Granular activated carbon stimulates biogas production in pilot-scale anaerobic digester treating agro-industrial wastewater

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Abstract

This work examines the continuous addition (5 g/L) of conductive granular activated carbon (GAC) in an integrated pilot-scale unit containing an anaerobic digester (180 L) and an aerobic submerged membrane bioreactor (1600 L) connected in series for the treatment of agro-industrial wastewater. Biogas production increased by 32% after the addition of GAC. *Methanosaeta* was the dominant methanogen in the digester, and its relative abundance increased after the addition of GAC. The final effluent after post-treatment with the aerobic membrane bioreactor had a total solids content less than 0.01 g/L and a chemical oxygen demand between 120-150 mg/L. A simple cost analysis showed that GAC addition is potentially profitable, but alternatives ways of retaining the GAC in the system need to be found. Overall, this study provides useful scientific data for the possible application of GAC in full-scale biogas projects.

Keywords: *archaea*, conductive materials, direct interspecies electron transfer, manure, membrane bioreactor,

1. Introduction

Global climate goals are encouraging more and more political authorities and policy makers to take more initiatives as far as investment in energy derived from renewable sources is concerned. Increased participation of renewable energy into the energy mix supports energy security, protects the environment, and mitigates the effect of climate change. The conversion of organic matter into a renewable energy source (biogas/methane) via anaerobic digestion (AD) is gaining momentum.

A category of raw material that can be used as a feedstock in AD is waste generated from the agro-industrial sector. The fact that these types of waste can cause high levels of pollution, and that food production has to meet increasing demand as population grows, highlight the necessity for proper management. For example, the co-digestion of manure with other agro-industrial wastewaters, such as dairy wastewater and olive mill wastewater (OMW), has been examined in the past (Scano et al., 2021). It seems, that co-digestion could (1) provide a better nutrient balance, and (2) dilute possible toxic compounds, such as ammonia in manure and phenols in OMW, resulting in increased biogas production (Rubio et al., 2022)

During the AD process, microbial communities share electrons among themselves, creating a set of interactions, known as “interspecies electron transfer” (IET) (Kato et al., 2012). These sets of interactions contribute to the production of methane with electron transfer from alcohols or fatty acids to CO_2. In this case, hydrogen plays the role of electron carrier, and any problems related to its partial pressure affect the efficiency, and finally the success, of the process (Park et al., 2018). Over the last 15 years, research interest has shifted to another process of electron transfer, *i.e.*, direct interspecies electron transfer (DIET), where electrons are transferred between donors and receivers, without reduced molecules or carriers, such as hydrogen (Gahlot et al., 2020). Conductive materials (CM) can act as electric connection bridges between microorganisms, and promote efficient DIET (Kutlar et al., 2022).

The addition of CM, such as biochar, GAC, carbon-fiber and graphite, accelerates the growth of methanogens (Wu et al., 2020). This accelerated growth takes place because of the high conductivity, the porous structure and the resistance in corroding conditions of the CM (Park et al. 2018). The selection of appropriate CM and its concentration inside the system should deliberately meet several criteria. First, it should be financially viable for both the experimental stage and on a larger scale. Second, it should not cause an excessive rise in solids within the reactor. Third, the environmental impact of the digestate (due to the presence of CM) should be limited.(Nguyen et al., 2021).

The most interesting carbon-based CMs in terms of the efficiency and applicability seems to be biochar and GAC. Both of them can enhance the anaerobic digestion process by a) providing extra surface area to microorganisms b) improving pH buffer capacity, c) absorbing possible toxic compounds and d) promoting DIET (Xu et al. 2022). The cost of biochar is usually lower than that of GAC. However, GAC has significantly higher electrical conductivity, adsorption capacity and specific surface area in comparison with biochar. As a result, its addition in anaerobic reactors may have a completely different effect on the process (Wang et al., 2022).

. Several lab-scale studies regarding the possible addition of GAC (at doses ranging from 1 to 50 g/L) in anaerobic digesters have been conducted, mostly during the last 5 years. For example, Ryue et al. (2019) tested GAC addition (at a concentration of 25 g/L) in biochemical methane potential tests using food waste as feedstock and found that the maximum methane production rate increased by 26% while total cumulative methane production was almost double in comparison with the control (no GAC addition). In another study, He et al. (2021) reported an increase of methane production of about 13% in serum bottles containing GAC (10 g/L) using a mixture of fat, oil, grease and waste activated sludge as feedstock. Other studies focus on the effect of CM on the AD microbial community. Romero et al. (2020) found that the presence of GAC, in an anaerobic reactor treating swine manure, increased *Methanosaeta* abundance by 13.2%. Logan et al. (2022) found that GAC addition enriched the Synergistes class (0.8% to 29.2%) and *Geobacter* genus (0.4% to 11.3%).

Until now, existing studies regarding GAC addition have focused on lab scale experiments. In contrast, to our knowledge, there is only one study (Zhang et al., 2018) at pilot-scale level that examined the supplementation of PAC in a digester for the treatment of food waste. Here, the effect of GAC addition on a pilot scale AD system treating agro-industrial wastewater was investigated (1) to evaluate how the addition of GAC affects the biogas productivity, the characteristics of digestate and the microbial community of the anaerobic reactor and (2) to determine the effect on the downstream process (aerobic MBR). It is expected that this study will provide a foundation for future full-scale projects involving the addition of GAC in anaerobic bioreactors, as well as to make more accurate estimates of expected benefits and limitations.

