BioEnergy Research (2023) 16:2168–2177
https://doi.org/10.1007/s12155-023-10593-8

Enhancement of Water Hyacinth Juice Treatment in an Anaerobic Sequential Batch Reactor with Coffee Husk–Derived Biochar

Daiki Ogasawara Maruyama[1] · Shin-ichi Akizuki[2] · Mutsumi Sekine[3] · Akinori Fujita[1] · Nigus Gabbiye Habtu[4] · Shinjiro Sato[3] · Tatsuki Toda[1,3]

Received: 1 December 2022 / Accepted: 30 March 2023 / Published online: 4 May 2023

Abstract

The proliferation of water hyacinths is a global issue with significant environmental and social implications, and its proper management is a critical issue. Anaerobic digestion (AD) of compressed water hyacinth juice (WHJ) is key to efficiently utilizing water hyacinth biomass, but a simpler and more cost-effective method has yet to be established. In this study, the effectiveness of biochar carriers derived from local waste biomass (i.e., coffee husk) for WHJ treatment was evaluated in a sequential batch reactor. This was compared to conventional AD carriers (polyurethane sponge) and no-carrier conditions. The no-carrier condition resulted in process failure after 40 days due to the accumulation of volatile fatty acids from the substrate overload. In contrast, the biochar condition showed a significant CH_4 yield (472 mL/g-VS) and total organic carbon removal (88.6%), comparable to the sponge carrier condition. Scanning electron microscope observation revealed an aggregation of mainly rod-shaped microorganisms in the biochar pores, indicating biofilm formation and a rise in microbial concentration. Nano-archaea (*Candidatus Diapherotrites* archaeon ADub.Bin253), which have a symbiotic relationship with methanogens, were detected, particularly in carrier-filled conditions, with a relative archaea abundance of 12.9–28.6%. This study highlights the effectiveness of using coffee husks to treat WHJ, which can both exist in the same region, and suggests an alternative way of using locally generated biomass for local waste treatment.

Keywords Semicontinuous reactor · Biofilm carrier · Nano-archaea · Microbial immobilization · Agricultural waste

Introduction

Water hyacinths (*Eichhornia crassipes*) are floating aquatic weeds native to South America that are widely cultivated and used as ornamental throughout the world because of their beautiful light-purple flowers. On the other hand, due to human runoff into the environment and how incredibly fast they multiply, they have overgrown in rivers and lakes and are now a global issue [1]. This overgrowth hinders fishing and increases poverty [1], blocks waterways and inhibits agricultural activities [2], and has a serious impact on aquatic ecosystems by displacing native vegetation and reducing dissolved oxygen concentration [3]. The large population of water hyacinths in the environment and their rapid growth rate necessitate the development of low-cost long-term control strategies.

Our research group has developed an efficient method to utilize overgrown water hyacinths (Japanese Patent No. 7204263). This method involves crushing and compressing the water hyacinths, recovering biogas from compressed water hyacinth juice (WHJ) using anaerobic digestion (AD), and producing biochar from compressed solid residue for fuel and soil conditioning. The AD effluent can be used for purposes such as growing nutritional microalgae and vegetables using hydroponics. To reduce the size of the AD

✉ Daiki Ogasawara Maruyama
daikimaruyama@soka.gr.jp

1 Laboratory of Restoration Ecology, Graduate School of Science and Engineering, Soka University, 1-236 Tangi-machi, Hachioji, Tokyo 192-8577, Japan

2 Institute of Plankton Eco-engineering, Soka University, 1-236 Tangi-machi, Hachioji, Tokyo 192-8577, Japan

3 Department of Science and Engineering for Sustainable Innovation, Faculty of Science and Engineering, Soka University, 1-236 Tangi-machi, Hachioji, Tokyo 192-8577, Japan

4 Faculty of Chemical and Food Engineering, Bahir Dar Institute of Technology, Bahir Dar University, P.O. Box 26, Bahir Dar, Ethiopia

reactor and the operating costs, it is necessary to treat WHJ as quickly as possible. Previous studies have confirmed that an up-flow anaerobic sludge blanket reactor with internal circulation is effective for WHJ treatment [4, 5]. However, the installation of these complex reactors in developing countries may be a challenge due to their high cost [6]. Therefore, the development of simpler and economical methods is needed.

One potential method is to add biochar carriers to the reactor to improve AD treatment performance. Biochar, which is produced from biomass pyrolysis, is a promising option due to its high performance and low production cost [7]. The high porosity and adsorption capacity of biochar can support microbial growth, toxic substance removals, and buffer pH levels [8]. Additionally, the filling of carriers can promote early biofilm formation [9], which can increase microorganism density [10] and protect them from adverse environments, such as acidic conditions [11]. Carrier features have been used in various types of reactors [10] and are highly applicable to existing reactors. Coffee husk is a suitable raw material for producing biochar, as they are readily available in tropical and subtropical countries [12, 13]. Coffee husks are waste products generated in large quantities during coffee production, and their disposal, processing, and possible recycling are important environmental and economic concerns [14].

Using coffee husk-derived biochar carriers to enhance AD treatment of WHJ is an important initiative from the viewpoint of making effective reuse of the overgrown water hyacinth themselves locally. This approach has several benefits, including easy introduction into local digesters, reducing purchase and transportation costs for carriers compared to the use of conventional commercial carriers, and reducing environmental impact from coffee husk disposal. However, research on the use of carriers in treating WHJ is limited and requires further investigation. This study aims to evaluate the performance of WHJ treatment using coffee husk–derived biochar carriers in a sequential batch reactor (SBR).

Materials and Methods

Substrate and Inoculum Preparation

The water hyacinths were collected from swamp fields in November 2020 in Kazo City, Saitama Prefecture, Japan. The whole plant of water hyacinth (i.e., leaves, stem, and roots) was then shredded and compressed by a milling and dewatering machine (RSC-2500MC/RSC-250S, R-ing Co. Ltd, Japan), and the WHJ was filtered through a 106-µm mesh and stored in a refrigerator at 4 °C. The filtered WHJ had the following characteristics: pH 4.02 ± 0.06, 12,878 ± 3139 mg/L of suspended solid (SS) concentration, 9818 ± 161 mg/L of total organic carbon (TOC) concentration, 29,617 ± 369 mg/L of chemical oxygen demand (COD) concentration, 35,093 ± 384 mg/L of total solid (TS) concentration, 26,013 ± 380 mg/L of volatile solid (VS) concentration, 257.6 ± 5.4 mg/L of total nitrogen (TN) concentration, 38.1 ± 0.7 of carbon-to-nitrogen (C/N) ratio, and 3538 ± 27 mg/L of volatile fatty acid (VFA) concentration.

