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Biochar addition to mitigate oil inhibition in anaerobic digestion of food wastewater: Microbial insights from biochemical methane potential tests

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ABSTRACT

The high oil content in food wastewater often limits the process of anaerobic digestion by suppressing microbial activity and growth, both essential for organic conversion and consequently methane production. The objective of this study was to optimize biochar dosage for enhancing anaerobic digestion of food wastewater. Thus, the synthetic food wastewater containing 4 g/L of sodium oleate (oleate-Na) and an initial organic concentration of 5000 mg/L COD was used. Results show that methane production was severely suppressed during the anaerobic digestion of food wastewater with the addition of oleate-Na, reducing cumulative methane yield by 36.61 %. The inhibitory effect was mitigated with biochar addition, particularly at 5 g/L, which facilitated oleate-Na biodegradation to increase the cumulative methane production by 196.50 %. Sludge characterization and microbial analysis indicated that adding 5 g/L of biochar significantly enhanced biomass growth and selectively enriched functional bacteria, such as Thermotogae and Bacteroidetes, along with archaea like *Methanoculleus* and *Methanosarcina*, promoting hydrogenotrophic methanogenesis for methane production. Nevertheless, this benefit was reduced when biochar addition was increased from 5 g/L to 8 g/L, likely due to its excessive adsorption of compounds, like volatile fatty acids, to limit further methanogenesis.

1. Introduction

Anaerobic digestion has been widely employed for treating food wastewater and recovering valuable resources (Chew et al., 2021). Anaerobic digestion converts the organic matter in food wastewater into methane, a renewable energy source (Chen et al., 2020). However, the process of methane generation in anaerobic digestion is frequently hindered as a result of elevated levels of organic compounds, especially oils (Liu et al., 2023). Although food wastewater has substantial potential for methane production, its elevated oil content often causes the rapid accumulation of inhibitors like ammonium, long-chain fatty acids, and VFAs, which together restrained methane generation through anaerobic digestion (Liu et al., 2017).

Biochar has often been applied to increase the tolerance of anaerobic digestion in response to challenging wastewater environments

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(Liu et al., 2017). Its porous structure is well known for providing a substantial surface area for microbial attachment and colonization, which, in turn, promotes biomass growth as well as enhancing organic matter degradation (Masebinu et al., 2019a; Salma et al., 2023). Furthermore, the conductivity of biochar facilitates direct interspecies electron transfer (DIET) between methanogenic and exoelectrogenic bacteria, thereby enhancing methane production in anaerobic digestion (Lee et al., 2022a). Additionally, biochar can further optimize anaerobic digestion by regulating sludge pH and adsorbing inhibitory compounds, such as volatile fatty acids (VFAs) and ammonium (Tsui et al., 2021).

Studies have previously shown the efficacy of biochar in modulating anaerobic wastewater treatment processes. As reported by Sugiarto et al. (2021), the addition of biochar to the anaerobic digestion of food wastewater increased cumulative methane yield by 46.90 % and daily methane production by 43.00 %, primarily due to improved VFA degradation and the proliferation of *Clostridia* and *Methanosaeta* populations. Similarly, Wang et al. (2023) reported that biochar could promote DIET by upregulating critical genes involved in methane production, leading to a 26.90 %-40.80 % increase in methane yield during swine manure anaerobic digestion. However, an excessive amount of biochar was found to inhibit the digestion process. Shi et al. (2022) found that adding 4.80 g/g volatile solids (VS) of biochar to oily sludge (OS) containing both naphthalene and starch caused a 32.50 % reduction in methane production efficiency and extended the lag phase by 5.72 hours. Nevertheless, the optimal biochar dosage for enhancing methane production in food wastewater anaerobic digestion remains unidentified, and how varying dosages influence digestion efficiency and microbial response mechanisms remains unclear.

This study aimed to evaluate the effectiveness of biochar for mitigating oil inhibition within the anaerobic treatment of food wastewater. To simulate oil residue in synthetic food wastewater, 4 g/L of oleate-Na was introduced, which impeded the anaerobic digestion process. Biochar was then added to the digester at varying concentrations between 0 and 8 g/L. Biogas production and organic decomposition were measured to determine the optimal biochar concentration. Additionally, microbial dynamics and methanogenesis pathways were examined to elucidate biomass responses to elevated oil content and biochar addition. The findings provide crucial insights for developing effective strategies to optimize the anaerobic digestion of food wastewater in order to enhance resource recovery.