2. Materials and methods

2.1 Pilot-scale treatment plant

The treatment unit (Figure 1) consisted of a feeding tank (100 L), a continuously stirred anaerobic digester (180 L), a digestate tank for temporary storage of digestate (100 L) and an aerobic MBR for the post-treatment of digestate (1600 L) (see supplementary material). The system included a programmable logic controller (PLC) program, pumps, motors, flow meters, valves, and transmitters to control and monitor the operation. The anaerobic digester was maintained at mesophilic temperatures (34-38 ^{o}C) by recirculating hot water through a double wall. For the start-up of the digester, a mixture of anaerobic inoculum from a full-scale mesophilic anaerobic digester and a pilot-scale up-flow anaerobic sludge blanket reactor (UASB) (treating manure and municipal wastewater respectively) was used. The hydraulic retention time (HRT) in the anaerobic digester was 56 d for the first 104 days (phase I) and was then reduced to 25 d (phase II). At the final phase (lasting 41 days) of the experiment (phase III), GAC (Ravasol GAC B-830, Inaqua, Germany, effective size: 0.8-1.0 mm, apparent density: 475 ± 25 kg/m^3) was added to the digester and in the feedstock (continuously) at a concentration of 5 g/L. This dose was selected in order to be within the range of optimal dosages reported from previous lab-scale experiments. The MBR unit was equipped with a submerged polyethersulfone (PES) flat-sheet membrane module (Microdyn Nadir, Germany). The size of the pores was 0.04 μm while the surface area was 10 m^2. The organic loading rate (OLR) applied in the aerobic MBR during phase II and Phase III was 0.03 kg COD/m^3/d.

2.2 Feedstock

A mixture of OMW, dairy wastewater and sheep manure was used during the experiments. The OMW was obtained during the olive harvest season from a medium-size olive mill located in Mytilene, Greece. Cheese manufacturing wastewater was collected on a biweekly basis from a local cheese-processing factory. Sheep manure was collected from a local sheep farm. Raw materials were added and mixed in the feeding tank every 10-15 days. The mixing ratio used in the experiment was: 15 L of OMW, 10 L of cheese-processing wastewater, 2 kg of sheep manure and 75 L of permeate from the MBR system. The permeate from the MBR was used as re-circulated process water to dilute the high total solids content of sheep manure and the high phenols content (toxic to methanogens) of OMW. The physicochemical characteristics of the agro-industrial wastewater mixture that was fed into the anaerobic digester are presented in Table 1.

2.3 Analytical methods

Samples from the influent and the effluent of the anaerobic digester as well as from the effluent of aerobic MBR were collected every 2-3 days and analyzed for total solids (TS), volatile solids (VS), total COD and dissolved COD according to APHA (2005) using gravimetric and closed reflux method, respectively. The pH of the samples was recorded using a portable pH-meter (C932, Consort), while the turbidity of the final effluent was recorded using a 2100Q portable turbidimeter (Hach). The analysis of total phenols in the feedstock was conducted using the Folin–Ciocalteu method. Biogas production was recorded every 2-3 days using a 2-L gas bag. Specifically, the biogas collected in the bag in a period of 1 hour was measured. Methane content was determined using an Agilent 6890N gas chromatograph supplied with a Thermal Conductivity Detector (TCD) and a capillary column (HP-Plot Q, Agilent, USA). The oven temperature was 40 ^{o}C for the first 1.5 min and then increased gradually (50 ^{o}C/min) to 220 ^{o}C. The temperature of the injector and the detector was set at 200 ^{o}C and 230 ^{o}C, respectively.

2.4 Microbial community analysis

Samples were collected from the anaerobic digester on the last day before the addition of GAC, as well as 38 days after the addition of GAC, and stored at -20°C until DNA extraction. The DNA extraction was performed using bead beating with a PowerLyzer (Qiagen, Venlo, the Netherlands) and phenol/chloroform extraction (De Paepe et al., 2017). Following a quality check through a 1% agarose gel electrophoresis, 10 µL of the DNA extract was sent to LGC genomics GmbH (Berlin, Germany) for library preparation and amplicon sequencing on an Illumina MiSeq platform (V3 chemistry), using the universal primers U341F (5’- CCTAYGGGRBGCASCAG -3’) and U806R (5’- GGACTACNNGGGTATCTAAT -3’) (Sundberg et al. 2013). The amplicon sequence data were processed using the DADA2 R package according to the pipeline tutorial (Callahan et al., 2016). The detailed description of amplicon sequencing as well as amplicon sequencing data processing are described in a previous work (Van Landuyt et al., 2022). The fastq files obtained from this study were submitted to the National Center for Biotechnology Information (NCBI) under the Accession number SRP414262.

2.5 Data analysis

The data were analyzed through one-way analysis of variance (ANOVA) to compare the effect of GAC addition on the performance of the pilot unit. Differences between means were tested for significance ($p<0.05$) by Tukey’s test. The assumptions of ANOVA were checked by the Shapiro-Wilk test (normality) and Levene’s test (homogeneity). Statistical analysis and graphics were performed using OriginPro 2022 (Originlab, USA).