Mesophilic anaerobic sludge was collected from a full-scale anaerobic digester that treated dewatered sewage sludge at the Hokubu Sludge Treatment Center in Yokohama City, Kanagawa Prefecture, Japan. Then, the sludge was stored in a temperature-controlled room at 37 °C for 10 days to remove residual organic compounds from the sludge [15]. The SS and volatile SS concentrations in the sludge were 16,542 and 12,594 mg/L, respectively.

Biofilm Carrier Preparation

Two different biofilm carriers (biochar and sponge) were prepared in this study. The coffee husks were purchased from the Matayoshi Coffee Plantation in Kunigami District, Okinawa Prefecture, Japan. The coffee husks were dried at 105 °C for 24 h and then pyrolyzed in a muffle furnace at a heating rate of 5 °C/min to 600 °C with a retention time of 2 h. For the operation of the muffle furnace, it was the same as Ahmed et al. [16]. The pyrolyzed biochar was washed for 8 h by mixing it with pure water on a shaker at 80 rpm and was used as a biochar carrier. The biochar carrier was sieved to obtain particle sizes of 3.2–5.6 mm. A commercially available polyurethane sponge was used as a control carrier and cut into 5-mm cubes. The cut sponge was washed with pure water and then dried at room temperature and then used as a sponge carrier. Carrier size was determined based on the report of Cayetano et al. [10].

Bioreactor and Operating Conditions

Medium bottles with an effective volume of 2.0 L were used as reactors. Three conditions were set up: Two bottles were filled with different carriers (biochar and sponge), and the third bottle was left without a carrier. The apparent filling rate of the carriers was 20% (v/v) [17]. In the carrier-filled conditions, the apparent volume of each carrier was measured using a 0.5-L female cylinder and then pure water was added to the carrier to bring the volume to 0.4 L, which was transferred to a medium bottle along with 1.6 L of seed sludge. Specific amounts of biochar and sponge carrier were 41 g and 7 g, respectively. In the control condition without a carrier, 0.4 L of pure water and 1.6 L of seed sludge were added in a medium bottle. The experiment was conducted under anaerobic conditions at 37 °C ± 1 °C with shaking at 100 rpm. The SBR operation was conducted by repeating the following processes: 15 min for substrate supply, 21.5

h for reaction, 2 h for settling, and 15 min for effluent discharge (Fig. 1). At the start-up of the experiment, only seed sludge was added to the reactors until the effective volume was filled. The HRT and the OLR were set to 10 days and 2.96 g-COD/L/day, respectively. The produced biogas was collected using aluminum gas bags. The sludge and effluent samples were collected using syringes once every 2 days. The substrate was supplied using syringes daily.

Analytical Methods

The pH was measured using a benchtop pH meter (Seven-Compact pH/Ion meter S220, Mettler Toledo, USA). The concentrations of TS, VS, and SS were measured according to the method defined by the American Public Health Association (2006) [18]. The concentrations of TOC, TN, dissolved organic carbon (DOC), and total dissolved nitrogen were measured using a TOC analyzer (TOC-L CPH/CPN, Shimadzu, Japan). The VFA concentrations were measured using a spectrophotometer (DR-3900, HACH, USA) according to the esterification method (HACH method 8196). The COD concentrations were measured using a spectrophotometer (DR-3900, HACH, USA) in accordance with the USEPA reactor digestion method (HACH method 8000). The volume of the biogas produced was measured using a syringe at room temperature (approximately 25 °C) as the gas was collected in a gas bag. The CH_4 content was measured following the procedure described by Salangsang et al. [19]. The TOC removal efficiency was calculated using the following equation:

$$\text{TOC removal efficiency } (\%) = \frac{\text{TOC}_{\text{in}} - \text{DOC}_{\text{eff}}}{\text{TOC}_{\text{in}}} \times 100 \quad (1)$$

where TOC_{in} is the TOC concentration in the substrate that is supplied to the reactor, and DOC_{eff} is the DOC concentration in the effluent.

The carbon balance was calculated using the following equations:

$$C_{\text{CH}_4}\,(\text{mg}) = \frac{V_{\text{CH}_4} \times C \times K_0 \times 1000}{V \times K_1} \quad (2)$$

$$C_{\text{CO}_2}\,(\text{mg}) = \frac{V_{\text{CO}_2} \times C \times K_0 \times 1000}{V \times K_1} \quad (3)$$

$$C_{\text{Others}}\,(\text{mg}) = \text{TOC}_{\text{in}} - \left(\text{DOC}_{\text{eff}} + C_{\text{CH}_4} + C_{\text{CO}_2}\right) \quad (4)$$

where C_{CH4} is the amount of carbon contained in CH_4 produced in each reactor, V is 22.4 L/mol, which is the volume of 1-mol standard state gas, and V_{CH4} is the volume of CH_4 produced in each reactor. C is the amount of carbon (12 g/mol), K_0 is 273 K, and K_1 is 298 K at room temperature. C_{CO2} is the amount of carbon contained in CO_2 produced in each reactor, and V_{CO2} is the volume of CO_2 produced in each reactor. C_{Others} includes carbon used for microbial growth in the reactor and undegraded substrate carbon retained in the reactor. The experimental period covered for the mass balance calculation was days 35–49.

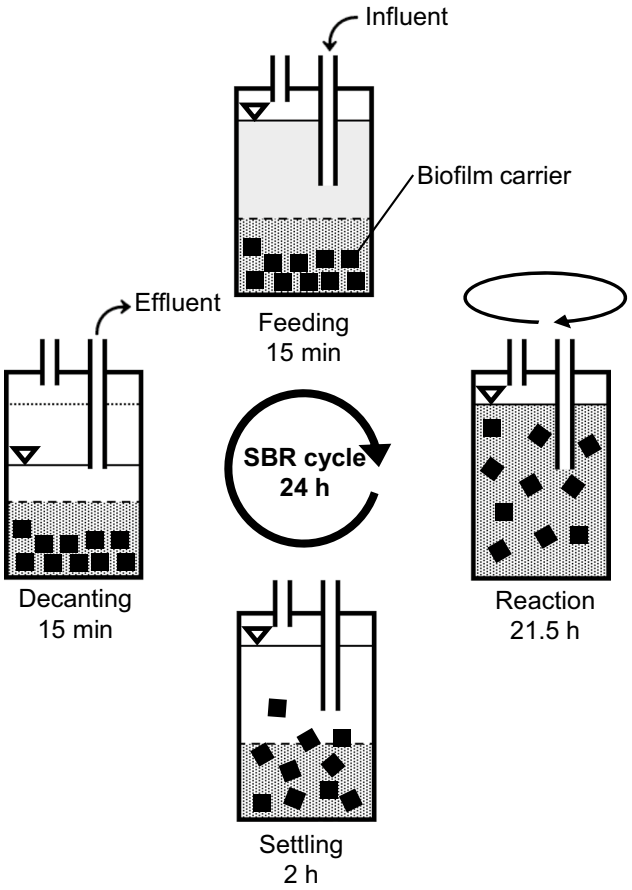

Fig. 1 Schematic of sequencing batch reactor (SBR) operation. This experiment was conducted in reactors (effective volume: 2.0 L) filled with carriers and in reactors without carriers. Biogas is always collected in a gas bag through the port

