, F.E.; Andrade-Bustamante, G.; Holguín-Peña, R.J.; Renganathan, P.; Gaysina, L.A.; Sukhanova, N.V.; Puente, E.O.R. Microalgae as functional food ingredients: Nutritional benefits, challenges, and regulatory considerations for safe consumption. *Biomass* **2025**, *5*, 25. [\[CrossRef\]](#)
16. Dimopoulou, M.; Kolonas, A.; Stagos, D.; Gortzi, O. A review of the sustainability, chemical composition, bioactive compounds, antioxidant and antidiabetic activity, neuroprotective properties, and health benefits of microalgae. *Biomass* **2025**, *5*, 11. [\[CrossRef\]](#)
17. Ziganshina, E.E.; Yureva, K.A.; Ziganshin, A.M. Poultry slaughterhouse wastewater treatment by green algae: An eco-friendly restorative process. *Environments* **2025**, *12*, 331. [\[CrossRef\]](#)
18. Bulynina, S.S.; Ziganshina, E.E.; Terentev, A.D.; Ziganshin, A.M. Treatment of wastewater from the fish processing industry and production of valuable algal biomass with a biostimulating effect. *Phycology* **2026**, *6*, 2. [\[CrossRef\]](#)
19. Elisabeth, B.; Rayen, F.; Behnam, T. Microalgae culture quality indicators: A review. *Crit. Rev. Biotechnol.* **2021**, *41*, 457–473. [\[CrossRef\]](#) [\[PubMed\]](#)
20. Guieysse, B.; Plouviez, M. Microalgae cultivation: Closing the yield gap from laboratory to field scale. *Front. Bioeng. Biotechnol.* **2024**, *12*, 1359755. [\[CrossRef\]](#) [\[PubMed\]](#)
21. Park, J.; Kumar, A.N.; Cayetano, R.D.A.; Kim, S.H. Assessment of *Chlorella* sp. as a potential feedstock for biological methane production. *Bioresour. Technol.* **2020**, *305*, 123075. [\[CrossRef\]](#) [\[PubMed\]](#)
22. Torres, A.; Padrino, S.; Brito, A.; Diaz, L. Biogas production from anaerobic digestion of solid microalgae residues generated on different processes of microalgae-to-biofuel production. *Biomass Convers. Biorefinery* **2023**, *13*, 4659–4672. [\[CrossRef\]](#)
23. Cheenakula, D.; Hoffstadt, K.; Krafft, S.; Reinecke, D.; Klose, H.; Kuperjans, I.; Grömping, M. Anaerobic digestion of algal–bacterial biomass of an Algal Turf Scrubber system. *Biomass Convers. Biorefinery* **2023**, *13*, 13605–13619. [\[CrossRef\]](#)
24. Ziganshina, E.E.; Bulynina, S.S.; Yureva, K.A.; Ziganshin, A.M. Growth parameters of various green microalgae species in effluent from biogas reactors: The importance of effluent concentration. *Plants* **2022**, *11*, 3583. [\[CrossRef\]](#) [\[PubMed\]](#)
25. Kumar, P.; Bhattacharya, A.; Prajapati, S.K.; Malik, A.; Vijay, V.K. Anaerobic co-digestion of waste microalgal biomass with cattle dung in a pilot-scale reactor: Effect of seasonal variations and long-term stability assessment. *Biomass Convers. Biorefinery* **2022**, *12*, 1203–1215. [\[CrossRef\]](#)
26. Kumar, V.; Jaiswal, K.K.; Tomar, M.S.; Rajput, V.; Upadhyay, S.; Nanda, M.; Vlaskin, M.S.; Kumar, S.; Kurbatova, A. Production of high value-added biomolecules by microalgae cultivation in wastewater from anaerobic digestates of food waste: A review. *Biomass Convers. Biorefinery* **2023**, *13*, 9625–9642. [\[CrossRef\]](#)
27. Weber, S.; Grande, P.M.; Blank, L.M.; Klose, H. Insights into cell wall disintegration of *Chlorella vulgaris*. *PLoS ONE* **2022**, *17*, e0262500. [\[CrossRef\]](#) [\[PubMed\]](#)
28. Hansen, K.H.; Angelidaki, I.; Ahring, B.K. Anaerobic digestion of swine manure; inhibition by ammonia. *Water Res.* **1998**, *32*, 5–12. [\[CrossRef\]](#)

29. Bolyen, E.; Rideout, J.R.; Dillon, M.R.; Bokulich, N.A.; Abnet, C.C.; Al-Ghalith, G.A.; Alexander, H.; Alm, E.J.; Arumugam, M.; Asnicar, F.; et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat. Biotechnol.* **2019**, *37*, 852–857, Correction in *Nat. Biotechnol.* **2019**, *37*, 1091. [[CrossRef](#)] [[PubMed](#)]