3. Results and discussion

3.1 Performance of the pilot unit

3.1.1 Anaerobic digester performance

Solids and organic matter removal

No significant differences were found in the pH of the digester and in the VS and COD concentrations of its effluents between phase II and phase III (Figure 2). Specifically, pH values in the anaerobic reactor ranged from 7.3 to 7.9 during phase II and from 7.4 to 7.8 during phase III. In both cases, the microorganisms established in the reactor can neutralize the acidity of the incoming influent (mean pH value: 5.9 ± 0.8), resulting in a stable operation within the acceptable pH range for the AD process. The mean VS concentration in the digester was 4.2 g/L in both examined phases (II and III) corresponding to an average VS removal of 48% during the anaerobic digestion process (Table 2). Similarly, the COD concentration decreased from 20.9 ± 7.9 g/L in the inlet to 6.8 ± 1.9 g/L and 7.0 ± 1.2 g/L in the outlet during phase II and phase III, respectively. The average COD removal was 65-66 % in both phases (Table 2). The VS and COD removal performance are in accordance with previous findings in the operation of AD systems using agro-industrial waste and wastewater as feedstock. Thanos et al. (2021) examined a mixture of OMW, pig manure and cheese whey as a feedstock in a pilot-scale anaerobic digester (reactor volume: 220 L) operating at an HRT of 30 days and found a VS and COD removal from 50 to 57 % and 50 to 58%, respectively. A study in Spain (Rubio et al., 2022) found that the average VS and dissolved organic carbon removal performance in a lab-scale anaerobic digester treating a feedstock containing two-phase olive mill waste and cow manure (HRT: 30 d) were 45% and 70%, respectively.

To our knowledge, there is no data regarding the effect of GAC addition on VS and COD removal efficiency in anaerobic digesters treating agro-industrial wastewater in general.

Previous lab-scale studies with other types of feedstocks reported contradictory results. The addition of GAC, biochar and carbon cloth seems to increase VS and COD removal in batch reactors treating municipal biowaste or sewage sludge (Dang et al., 2017; Guo et al., 2022). In contrast, a recent article on the effect of carbon cloth addition in an anaerobic digester treating source-separated organic waste reported no significant effects on VS and COD removal similar to this study (de Albuquerque et al., 2022).

Biogas Production

The mean biogas production rate increased from 24.7 ± 6.8 L/d before GAC addition to 32.7 ± 8.9 L/d after GAC addition (Figure 3), corresponding to an average increase of 32%, though this increase was not significant (p-value = 0.08). The methane content in the biogas also increased from 61.8 ± 1.1% before addition to 68.6 ± 2.2% after addition. The methane yield in correlation with COD added was 95 mL/gCOD$_{added}$/d during phase II and 158 mL/gCOD$_{added}$/d during phase III. Current knowledge regarding the effect of the addition of activated carbon on methane productivity at pilot scale level is very limited. Only the Zhang et al. (2018) examined the addition of PAC (15 g/L) in a 700-L mesophilic anaerobic digester treating food waste. They reported an increase of methane yield by 41% after the addition of PAC. In addition, there are several studies that tested GAC addition in lab scale experiments (Xu et al., 2022). The great majority of these works was conducted in serum bottles under batch operation using different feedstocks, such as sewage sludge (He et al., 2021), food waste (Ryue et al., 2019), domestic wastewater (Park et al., 2020), manure (Romero et al., 2020) and pharmaceutical wastewater (Dai et al., 2022). The dosage used was between 1 g/L and 25 g/L. Recently, Dang et al. (2022) examined the continuous operation of a 1L UASB reactor supplemented with GAC using blackwater as feedstock. In these lab-scale experiments, a positive effect of GAC addition on methane production was recorded, with an increase of between 8 and 26%.

The possible mechanisms which could be related to the enhancement of methane production due to GAC addition in this study are illustrated in Figure 4. The increase of methane production in the digesters supplemented with GAC is often related to the promotion of the DIET process, due to its high electrical conductivity, which can substitute e-pili for electron transfer (Zhang et al., 2020). It is reported that the electrical conductivity of GAC is 100-fold to 1000-fold higher than the electrical conductivity of e-pili (Kutlar et al., 2022) while its electrochemical characteristics could permit long -distance electron exchange (Jin et al., 2022). Liu et al. (2012) proved that the high conductivity of GAC allows the electron exchange between microorganisms. As a result, the increased methane production in an anaerobic digester was related to the accelerating electron transfer to methanogens (*Methanosaeta sp.* or *Methanosarcina sp.*).

Other characteristics of GAC may also play an important role in the enhanced methane production.For example, the porous structure of GAC promotes the establishment of biofilms on its surface, resulting in fostered syntrophic interactions between microbes and significant changes in the established microbial community composition (Dai et al., 2022). Results have shown that GAC promote the enrichment of both electro-active bacteria and methanogens on its surface (Xiao et al., 2022). Moreover, the enrichment of acetoclastic methanogens on the surface of GAC could enhance the methane production without the presence of DIET.

GAC is known to have the ability to absorb toxic compounds, due its high absorption capacity (Xiao et al., 2022). In this study, a possible inhibition of the methanogenesis may occur, due to the presence of ammonia in sheep manure or/and phenolic compounds in OMW. Previous studies have shown that GAC reduced the concentration of ammonia (Chowdhury et al., 2019) and phenols (Bertin et al., 2010) in anaerobic digesters, resulting in increased methane productivity. Specifically, Chowdhury et al. (2019) reported that the reduction of ammonia concentration in an anaerobic digester treating fat, oil, grease and food waste may be related to the adsorption of ammonia in GAC pores. Moreover, a common problem for the anaerobic digestion of OMW is the presence of phenols which inhibits the methanogenesis step. Jiang et al. (2021) found that GAC adsorbs 67-76% of phenols (in terms of peak area) contained in a sludge hydrolysate resulting in a significant acceleration of methanogenesis. It should be also mentioned that carbon materials such as GAC could also adsorb the hydrogen produced by oxidizing-bacteria then consumed by the biofilm of hydrogenotrophic methanogens. As a result, the partial pressure of hydrogen in the digester could be reduced.