Scanning Electron Microscope (SEM)

The carrier and the microorganisms on the carrier were observed by a SEM. The microbial carriers were collected from the reactor on day 49 of the experiment and washed with a phosphate buffer solution (pH = 7.2) to remove excess sludge. Afterward, the carriers were fixed overnight in a 2.5% (v/v) glutaraldehyde solution [20]. The samples for SEM were prepared based on freeze-drying [21]. The SEM samples were washed with pure water, dried using a freeze dryer (FD-6510, SUN Technologies), and coated with osmium using a metal coating device (HPC-1S, Vacuum

Device). The micrographs of these samples were taken using a SEM (JSM-7500F, JEOL).

Microbial Community Analysis

The sludge samples were collected from the reactor on day 49 of the experiment and stored at −20 °C until analysis. The DNA was extracted using Extrap Soil DNA Kit Plus ver. 2 (NIPPON STEEL Eco-Tech, Japan) according to the manufacturer's instructions. Library preparation and sequencing analysis were outsourced to Bioengineering Lab Co., Ltd. (Kanagawa, Japan). The 16S rRNA gene V4 region of the extracted DNA sample was amplified through the MiSeq (Illumina, San Diego, USA) sequencing platform. In the Quantitative Insights Into Microbial Ecology 2 software package (2021.11 release), all the effective sequences were grouped into operational taxonomic units (OTUs). Each OTU was classified using a 97% OTU reference 16S rRNA database of Silva (ver. 132).

Statistical Analysis

Substrate and seed sludge characteristics were performed at least three times, and the average values were presented. These results were expressed as mean value ± standard deviation of the carried-out analyses for all the measurements (as shown in the section—"Materials and Methods"). The CH_4 production of the reactors over the experimental period was statistically analyzed by one-way ANOVA followed by the Tukey–Kramer comparison test using Statcel3 software program (OMS publishing, Inc., Japan) [15].

Results and Discussion

Overall Reactor Performance

Reactor performance in terms of pH and CH_4 production rate largely varied depending on the conditions (Fig. 2a and b). The pH value in an AD reactor is used as a simple indicator of process stability, and the appropriate pH range is 6.5–8.2 [22]. In this experiment, the pH values ranged from 6.8 to 7.5 for both the biochar- and sponge-operated reactors throughout the whole experimental period (Fig. 2a). Similarly, the CH_4 production in both carrier-filled reactors was generally stable between 1.0 and 1.3 L/L/day (Fig. 2b). In contrast, the pH and CH_4 production rate in the control condition suddenly dropped around day 40 and continued to decrease until the end of the experiment.

Table 1 presents the average CH_4 production rates during the experiment compared using one-way ANOVA followed by Tukey–Kramer's method. The conditions were compared from day 31, when the operation was relatively stable, until day 49. The control condition had an average CH_4 production of 0.44 ± 0.42 L/L/day, with values fluctuating widely

Table 1 Methane production during stable periods at days 31–49. All values are averages with standard deviation (*n*). Letters represent significant differences within each condition by one-way ANOVA followed by Tukey–Kramer's method ($p < 0.01$)

Conditions	Methane production (L/L/day)
Control	$0.44 \pm 0.42\ (8)^{A}$
Biochar	$1.13 \pm 0.09\ (8)^{B}$
Sponge	$1.08 \pm 0.15\ (8)^{B}$

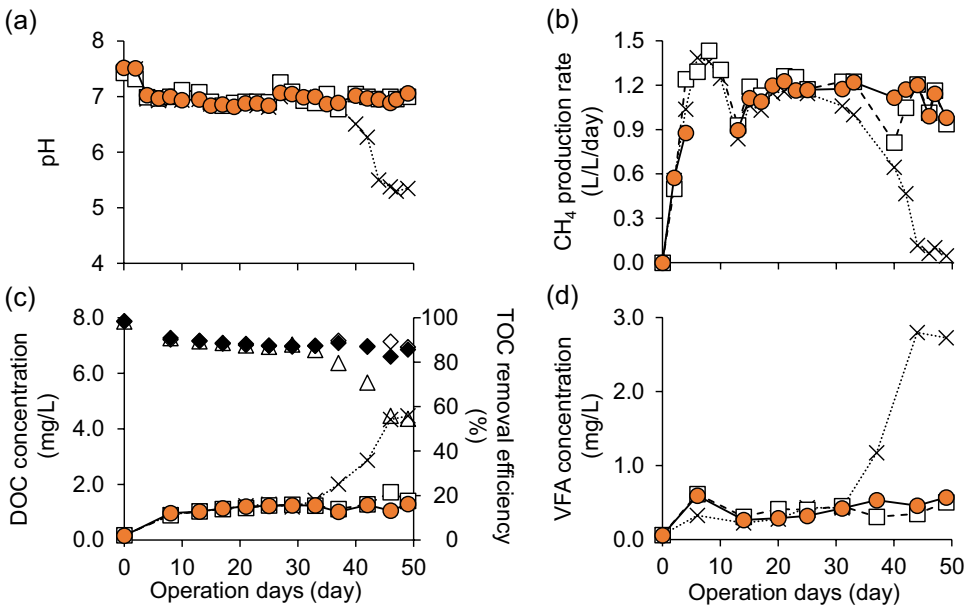

Fig. 2 Time courses of pH (**a**), CH_4 production rate (**b**), DOC concentration of effluent and TOC removal efficiency (**c**), and VFA concentration of effluent (**d**). Orange circle (●) and black diamond (◆): biochar condition; white square (□) and white diamond (◇): sponge condition; black cross (×) and white triangle (△): control condition

throughout the period. In contrast, the biochar and sponge conditions were stable at 1.13 ± 0.09 and 1.08 ± 0.15 L/L/day, respectively, and significantly higher than the control condition ($p < 0.01$). No significant differences were found between the biochar and sponge conditions, indicating that each carrier's performance was almost identical. Although there are no reported studies using coffee husk–derived biochar for AD treatment, the results confirm that biochar performs comparably to other carriers in treating WHJ.