30. Dueholm, M.K.D.; Andersen, K.S.; Korntved, A.K.C.; Rudkjøbing, V.; Alves, M.; Bajón-Fernández, Y.; Batstone, D.; Butler, C.; Cruz, M.C.; Davidsson, Å.; et al. MiDAS 5: Global diversity of bacteria and archaea in anaerobic digesters. *Nat. Commun.* **2024**, *15*, 5361. [[CrossRef](#)] [[PubMed](#)]
31. Meneses-Reyes, J.C.; Hernández-Eugenio, G.; Huber, D.H.; Balagurusamy, N.; Espinosa-Solares, T. Biochemical methane potential of oil-extracted microalgae and glycerol in co-digestion with chicken litter. *Bioresour. Technol.* **2017**, *224*, 373–379. [[CrossRef](#)] [[PubMed](#)]
32. Yang, Y.; Zhang, Y.; Li, Z.; Zhao, Z.; Quan, X.; Zhao, Z. Adding granular activated carbon into anaerobic sludge digestion to promote methane production and sludge decomposition. *J. Clean. Prod.* **2017**, *149*, 1101–1108. [[CrossRef](#)]
33. Capson-Tojo, G.; Moscoviz, R.; Ruiz, D.; Santa-Catalina, G.; Trably, E.; Rouez, M.; Crest, M.; Steyer, J.P.; Bernet, N.; Delgenes, J.P.; et al. Addition of granular activated carbon and trace elements to favor volatile fatty acid consumption during anaerobic digestion of food waste. *Bioresour. Technol.* **2018**, *260*, 157–168. [[CrossRef](#)] [[PubMed](#)]
34. Li, L.L.; Tong, Z.H.; Fang, C.Y.; Chu, J.; Yu, H.Q. Response of anaerobic granular sludge to single-wall carbon nanotube exposure. *Water Res.* **2015**, *70*, 1–8. [[CrossRef](#)] [[PubMed](#)]
35. Salvador, A.F.; Martins, G.; Mellefranco, M.; Serpa, R.; Ajm, S.; Cavaleiro, A.J.; Pereira, M.A.; Alves, M.M. Carbon nanotubes accelerate methane production in pure cultures of methanogens and in a syntrophic coculture. *Environ. Microbiol.* **2017**, *19*, 2727–2739. [[CrossRef](#)] [[PubMed](#)]
36. Ponzelli, M.; Koch, K.; Drewes, J.E.; Radjenovic, J.; Vinardell, S. The ambivalent role of graphene oxide in anaerobic digestion: A review. *Bioresour. Technol.* **2024**, *414*, 131663. [[CrossRef](#)] [[PubMed](#)]
37. Liu, T.; Liu, C.; Chang, H.; Wu, Q.; Wang, W.; Wu, G. Potential microbial mechanisms driving granular activated carbon-enhanced syntrophic propionate degradation under elevated organic loading rates. *J. Environ. Chem. Eng.* **2026**, *14*, 121343. [[CrossRef](#)]
38. Weiss, S.; Zankel, A.; Lebuhn, M.; Petrak, S.; Somitsch, W.; Guebitz, G.M. Investigation of microorganisms colonising activated zeolites during anaerobic biogas production from grass silage. *Bioresour. Technol.* **2011**, *102*, 4353–4359. [[CrossRef](#)] [[PubMed](#)]
39. Nissilä, M.E.; Li, Y.C.; Wu, S.Y.; Lin, C.Y.; Puhakka, J.A. Hydrogenic and methanogenic fermentation of birch and conifer pulps. *Appl. Energy* **2012**, *100*, 58–65. [[CrossRef](#)]
40. Jensen, M.B.; de Jonge, N.; Dolriis, M.D.; Kragelund, C.; Fischer, C.H.; Eskesen, M.R.; Noer, K.; Møller, H.B.; Ottosen, L.D.M.; Nielsen, J.L.; et al. Cellulolytic and xylanolytic microbial communities associated with lignocellulose-rich wheat straw degradation in anaerobic digestion. *Front. Microbiol.* **2021**, *12*, 645174. [[CrossRef](#)] [[PubMed](#)]
41. Imachi, H.; Sakai, S.; Lipp, J.S.; Miyazaki, M.; Saito, Y.; Yamanaka, Y.; Hinrichs, K.U.; Inagaki, F.; Takai, K. *Pelolinea submarina* gen. nov.; sp. nov.; an anaerobic, filamentous bacterium of the phylum Chloroflexi isolated from subseafloor sediment. *Int. J. Syst. Evol. Microbiol.* **2014**, *64*, 812–818. [[CrossRef](#)] [[PubMed](#)]