The GAC dosage used in this pilot-scale study (5 g/L) was within the range of dosages examined in previous lab-scale studies (1-50 g/L). Contradictory results regarding the effect of the dosage on methane productivity were reported in the past (Kutlar et al., 2022). Some researchers found that the application of doses higher than 10 g/L enhanced the methanogenesis (He et al., 2021), while others reported that higher doses had no effect on the process (Zhang et al., 2018). The application of high doses may affect the existing mechanical equipment of full-scale AD plants. In this study, the addition of GAC (5 g/L) twice caused blockage of the feeding system, due to higher density of GAC, which tended to settle in the feeding tank.

3.1.2 Aerobic MBR performance

The average pH in the effluent of the MBR during phase II and III was significantly higher (p-value < 0.0001) in comparison with the pH in the digestate (Table 2, Figure 5). A similar slight increase of pH was also observed in previous work regarding the operation of MBRs for liquid digestate treatment (Lee et al., 2021). The pH increase during the aerobic treatment of the digestate may be correlated with the biodegradation of volatile fatty acids (Mohamed et al., 2008). The mean turbidity in the oulet of the MBR was the same during phase II and phase III (Figure 5). Despite the removal of solids by ultrafiltration, the permeate was still dark colored. Several organic compounds which are usually present in the effluent, such as humic substances, seems to be responsible for the colored effluent (Fernandes et al., 2020). This will be a notable problem for its final disposal in the environment or its utilization for microalgae cultivation.

Solids removal in the effluent of MBR was very efficient (> 99%). High removal efficiency was also observed for COD (96% during phase II and 98% during phase III), as presented in Table 2. The mean COD concentration in the permeate was 123 ± 10 mg/L during phase II and 152 ± 15 mg/L during phase III, which was significant lower (p-value <0.0001) than the influent (6776 ± 1910 mg/L and 6956 ± 1223 mg/L, respectively). The results regarding COD removal are in accordance with the literature. Specifically, Gong et al. (2010) examined the efficiency of an anoxic/aerobic MBR to treat the effluent of an anaerobic reactor (feedstock: manure) and found a COD removal of about 96%. Similarly, Lee et al. (2021) reported a COD removal of 96% from an aerobic MBR treating food-waste liquid digestate. The occurrence of GAC in the anaerobic digestate seems to have a slightly negative effect on COD concentration in the permeate. Previous works showed that the direct addition of PAC in aerobic MBRs enhanced the performance of the systems, due to the absorption of organic matter (Lin et al. 2011). However, in this work, the GAC was added in the anaerobic digester and in the feeding tank. Some of absorbed organic compounds could be released when the GAC was transferred to the aerobic MBR. The increase of pH during phase III could promote the desorption of the organic compounds from the surface of GAC (Feng et al., 2020).

3.2 Microbial community in the anaerobic digester

The microbial community was dominated by *Methanosaeta* genus in the pilot reactor, and their relative abundance increased from 18.4% in phase II to 25.2% in phase III. Similar, the relative abundance of *Methanospirillum* species increased due to GAC supplementation (phase II: 3.1%, phase III: 4.6%). The most abundant bacterial genera were Family Bacteroidetes *vadinHA17, DMER64,* Family Synergistaceae, *RBG-16-49-21, Christensenellaceae R-7* and *Anaerovorax* (Figure 6). The addition of GAC resulted in a considerable increase of DMER64 (from 3.1 % to 4.6%), SBR1031 (from 0.6% to 2.7%) and SB-5 (from 0.9% to 1.4%). Regarding, the phylum, the relative abundance of *Bacteriodota* and *Firmicutes* decreased after GAC addition, while *Chloroflexi* increased.

The increase of *Methanosaeta* species may be related to the promotion of DIET, as these species can directly accept electrons from electro-active bacteria or conductive materials (Rotaru et al., 2014). Several previous lab-scale studies reported increased *Methanosaeta* population in GAC-amended anaerobic digesters (Park et al., 2018; Kutlar et al. 2022). Specifically, Romero et al. (2020) found that the relative abundance of *Methanosaeta* was enhanced by 13% in batch reactors containing GAC in comparison with controls. Zhang et al. (2020) examined the effect of GAC supplementation in anaerobic UASB digester treating synthetic domestic wastewater. They found that GAC addition resulted in a 16% increase of *Methanosaeta. Methanosaeta* species are mainly acetate-consuming methanogens.They can, however, also reduce CO_2 to methane using electrons provided via conductive materials

(Rotaru et al., 2014), which could help to explain the higher methane content found in biogas during phase III in comparison with phase II.

In terms of bacteria, the promotion of the DIET process in AD is often linked with the enrichment of *Geobacter* (Nguyen et al., 2019). Nevertheless, this observation was mainly reported in experiments conducted with single substrates, while in several other studies they are present in low relative abundance or absent (Kutlar et al., 2022), similar with this work. Other bacteria may also participate in DIET. For example, the increased population of *DMER64* after the addition of GAC could be an indication of DIET promotion. Lee et al. (2019) stated that *DMER64* (phylum *Bacteriodota*) could establish magnetite-mediated DIET with *Methanosaeta*. Moreover, Wang et al. (2019) concluded that *Choroflexi* could act as electron-donating partner in DIET during AD of sucrose with the supplementation of conductive magnetite. In this study, the microbial communities of genus SBR1031 and in general of class of *Anaerolineae* (phylum *Chloroflexi*) increased 4.5-fold and 2.3-fold, respectively, during AD with GAC supplementation. The possible electro-activity of *Choroflexi* was firstly stated by Blanchet et al. (2014), as it was the dominant phylum of electroactive biofilms in electrochemical reactors fed with acetate. In that work, *Anaerolinea sp.* was identified on the surface of electrodes.