The effluent's DOC concentration was considerably stable at around 1.0–1.2 g/L in the biochar- and sponge-filled conditions, resulting in high TOC removal efficiencies of >80% (Fig. 2c). These TOC removal values were similar to those reported by Liu et al., indicating good reactor start-up and performance [5]. In the control condition, the DOC concentration increased from day 33 onward, decreasing TOC removal efficiency. VFAs are the main contributors to acidification in AD. The pH decreases when the VFA production rate exceeds its decomposition rate and can result in process instability or failure [22, 23]. In the biochar- and sponge-filled conditions, the VFAs did not accumulate and were stable between 0.3 and 0.6 g/L until day 49 (Fig. 2d). In contrast, the accumulation of VFAs began after day 30 in the control condition and increased exponentially until day 44, after which it stabilized.

The carbon mass balance calculated from the results on days 35–49 showed the difference in the performance of each reactor (Fig. 3). In the biochar- and sponge-filled conditions, biogas accounted for ~90% of the total carbon, and the substrate was well converted into gas. In contrast, the control condition showed a high value for the "others" component (36%), indicating inefficient substrate conversion to biogas. These results indicate that biochar and sponge were appropriate carriers for stable AD of WHJ.

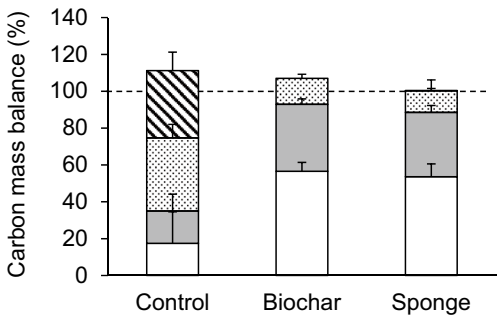

Fig. 3 Carbon mass balance of days 35–49 in this experiment. White bar (□): CH_4; gray bar (▩): CO_2; dotted bar (▨): effluent DOC; hatched bar (▧): others

SEM Observation

The results of the SEM observation of each carrier before experimental use are shown in Fig. 4. The surface of the biochar carrier was covered with numerous latticelike irregularities, and the size of the pores was about 20 µm (Fig. 4a and b). The sponge carrier had very large voids (about 200–600 µm) and a smooth fiber surface (Fig. 4c and d). SEM observation of the carriers on day 49 showed biofilm formation on

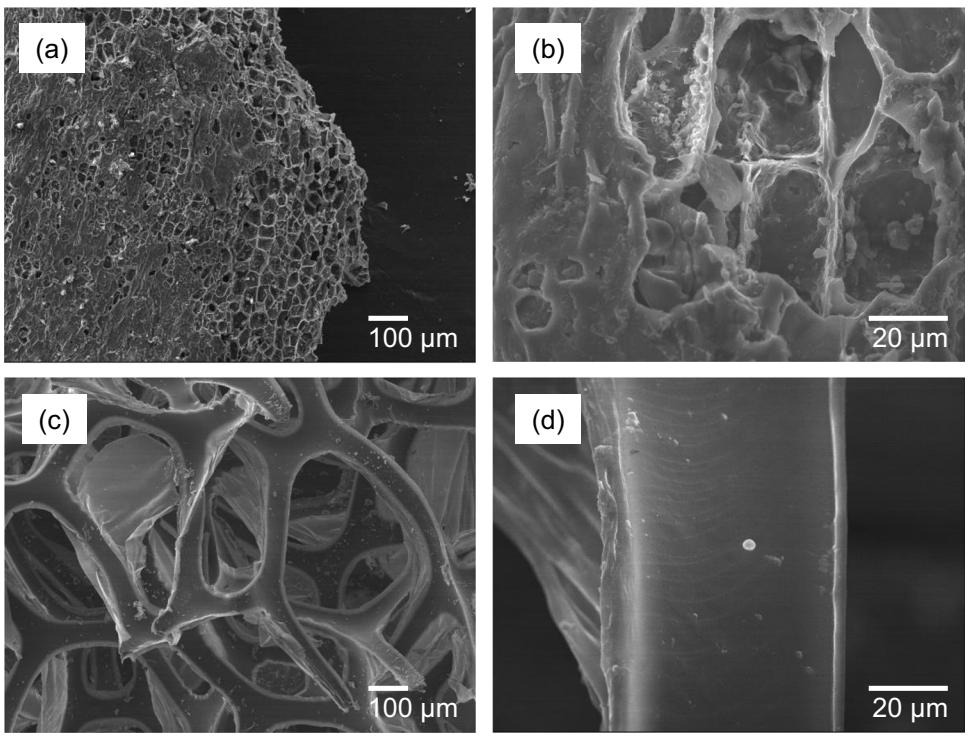

Fig. 4 SEM photos of the carriers before experimental. Low (**a**) and high (**b**) magnification of biochar. Low (**c**) and high (**d**) magnification of sponge

both carriers (Fig. 5). On the surface of the biochar, aggregation of microorganisms was observed, mainly rod-shaped microorganisms in the pores (Fig. 5a and b). In particular, the pores of the biochar held microorganisms. Similarly, Ma et al. [24] reported that the pores of biochar acted as a shelter for microorganisms. Microorganisms and biochar are easily bound by van der Waals forces due to their hydrophobic surfaces [25]. In the sponge carriers, clumps of sludge were held in their voids, and the filamentous microorganisms were agglomerated (Fig. 5c and d). The tight attachment of the microorganisms to the sponge carriers was attributed to the hydrophilic groups and cationic surfactant active groups of polyurethane [26]. Although the microbial aggregation process of the biochar and sponge carriers may have been different, both successfully formed biofilms, indicating high treatment performance.

Factors Affecting Reactor Performance

The control condition experienced acidification due to the high acidity and C/N ratio of the WHJ substrate, which reduced the activity of the methanogenic archaea and acetogenic bacteria responsible for AD. This resulted in the accumulation of VFAs and decreased efficiency of substrate degradation, leading to treatment failure. The pH of WHJ, which was 4.02, was below the suitable range of 6.5–8.2 for microorganisms involved in AD [22]. Additionally, the C/N ratio of WHJ was 38, higher than the recommended range of 20–30 for AD [27]. Substrates with high C/N ratios inhibit microbial growth because there is insufficient nitrogen for microbial cells to function properly, leading to VFA accumulation and reduced pH [28, 29]. The acidity and high C/N ratio of WHJ may have reduced the activity of microorganisms in the reactor, leading to decreased substrate degradation efficiency, growth rate, and eventual failure of the control condition operation.