2. Materials and methods

2.1. Experimental materials

Due to the significant fluctuations in oil content and solid components in actual food wastewater, synthetic food wastewater with similar characteristics was used in this study to ensure experimental conditions were controllable and reproducible (Table S1, Supplementary Data). Glucose, potassium dihydrogen phosphate, and urea were used as sources of carbon, phosphorus, and nitrogen, respectively. Previous reports indicate that the chemical oxygen demand (COD) of food wastewater ranges from 500 to 10,000 mg/L, with typical values around 5000 mg/L (He et al., 2005). Accordingly, the COD level in the synthetic wastewater was adjusted to 5000 mg/L. The C: N: P ratio was set at 100:4:1 to replicate the low nitrogen levels of wastewater following oil extraction and recovery in industrial applications. In addition, inorganic salts and trace elements required for microbial growth and activity were supplemented. Oleate-Na with a purity of 98 % (Sigma-Aldrich, USA) was added to the synthetic wastewater as a representative of long-chain fatty acids to simulate the inhibitory impacts from oils in actual food wastewater.

Biochar sourced from a local supplier in Beijing, China, was used to alleviate oil inhibition during anaerobic digestion. This biochar was derived from corn stalks that were pyrolyzed at 500°C to obtain an ash content of 7.23 % and a pH of 9.46. The biochar was then sieved through an 80-mesh screen (0.20 mm, GB/T6003.1–2022) to obtain a particle size below 0.20 mm.

2.2. Experimental equipment and protocol

A single anaerobic respirator (AER–800, CHALLENGE , USA) with ten individual anaerobic digesters were used in this study (Fig. S1, Supplementary Data). All digesters had a sampling port in the cap and were placed in a thermostatic magnetic stirring water bath (MS–308, CHALLENGE, USA) with the temperature maintained at 35°C. A magnetic stirring device was placed inside the anaerobic digester bottles to agitate the mixed liquor at a rotational speed of 240 r/min. The biogas produced in each anaerobic digester was directed to a real–time gas meter for quantification.

Anaerobic sludge from a laboratory anaerobic membrane bioreactor (AnMBR) system was used to inoculate all anaerobic digesters. The AnMBR had been stably operated for several months with the same synthetic wastewater as the feed solution used in this study. The sludge from the AnMBR was rinsed three times using distilled water and then equally distributed to each digester. The synthetic food wastewater was mixed with the sludge to make up an effective digester volume of 150 mL and a corresponding mixed liquor suspended solids (MLSS) concentration of approximately 10 g/L. To replicate the oil residue in food wastewater, oleate-Na at a concentration of 4 g/L was added, simulating conditions known to inhibit anaerobic digestion as previously reported (Zhang et al., 2023a). The pH of mixed liquor was initially adjusted to 7.10–7.20 using $NaHCO_3$. Biochar was introduced to the digesters at dosages of 0, 2, 5, and 8 g/L. A control treatment, excluding both oleate-Na and biochar, was also included to assess the impact of oil inhibition on anaerobic digestion. Thus, the five treatments, denoted as T0–0, T4–0, T4–2, T4–5, T4–8 (i.e. T[$C_{oleate\text{-}Na}$]-[$C_{biochar}$]), were continuously operated in parallel for 26 days until the biogas production was negligible. All treatments were performed in triplicate to ensure result reliability.

2.3. Analytical methods

2.3.1. Physicochemical analysis

The standard dichromate method, along with a high-range spectrophotometer (HACH, USA), was employed to determine COD. A TOC/TN analyzer (Elementar, Germany) was used to measure total organic carbon (TOC) and total nitrogen (TN). The monitoring of electrical conductivity and pH was performed using a pH/conductivity meter (SA3100M, OHAUS, USA). Real-time monitoring of biogas production was conducted using an anaerobic respirator. Gas chromatography with a thermal conductivity detector was used to quantify the methane content in biogas. The measurement of VFAs was performed using gas chromatography (GC-7890, Agilent, USA), equipped with a flame ionization detector and a DB-FFAP column (30 m × 0.25 mm × 0.25 μm, Agilent, USA) as described by Li et al. (2017). Before analysis, the samples were filtered through a 0.45 μm membrane, and the VFAs were quantified using calibration curves prepared from known standards.