3.3 Simple cost analysis

A full-scale anaerobic digester with a typical volume of 2.500 m^3 at a GAC dose of 5 g/L and an HRT of 25 days (as this study) will require 182.5 tonnes of GAC per year plus 12.5 tonnes in the reactor at the beginning. The cost of GAC used in this study was 3.2 US$/kg. Therefore, the cost for GAC addition in the digester will be 585,825 US$/per year (plus 40,125 US$/ at the beginning). According to this study, the excess of methane produced from the pilot-scale digester (volume: 0.18 m^3) due to GAC addition was 7.16 L/d or 39.8 L/m^3 reactor/d. As a result, it is estimated that the excess of methane produced in the scenario of the full-scale reactor will be 36,290 m^3 per year. Assuming that the inferior calorific power of methane is 9.89 kWh/m^3 (Tiwari et al. 2021), and the electricity produced from a CHP unit is 35%, the addition of GAC will produce 125,617 kWh of electricity per year. For the addition of GAC to show profitability, the price of electricity must be higher than 5.09 US$//kWh. This price is far away from current electricity prices all over Europe (0.064-0.488 US$//kWh) (https://ec.europa.eu/eurostat/statistics-explained/index.php?title=Electricity_price_statistics). Despite the large number of articles published in the past regarding conductive materials addition in AD, the economic feasibility of the process is rarely mentioned. Tiwari et al. (2021) examined GAC and biochar addition in lab-scale digesters and stated that the addition of these materials is not economically feasible at full-scale similar with this study. The retention of GAC or any other conductive material in AD is crucial for the successful application of this promising technology. This could be achieved with the use of anaerobic membrane bioreactors, up-flow anaerobic sludge blanket reactors or newly developed conductive biocarriers.

Conclusions

The addition of GAC resulted in notable increase in biogas production and methane content. The use of an aerobic MBR for the post-treatment of digestate could achieve high removal efficiencies for solids and COD. The addition of GAC in the anaerobic digester resulted in considerable changes in the microbial community of the reactor, with an increased relative abundance of the archaea, which explains the increased biogas production. The enrichment of *Methanosaeta* could indicate the promotion of DIET. A simple cost-analysis showed that this process will be feasible, only if the GAC is retained in the digester.

"E-supplementary data of this work can be found in online version of the paper."

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564 **Table 1.** Characteristics of agro-industrial wastewater mixture during the entire experiment

Parameter	Mean values ± standard deviation (Number of samples)
pH	5.9 ± 0.8 (19)
Total Solids (g/L)	11.9 ± 2.3 (14)
Volatile Solids (g/L)	8.4 ± 2.1 (14)
COD total (g/L)	20.9 ± 7.9 (17)
Phenols (mg/L)	192 ± 13 (8)

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Table 2. Effluent quality and average removal performance (in parentheses) of the pilot unit during the experiment. ND = Not determined. NA – Not applicable. The values presented after slash indicates the number of samples.

Parameter	Anaerobic digester		Aerobic MBR	
	Phase II	Phase III	Phase II	Phase III
pH	7.6 ± 0.2/15	7.7 ± 0.1/8	8.1 ± 0.2/10	8.5 ± 0.2/9
TS (g/L)	6.9 ± 1.9 (40)/11	6.4 ± 1.1 (44)/7	<0.01 (>99)/10	<0.01 (>99)/9
VS (g/L)	4.2 ± 1.5 (48)/11	4.2 ± 1.2 (48)/7	<0.01 (>99)/10	<0.01 (>99)/9
COD (mg/L)	6,776 ± 1,910 (66)/11	6,956 ± 1,223 (65)/7	123 ± 10 (96)/10	152 ± 15 (98)/9
Turbidity (FNU)	ND	ND	1.5 ± 0.3/10	1.5 ± 0.1/9
Biogas (L/d)	24.7 ± 6.8/8	32.7 ± 8.9/8	NA	NA
Methane content (%)	61.8 ± 1.1/8	68.6 ± 2.2/8	NA	NA

MBR: membrane bioreactor, TS: Total Solids, VS: Volatile Solids, COD: Chemical Oxygen Demand, FNU: Formazin Nephelometric Unit,