In contrast, in carrier-filled conditions (biochar and sponge), the biofilm formed on the surface of the carrier plays a crucial role in maintaining high microbial density and protecting microorganisms from unfavorable environments. The biofilm has a microbial density 10 times higher than that of suspended sludge [30], significantly enhancing the efficiency of substrate degradation and VFA conversion to biogas. Furthermore, the biofilm protects the microorganisms from external acidity and other environmental factors through the extracellular polymeric substance matrix, preventing a decline in microbial activity [30]. These biofilm functions greatly contributed to the WHJ treatment performance in this experiment, with pH values consistently around 7 in the biochar and sponge conditions, indicating higher methane production and organic matter removal performance than in the control condition.

Table 2 summarizes the studies on the AD of water hyacinths or WHJ. Compared to other studies, the substrate used in this study had the lowest pH value. Moreover, the C/N ratio of the substrate in this study was 38, while in a previous study, it was 23 [5], which is suitable for AD. Normally, such low pH and high C/N ratio of substrates inhibit AD reactions

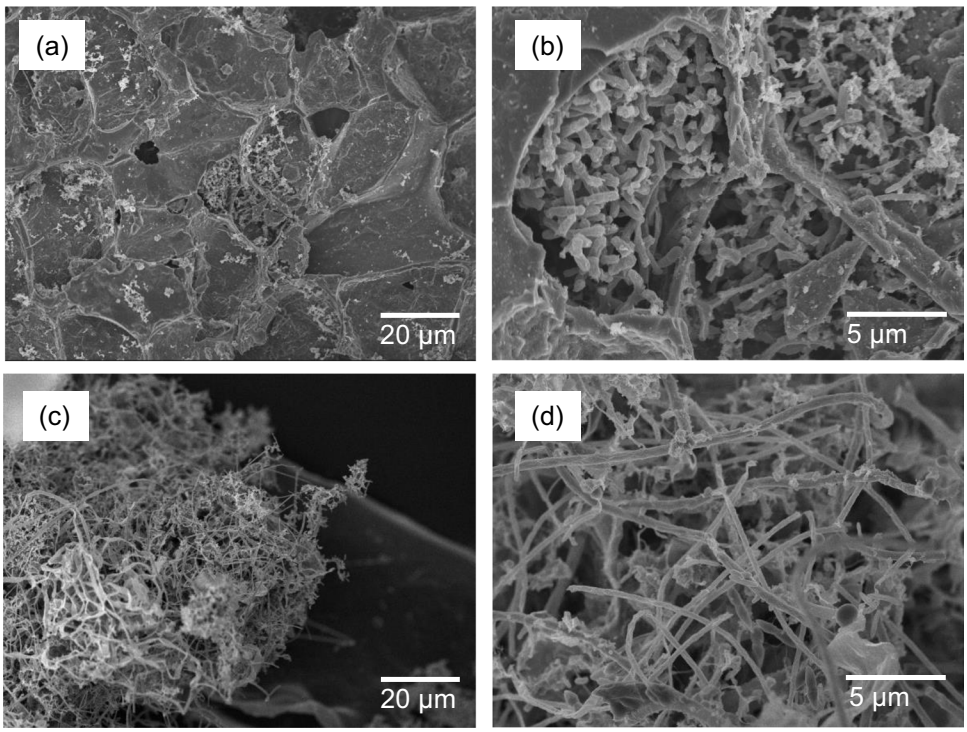

Fig. 5 SEM photos of the carriers on day 49 of the experiment. Low (**a**) and high (**b**) magnification of biochar. Low (**c**) and high (**d**) magnification of sponge

Table 2 Comparison with previous studies

Substrate	Reactor type	Substrate characteristics		OLR (g-COD /L/day)	CH_4 production rate		COD (TOC) removal (%)	References
		pH	COD (mg/L)		mL/g-COD	mL/g-VS		
WH	CSTR	n.d.	n.d.	0.73 (g-VS)	n.d.	240	n.d.	[31]
WH	Two-stage	5.8	n.d.	3.75	146.4	n.d.	73	[32]
Pretreated WH	Two-stage	5.8	n.d.	3.75	340.8	n.d.	82	[32]
WHJ	UASB	7.0–7.5	15,630–23,500	8.85	150	n.d.	86	[5]
WHJ	MIC	7.0–7.5	15,630–23,500	17.93	210	n.d.	82	[5]
WHJ	Batch	6.8	5960	n.d.	n.d.	635	n.d.	[33]
WHJ	SBR (biochar)	4.0	29,617	2.96	413.8	471	89 (TOC)	This study

WH water hyacinth, *WHJ* water hyacinth juice, *COD* chemical oxygen demand, *HRT* hydraulic retention time, *OLR* organic loading rate, *VS* volatile solid, *TOC* total organic carbon, *n.d.* not determined, *UASB* up-flow anaerobic sludge blanket, *MIC* modified internal circulation, *CSTR* continuous stirred tank reactor, *SBR* sequencing batch reactor

Reference: [31] Hanisak et al., 1980, [32] Barua and Kalamdhad, 2019, [5] Liu et al., 2020, [33] Hudakorn and Sritrakul, 2020

and reduce reactor performance [22, 28]. Nevertheless, this experiment yielded the highest CH_4 production rate of 413.8 mL/g-COD and a relatively high organic removal efficiency of 89% (TOC) compared to other studies. Even with substrates being unfavorable to AD, the high treatment performance suggests that using the coffee husk–derived biochar carrier may provide superior operating conditions in treating other WHJ.

Microbial Community

Fifteen bacterial phyla were detected to be more than 1% in the seed sludge with *Bacteroidetes* (25.8%), *Proteobacteria* (13.2%), *Cloacimonetes* (12.8%), and *Firmicutes* (12.2%) predominating (Fig. 6a). *Bacteroidetes* and *Firmicutes* were predominant in all the reactors on day 49 (58.0–83.4%). *Bacteroidetes* and *Firmicutes* are bacterial phyla commonly found in a typical AD reactor and are responsible for the degradation and hydrolysis of complex macromolecules by releasing extracellular enzymes [34]. The relative abundance of both phyla was significantly higher in the biochar- and sponge-filled conditions (58.8% and 47.2%, respectively). The pores of biochar have been reported to act as a shelter for microorganisms and enrich these species [35]. *Bacteroidetes* and *Firmicutes* which increased by the addition of the carriers may have contributed to the rapid degradation of SS in the WHJ. In addition, *Firmicutes* are capable of degrading acetate and the low presence of *Firmicutes* leads to the accumulation of acetate due to reduced consumption [36]. This is consistent with the lower relative abundance of *Firmicutes* in the control reactor compared to the other reactors and with the higher resultant concentration of VFAs.