42. Chen, H.; Hao, S.; Chen, Z.; O-Thong, S.; Fan, J.; Clark, J.; Luo, G.; Zhang, S. Mesophilic and thermophilic anaerobic digestion of aqueous phase generated from hydrothermal liquefaction of cornstalk: Molecular and metabolic insights. *Water Res.* **2020**, *168*, 115199. [[CrossRef](#)] [[PubMed](#)]
43. Lee, J.; Koo, T.; Yulisa, A.; Hwang, S. Magnetite as an enhancer in methanogenic degradation of volatile fatty acids under ammonia-stressed condition. *J. Environ. Manag.* **2019**, *241*, 418–426. [[CrossRef](#)] [[PubMed](#)]
44. McNerney, M.J.; Bryant, M.P.; Hespell, R.B.; Costerton, J.W. *Syntrophomonas wolfei* gen. nov. sp. nov.; an anaerobic, syntrophic, fatty acid-oxidizing bacterium. *Appl. Environ. Microbiol.* **1981**, *41*, 1029–1039. [[CrossRef](#)] [[PubMed](#)]
45. Roy, F.; Samain, E.; Dubourguier, H.C.; Albagnacet, G. *Syntrophomonas sapovorans* sp. nov.; a new obligately proton reducing anaerobe oxidizing saturated and unsaturated long chain fatty acids. *Arch. Microbiol.* **1986**, *145*, 142–147. [[CrossRef](#)]
46. Lei, Y.; Sun, D.; Dang, Y.; Chen, H.; Zhao, Z.; Zhang, Y.; Holmes, D.E. Stimulation of methanogenesis in anaerobic digesters treating leachate from a municipal solid waste incineration plant with carbon cloth. *Bioresour. Technol.* **2016**, *222*, 270–276. [[CrossRef](#)] [[PubMed](#)]
47. Wang, T.; Zhang, D.; Dai, L.; Dong, B.; Dai, X. Magnetite triggering enhanced direct interspecies electron transfer: A scavenger for the blockage of electron transfer in anaerobic digestion of high-solids sewage sludge. *Environ. Sci. Technol.* **2018**, *52*, 7160–7169. [[CrossRef](#)] [[PubMed](#)]
48. Nesbø, C.L.; Bradnan, D.M.; Adebuseyi, A.; Dlutek, M.; Petrus, A.K.; Foght, J.; Doolittle, W.F.; Noll, K.M. *Mesotoga prima* gen. nov.; sp. nov.; the first described mesophilic species of the Thermotogales. *Extremophiles* **2012**, *16*, 387–393. [[CrossRef](#)] [[PubMed](#)]
49. Vrieze, J.D.; Hennebel, T.; Boon, N.; Verstraete, W. *Methanosarcina*: The rediscovered methanogen for heavy duty biomethanation. *Bioresour. Technol.* **2012**, *112*, 1–9. [[CrossRef](#)] [[PubMed](#)]
50. Tongco, J.V.; Jannat, M.A.H.; Kim, S.; Park, S.H.; Hwang, S. Process performance and biogas output: Impact of fluctuating acetate concentrations on methanogenesis in horizontal anaerobic reactors. *Energies* **2025**, *18*, 3120. [[CrossRef](#)]

51. Wang, Z.; Gou, M.; Zheng, Q.; Xu, H.; Melhi, S.; El-Bahy, Z.M.; Elsharkawy, E.R.; Dang, Y.; Qiu, B. Enhanced biogas upgrading by photocatalytic conversion of carbon dioxide to methane by *Methanosarcina barkeri*–cadmium sulfide biohybrid. *Adv. Compos. Hybrid Mater.* **2024**, *7*, 111. [[CrossRef](#)]
52. Rotaru, A.E.; Shrestha, P.M.; Liu, F.; Shrestha, M.; Shrestha, D.; Embree, M.; Zengler, K.; Wardman, C.; Nevin, K.P.; Lovley, D.R. A new model for electron flow during anaerobic digestion: Direct interspecies electron transfer to *Methanosaeta* for the reduction of carbon dioxide to methane. *Energy Environ. Sci.* **2014**, *7*, 408–415. [[CrossRef](#)]
53. Rotaru, A.E.; Shrestha, P.M.; Liu, F.; Markovaite, B.; Chen, S.; Nevin, K.P.; Lovley, D.R. Direct interspecies electron transfer between *Geobacter metallireducens* and *Methanosarcina barkeri*. *Appl. Environ. Microbiol.* **2014**, *80*, 4599–4605. [[CrossRef](#)] [[PubMed](#)]
54. Zhou, J.; Smith, J.A.; Li, M.; Holmes, D.E. Methane production by *Methanothrix thermoacetophila* via direct interspecies electron transfer with *Geobacter metallireducens*. *mBio* **2023**, *14*, e00360-23. [[CrossRef](#)] [[PubMed](#)]

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