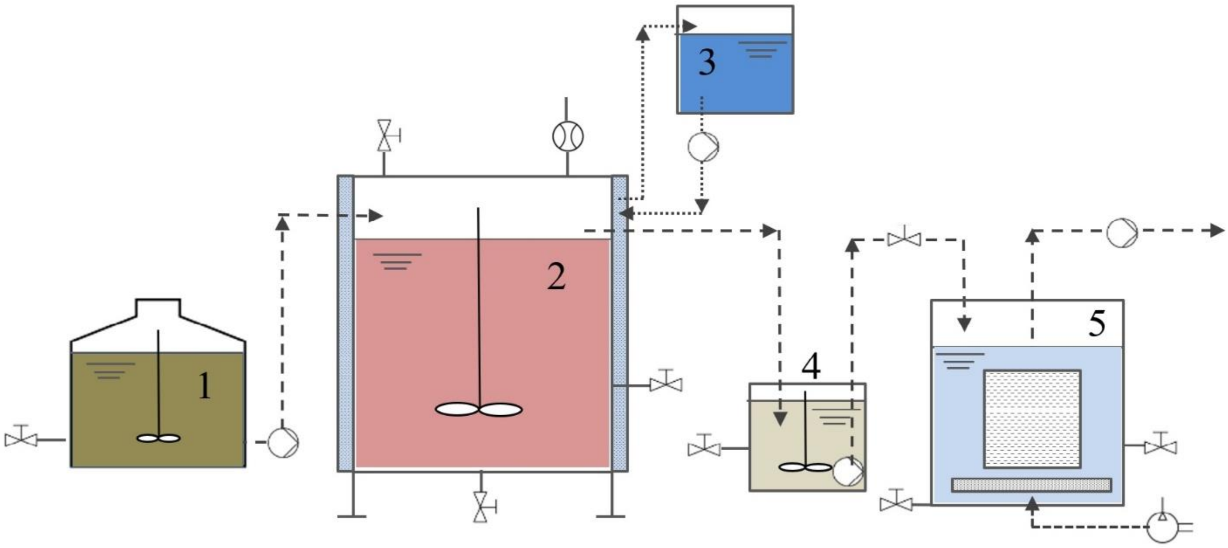

Figure 1. Schematic presentation of the pilot-scale treatment plant used in this study. 1: feeding tank, 2: anaerobic digester, 3: water heater tank, 4: digestate tank, 5: aerobic membrane bioreactor

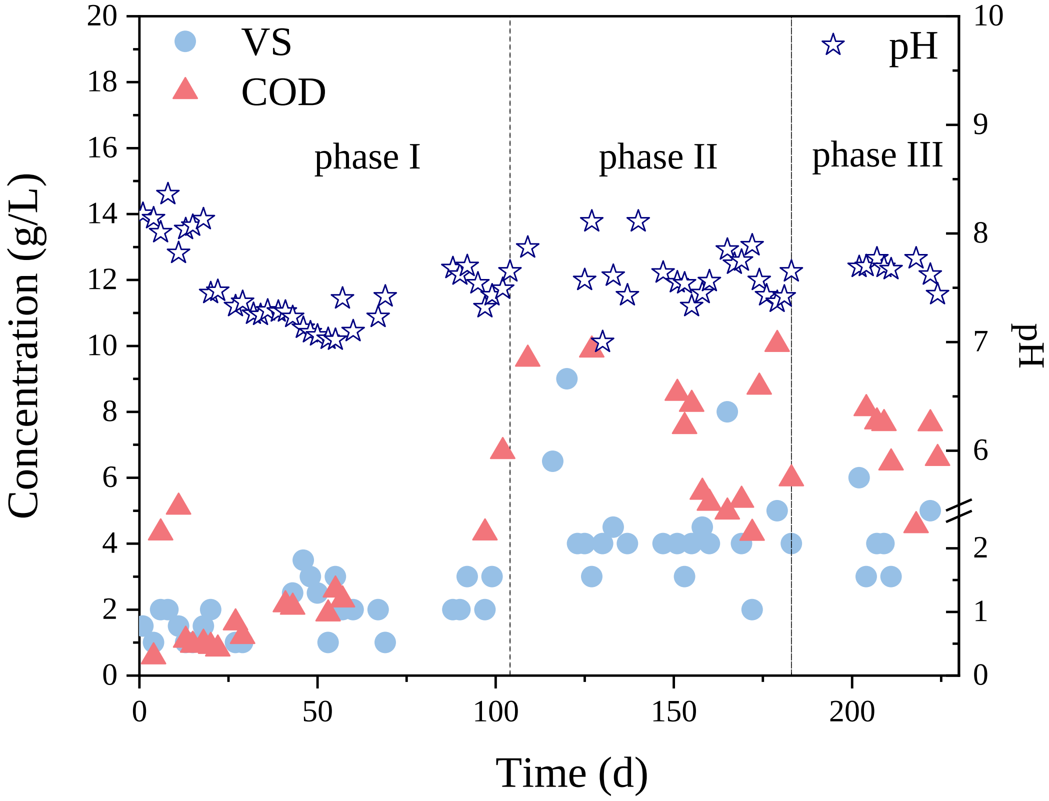

Figure 2. Volatile solids (VS), total chemical oxygen demand (COD) and pH variation in the anaerobic digester during phase I (start-up), phase II (without granular activated carbon (GAC)) and phase III (with GAC).