In total, 12 genera of archaea were detected to be more than 1% in the substrate, seed sludge, and reactors (Fig. 6b). *Methanosaeta* and *Methanolinea* were dominant in the seed sludge (23.5% and 38.2%, respectively). These two genera were also dominant in all the reactors, where their relative abundance was the highest in the control condition (82.0%) and moderate in the biochar- and sponge-filled conditions (53.0% and 51.5%, respectively). Biofilm carriers provide a habitat for microorganisms and retain them due to their porosity or large voids [37, 38]. In AD, archaeal diversity is directly related to reactor stability, and a decrease in diversity has been observed in loaded environments for microorganisms [39]. Differences in the relative abundance of *Methanosaeta* and *Methanolinea* occupying each reactor indicate that the addition of the carriers improves the archaeal diversity and performance stability. Unclassified *Woesearchaeia* (class level) and *Candidatus Diapherotrites* archaeon ADurb.Bin253, a type of *Woesearchaeia*, were also detected in the WHJ and reactors (Fig. 6b). *Woesearchaeia* is one of the classes in the phylum *Nanoarchaeaeota*. *Nanoarchaeaeota* have extremely small genomes and cell sizes and are generally known to live as parasites or symbionts with other archaea [40]. *Woesearchaeia* is thought to be a heterotroph that degrades organic matter to produce acetate and hydrogen and thought to live in symbiosis with methanogens and is more commonly detected in soil than in water [41–43]. *Woesearchaeia* was the dominant archaeon among the archaea in WHJ possibly because the water hyacinths in this experiment were rooted in a swampy area and had soil attached to them. *Candidatus Diapherotrites* archaeon ADurb.Bin253 detected in the seed sludge and reactors has never been reported to be detected in AD. *Candidatus Diapherotrites* archaeon ADurb.Bin253 was present in low relative abundance (1.1%) in the seed sludge, but its abundance significantly increased in the carrier-filled (biochar and sponge) conditions during the experiment (12.9–28.6%). These results indicate that *Candidatus Diapherotrites* archaeon ADurb.Bin253, which belongs to *Woesearchaeia*, may have contributed to organic matter degradation and reactor stability through symbiosis with archaea, and that

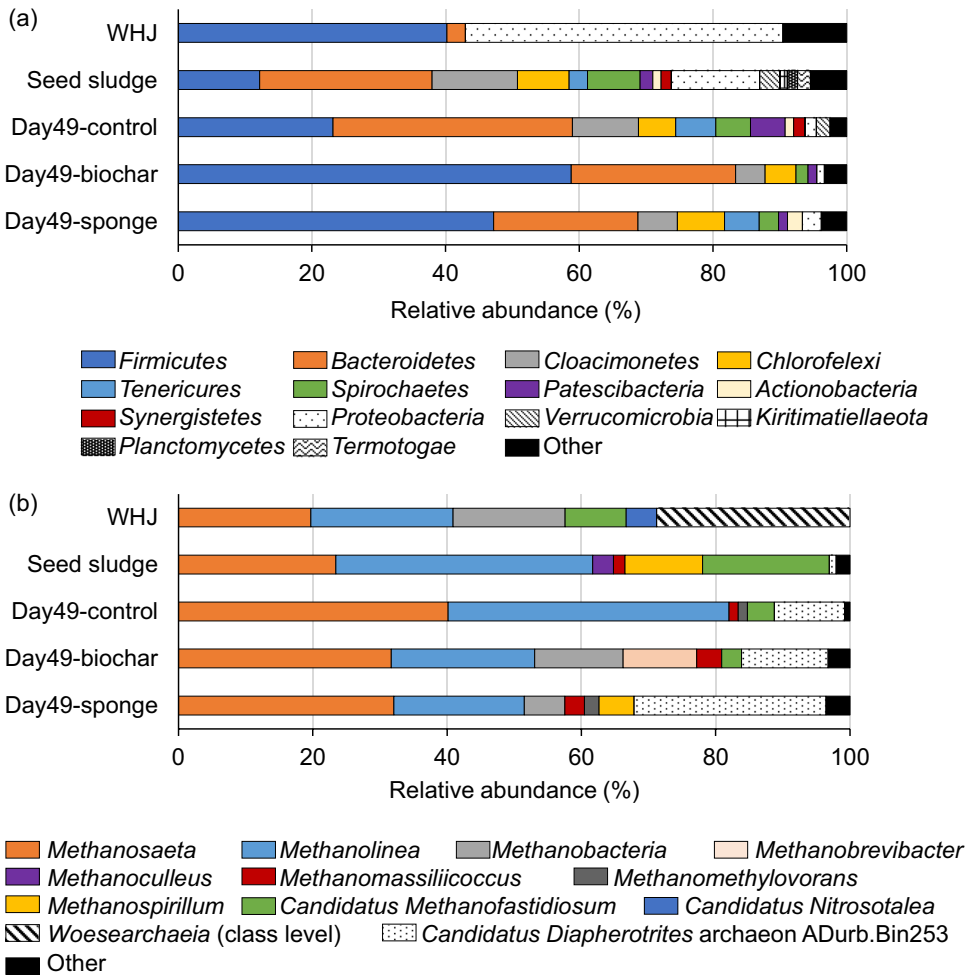

Fig. 6 Microbial community structure at the phylum level of bacteria (**a**) and genus level of archaea (**b**)

filling of carrier enhanced the relative abundance of *Candidatus Diapherotrites* archaeon ADurb.Bin253.

Conclusions

This study aimed to evaluate the effectiveness of coffee husk–derived biochar as a carrier for treating WHJ in an anaerobic SBR and compare its performance to that of sponge-filled and control conditions. The biochar carrier showed high CH_4 production (0.9–1.3 L/L/day), average TOC removal efficiency (88.6%), and pH stability (6.8–7.1). The result showed that the performance of the biochar was comparable to that of the sponge carrier. Hence, the coffee husk–derived biochar can be considered a sustainable and useful carrier for enhancing the AD of WHJ. Moreover, the carrier-filled conditions (12.9–28.6%) showed the presence of nano-archaea (*Candidatus Diapherotrites* archaeon ADub.Bin253), which are known to have a symbiotic relationship with methanogens, indicating their contribution to the substrate degradation and reactor stability. Further studies are needed to optimize the pyrolysis temperature for coffee husk biochar and to conduct pilot studies around Lake Tana, Ethiopia, to enhance WHJ treatment performance. Additionally, understanding the function of nano-archaea for WHJ treatment will be crucial for enhancing the proposed process.

Acknowledgements We gratefully acknowledge the Hokubu Sludge Treatment Center in Yokohama, Kanagawa Prefecture, Japan, for providing the anaerobic digestion inoculum and Kazo City, Saitama Prefecture, Japan, for the donation of the water hyacinths.

Author Contribution Data curation: Daiki Maruyama. Investigation: Daiki Maruyama and Akinori Fujita. Writing—original draft preparation: Daiki Maruyama. Writing—reviewing and editing: Shin-ichi Akizuki, Mutsumi Sekine, Nigus Gabbiye Habtu, and Shinjiro Sato. Project administration: Shin-ichi Akizuki. Resources: Tatsuki Toda. Supervision: Tatsuki Toda.

Funding This study was partially supported by Japan Science and Technology Agency (JST)/Japan International Cooperation Agency (JICA), Science and Technology Research Partnership for Sustainable Development (SATREPS) through the project for Eco-Engineering for Agricultural Revitalization toward Improvement of Human Nutrition (EARTH): Water Hyacinth to Energy and Agricultural Crops (grant number: JPMJSA2005).

Data Availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics Approval and Consent to Participate Not applicable

Consent for Publication All authors read and approved the final manuscript.

Competing Interests The authors declare no competing interests.

References

1. Malik A (2007) Environmental challenge vis a vis opportunity: the case of water hyacinth. Environ Int 33:122–138. https://doi.org/10.1016/j.envint.2006.08.004
2. Patel S (2012) Threats, management and envisaged utilizations of aquatic weed Eichhornia crassipes: an overview. Rev Environ Sci Biotechnol 11:249–259. https://doi.org/10.1007/s11157-012-9289-4
3. Gundupalli MP, Tantayotai P, Chuetor S, Cheenkachorn K, Joshi S, Bhattacharyya D, Sriariyanun M (2022) Improvement of water hyacinth bioconversion by different organic and mineral acid pretreatment and the effect of post-pretreatment washing. Bioenergy Res. https://doi.org/10.1007/s12155-022-10528-9
4. Liu J, Wang C, Wu K, Huang L, Tang Z, Zhang C, Wang C, Zhao X, Yin F, Yang B, Liu J, Yang H, Zhang W (2020) Novel start-up process for the efficient degradation of high COD wastewater with up-flow anaerobic sludge blanket technology and a modified internal circulation reactor. Bioresour Technol 308:123300. https://doi.org/10.1016/j.biortech.2020.123300
5. Liu J, Wang C, Wu K, Tang Z, Peng S, Huang J, Li F, Zhao X, Yin F, Yang B, Liu J, Yang H, Zhang W (2020) Comparison of long-term energy efficiency and microbial community dynamics of different reactors in response to increased loadings of water hyacinth juice. Sci Total Environ 744:140812. https://doi.org/10.1016/j.scitotenv.2020.140812
6. Vasco-Correa J, Khanal S, Manandhar A, Shah A (2018) Anaerobic digestion for bioenergy production: global status, environmental and techno-economic implications, and government policies. Bioresour Technol 247:1015–1026. https://doi.org/10.1016/j.biortech.2017.09.004
7. Zhao W, Yang H, He S, Zhao Q, Wei L (2021) A review of biochar in anaerobic digestion to improve biogas production: performances, mechanisms and economic assessments. Bioresour Technol 341:125797. https://doi.org/10.1016/j.biortech.2021.125797
8. Ahmad M, Rajapaksha AU, Lim JE, Zhang M, Bolan N, Mohan D, Vithanage M, Lee SS, Ok YS (2014) Biochar as a sorbent for contaminant management in soil and water: a review. Chemosphere 99:19–33. https://doi.org/10.1016/j.chemosphere.2013.10.071
9. Dias J, Bellingham M, Hassan J, Barrett M, Stephenson T, Soares A (2018) Influence of carrier media physical properties on start-up of moving attached growth systems. Bioresour Technol 266:463–471. https://doi.org/10.1016/j.biortech.2018.06.096
10. Cayetano RDA, Kim GB, Park J, Yang YH, Jeon BH, Jang M, Kim SH (2022) Biofilm formation as a method of improved treatment during anaerobic digestion of organic matter for biogas recovery. Bioresour Technol 344:126309. https://doi.org/10.1016/j.biortech.2021.126309
11. Montañés Alonso R, Pérez García M, Solera del Río R (2014) Performance of up-flow anaerobic fixed bed reactor of the treatment of sugar beet pulp lixiviation in a thermophilic range. Bioresour Technol 154:305–312. https://doi.org/10.1016/j.biortech.2013.12.071
12. Center TD (1994) Biological control of weeds: waterhyacinth and waterlettuce. In: Rosen D, Bennett FD, Capinera JL (eds) Pest management in the subtropics. biological control–a Florida perspective. Intercept Publishing Company, Andover
13. Murthy PS, Naidu MM (2012) Sustainable management of coffee industry by-products and value addition–a review. Resour Conserv Recycl 66:45–58. https://doi.org/10.1016/j.resconrec.2012.06.005
14. Fernández JV, Faria DN, Santoro MC, Mantovaneli R, Cipriano DF, Brito GM, Carneiro MTWD, Schettino MA Jr, Gonzalez JL, Freitas JCC (2022) Use of unmodified coffee husk biochar and ashes as heterogeneous catalysts in biodiesel synthesis. Bioenergy Res. https://doi.org/10.1007/s12155-022-10516-z
15. Fujiwara M, Koyama M, Akizuki S, Watanabe K, Ishikawa K, Ban S, Toda T (2020) Seasonal changes in the chemical composition and anaerobic digestibility of harvested submerged macrophytes. Bioenergy Res 13:683–692. https://doi.org/10.1007/s12155-019-10082-x
16. Ahmed M, Nigussie A, Addisu S, Belay B, Lehmann J, Sato S (2021) Valorization of animal bone waste for agricultural use through biomass co-pyrolysis and bio-augmentation. Biomass Conv Bioref. https://doi.org/10.1007/s13399-021-02100-w
17. Yang Y, Tada C, Miah MS, Tsukahara K, Yagishita T, Sawayama S (2004) Influence of bed materials on methanogenic characteristics and immobilized microbes in anaerobic digester. Mater Sci Eng C 24:413–419. https://doi.org/10.1016/j.msec.2003.11.005
18. Agborambang ME, Fujiwara M, Bhatia P, Kishi M, Sekine M, Salangsang MCD, Matsuyama T, Toda T (2022) Characterization of mixing by CFD simulation and optimization of mixing frequency to break scum and enhance methane yield in Chinese dome digester. Bioenerg Res. https://doi.org/10.1007/s12155-022-10519-w
19. Salangsang MCD, Sekine M, Akizuki S, Sakai HD, Kurosawa N, Toda T (2022) Effect of carbon to nitrogen ratio of food waste and short resting period on microbial accumulation during anaerobic digestion. Biomass Bioenergy 162:106481. https://doi.org/10.1016/j.biombioe.2022.106481
20. Liu J, Liu T, Chen S, Yu H, Zhang Y, Quan X (2020) Enhancing anaerobic digestion in anaerobic integrated floating fixed-film activated sludge (An-IFFAS) system using novel electron mediator suspended biofilm carriers. Water Res 175:115697. https://doi.org/10.1016/j.watres.2020.115697
21. Ota H, Abe K, Suzuki T, Toda T, Kuwata M (2011) A new method of freeze-drying using cooled water for SEM sample preparation. J Electron Microsc Technol Med Biol 25:9–13
22. Speece RE (1996) Anaerobic biotechnology for industrial wastewaters. Archae Press, Tennessee