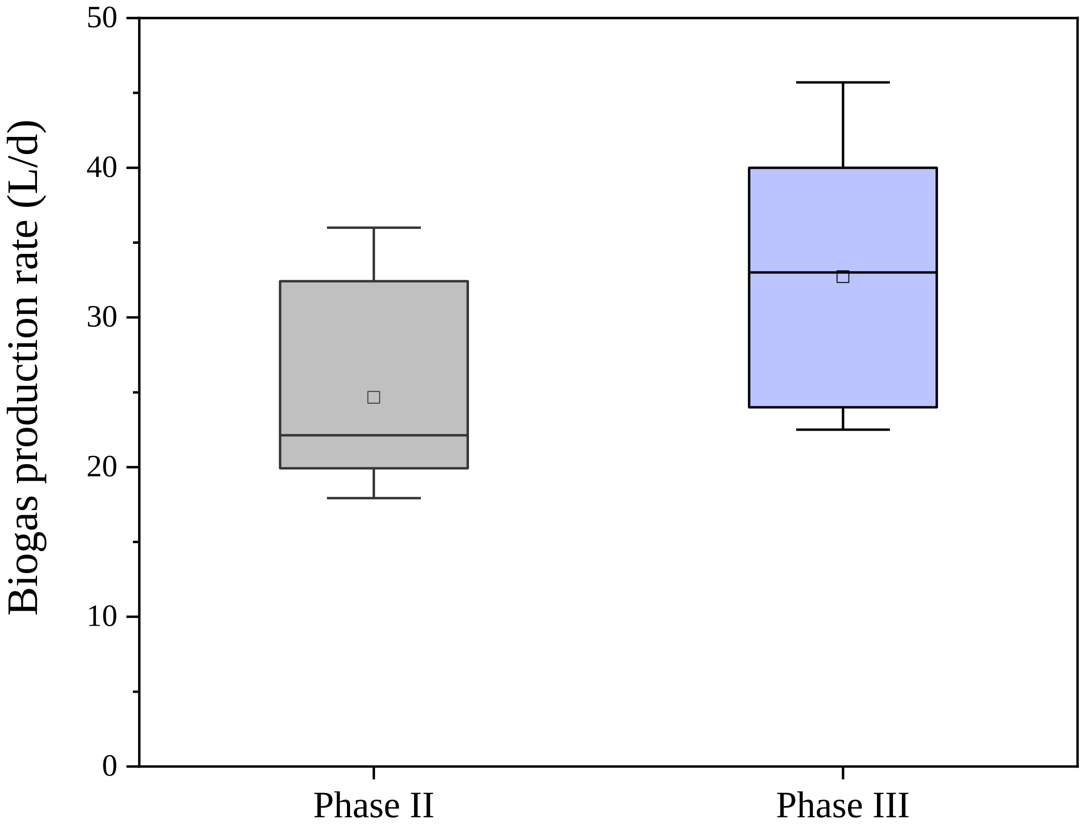

Figure 3. Biogas production during Phase II and Phase III of the experiment. Boxes represent median and lower and upper quartiles, while square points inside the box represent the mean values and whiskers represent 1.5× interquartile range. Number of samples: 8

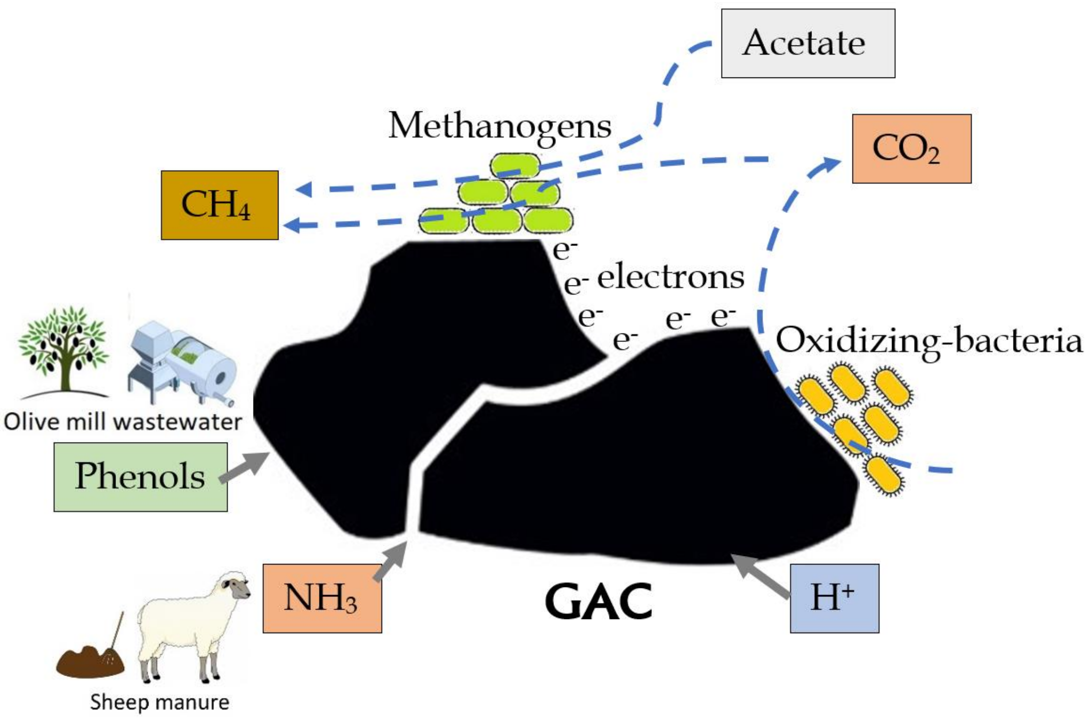

Figure 4. Possible effects of granular activated carbon (GAC) on the process: a) enrichment of electroactive bacteria and promotion of direct interspecies electron transfer (DIET), b) enrichment of methanogens (acetoclastic or/and hydrogenotrophic) on its surface and c) adsorption of toxic compounds (hydrogen, ammonia, and phenols).