23. Boe K, Batstone DJ, Steyer JP, Angelidaki I (2010) State indicators for monitoring the anaerobic digestion process. Water Res 44:5973–5980. https://doi.org/10.1016/j.watres.2010.07.043
24. Ma H, Hu Y, Wu J, Kobayashi T, Xu KQ, Kuramochi H (2022) Enhanced anaerobic digestion of tar solution from rice husk thermal gasification with hybrid upflow anaerobic sludge-biochar bed reactor. Bioresour Technol 347:126688. https://doi.org/10.1016/j.biortech.2022.126688
25. Nomura T, Nagao T, Yoshihara A, Tokumoto H, Konishi Y (2008) Selective immobilization of aceticlastic methanogens to support material. KONA Powder Part J 26:246–253. https://doi.org/10.14356/kona.2008022
26. Shi J, Xu C, Han Y, Han H (2019) Enhanced anaerobic biodegradation efficiency and mechanism of quinoline, pyridine, and indole in coal gasification wastewater. Chem Eng J 361:1019–1029. https://doi.org/10.1016/j.cej.2018.12.162
27. Li Y, Li L, Sun Y, Yuan Z (2018) Bioaugmentation strategy for enhancing anaerobic digestion of high C/N ratio feedstock with methanogenic enrichment culture. Bioresour Technol 261:188–195. https://doi.org/10.1016/j.biortech.2018.02.069
28. Jain S, Jain S, Wolf IT, Lee J, Tong YW (2015) A comprehensive review on operating parameters and different pretreatment methodologies for anaerobic digestion of municipal solid waste. Renew Sustain Energy Rev 52:142–154. https://doi.org/10.1016/j.rser.2015.07.091
29. Induchoodan TG, Haq I, Kalamdhad AS (2022) Factors affecting anaerobic digestion for biogas production: a review. In: Hussain C, Hait S (eds) Advanced Organic Waste Management, 1st edn. Elsevier, Amsterdam, pp 223–233
30. Langer S, Schropp D, Bengelsdorf FR, Othman M, Kazda M (2014) Dynamics of biofilm formation during anaerobic digestion of organic waste. Anaerobe 29:44–51. https://doi.org/10.1016/j.anaerobe.2013.11.013
31. Hanisak MD, Williams LVD, Ryther JH (1980) Recycling the nutrients in residues from methane digesters of aquatic macrophytes for new biomass production. Resour Recover Conserv 4:313–323. https://doi.org/10.1016/0304-3967(80)90037-2
32. Barua VB, Kalamdhad AS (2019) Biogas production from water hyacinth in a novel anaerobic digester: a continuous study. Process Saf Environ Prot 127:82–89. https://doi.org/10.1016/j.psep.2019.05.007
33. Hudakorn T, Sritrakul N (2020) Biogas and biomass pellet production from water hyacinth. Energy Rep 6:532–538. https://doi.org/10.1016/j.egyr.2019.11.115
34. Zhang Y, Cao L, Fu H, Zhang M, Meng J, Althakafy JT, Abo-Dief HM, El-Bahy SM, Zhang Y, Wei H, Xu BB, Guo Z (2022) Effect of sulfamethazine on anaerobic digestion of manure mediated by biochar. Chemosphere 306:135567. https://doi.org/10.1016/j.chemosphere.2022.135567
35. Wang S, Shi F, Li P, Yang F, Pei Z, Yu Q, Zuo X, Liu J (2022) Effects of rice straw biochar on methanogenic bacteria and metabolic function in anaerobic digestion. Sci Rep 12:1–14. https://doi.org/10.1038/s41598-022-10682-2
36. Jiang X, Yan Y, Feng L, Wang F, Guo Y, Zhang X, Zhang Z (2021) Bisphenol A alters volatile fatty acids accumulation during sludge anaerobic fermentation by affecting amino acid metabolism, material transport and carbohydrate-active enzymes. Bioresour Technol 323:124588. https://doi.org/10.1016/j.biortech.2020.124588
37. Luo C, Lü F, Shao L, He P (2015) Application of eco-compatible biochar in anaerobic digestion to relieve acid stress and promote the selective colonization of functional microbes. Water Res 68:710–718. https://doi.org/10.1016/j.watres.2014.10.052
38. Bu J, Hu BB, Wu HZ, Zhu MJ (2022) Improved methane production with redox-active/conductive biochar amendment by establishing spatial ecological niche and mediating electron transfer. Bioresour Technol 351:127072. https://doi.org/10.1016/j.biortech.2022.127072
39. Chen M, Liu S, Yuan X, Li QX, Wang F, Xin F, Wen B (2021) Methane production and characteristics of the microbial community in the co-digestion of potato pulp waste and dairy manure amended with biochar. Renew Energy 163:357–367. https://doi.org/10.1016/j.renene.2020.09.006
40. Wurch L, Giannone RJ, Belisle BS, Swift C, Utturkar S, Hettich RL, Reysenbach AL, Podar M (2016) Genomics-informed isolation and characterization of a symbiotic Nanoarchaeota system from a terrestrial geothermal environment. Nat Commun 7:12115. https://doi.org/10.1038/ncomms12115
41. Liu X, Li M, Castelle CJ, Probst AJ, Zhou Z, Pan J, Liu Y, Banfield JF, Gu JD (2018) Insights into the ecology, evolution, and metabolism of the widespread Woesearchaeotal lineages. Microbiome 6:1–16. https://doi.org/10.1186/s40168-018-0488-2
42. Wang H, Bier R, Zgleszewski L, Peipoch M, Omondi E, Mukherjee A, Chen F, Zhang C, Kan J (2020) Distinct distribution of archaea from soil to freshwater to estuary: implications of archaeal composition and function in different environments. Front Microbiol 11:576661. https://doi.org/10.3389/fmicb.2020.576661
43. Huang WC, Liu Y, Zhang X, Zhang CJ, Zou D, Zheng S, Xu W, Luo Z, Liu F, Li M (2021) Comparative genomic analysis reveals metabolic flexibility of Woesearchaeota. Nat Commun 12:1–14. https://doi.org/10.1038/s41467-021-25565-9