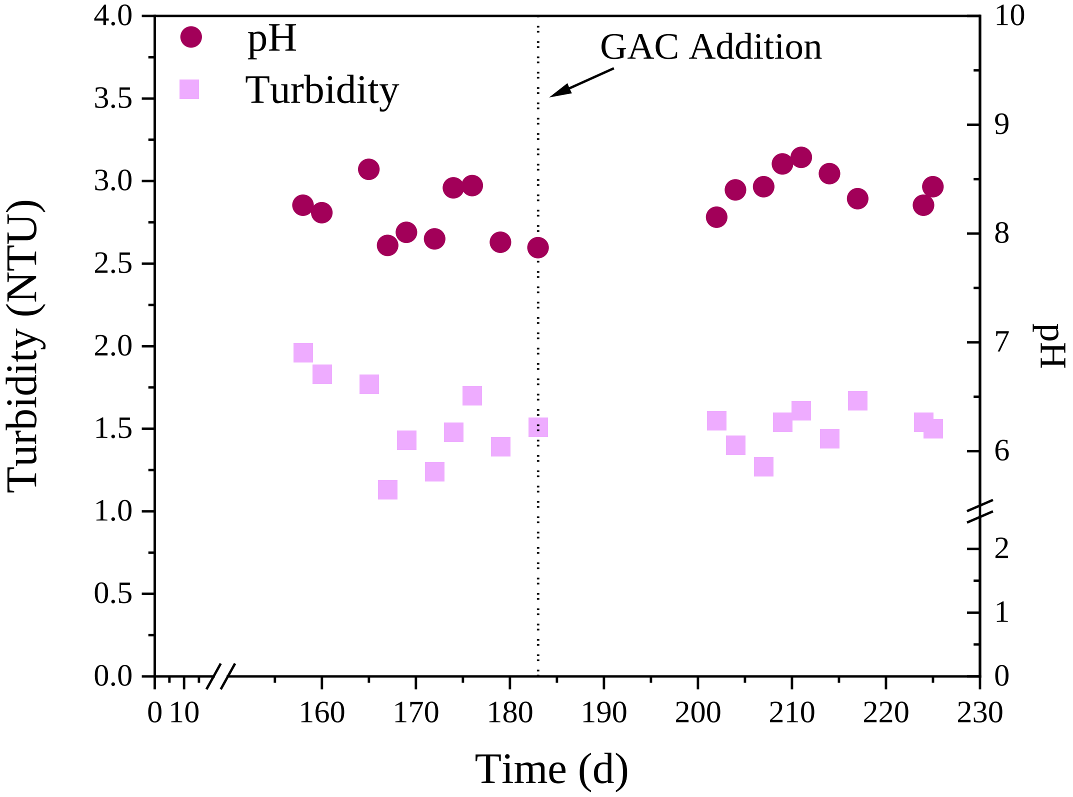

Figure 5. The pH and volatile solids variation in the inlet and the outlet of aerobic membrane bioreactor (MBR) during phase II and phase III of the experiment. NTU: Nephelometric Turbidity Units

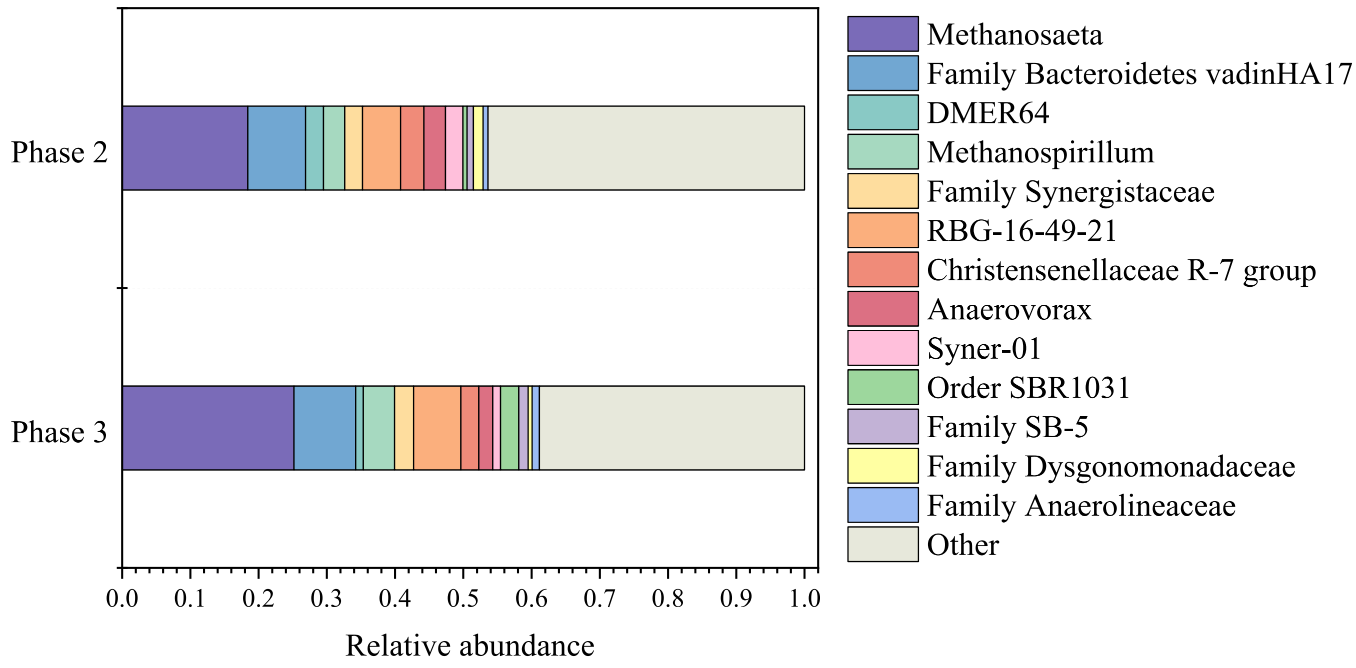

Figure 6. Taxonomic classification of the microbial community at the end of phase II (without granular activated carbon addition) and phase III (with granular activated carbon addition).