MLSS and mixed liquor volatile suspended solids (MLVSS) concentrations were measured following the procedures described in Standard Methods 2540 for water and wastewater analysis (Gilcreas, 1967; Rice et al., 2012). At the conclusion of anaerobic digestion, soluble microbial products (SMP) and extracellular polymeric substances (EPS) were quantified by measuring total protein and polysaccharide content using the Folin–phenol and phenol–sulfuric acid methods, respectively (Robles et al., 2018).

2.3.2. Microbial community analysis

Mixed liquor was collected at the end of anaerobic digestion for the analysis of microbial community structure and functional predictions. Pellets were obtained from the mixed liquor samples after centrifugation at 12,000 rpm for 20 min, freeze–dried in a freeze dryer (LABCONCO Free Zone, USA), and then stored at –20°C for later DNA extraction. The Power Soil DNA Isolation Kit (MO BIO, Norcross, Georgia, USA) was used to extract total DNA. Universal primers 338 F/806 R (5′–ACTCCTACGGAGGCAGCAG–3′ and 5'–GGACTTACGGAGCAG–3′) and Arch 344 F/806 R (5'–ACGGGYGCAGCAGGCGCGA–3′ and 5'–GGACTACVSGGGTATCTAAT–3′) were used to amplify bacterial and archaeal DNA, respectively.

2.3.3. Statistical analysis

Operational taxonomic units (OTUs) were formed by clustering sequences at a 97 % similarity level. Relative gene abundance was calculated using the Ribosomal Database Project (RDP) classifier. The study applied Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt2) to analyze enzyme gene abundance linked to different methane production pathways (Zheng et al., 2023). Using Statistical Product Service Solutions (SPSS), statistical analysis was carried out, and graphing was completed using Origin 2024.

3. Results and discussion

3.1. Biogas production

Oleate-Na addition initially facilitated biogas production but significantly inhibited the methanogenic performance in anaerobic digestion of food wastewater (Fig. 1). As shown in Fig. 1a, on the first day, the daily biogas production in the T4–0 digester increased to

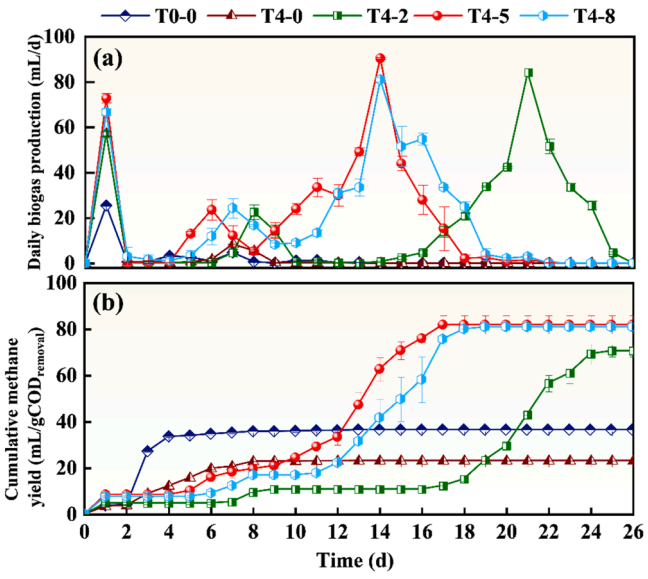

Fig. 1. (a) Daily biogas production and (b) cumulative methane yield under different concentrations of biochar and oleate-Na additions.

57.22 mL, 1.25 times higher than the T0–0 group (25.44 mL). This increase could be attributed to oleate-Na serving as a favorable carbon source for anaerobic digestion, enhancing biogas production (Cirne et al., 2007). However, after this initial phase, oleate-Na markedly inhibited methane production. The cumulative methane yield of T4–0 over 26 days was 23.13 mL/g COD $_{removal}$, which was 36.60 % lower than that of T0–0 (36.49 mL/g COD $_{removal}$). This strong inhibition might result from the buildup of organic acids due to oleate-Na degradation, which reduced methanogenic activity as detailed in Section 3.2.2 (Gaspari et al., 2021). As reported by Zhang et al. (2023), 2 g/L of oleate-Na enhanced biomass growth and activity, whereas 4 g/L caused a significant VFA buildup, leading to the failure of the AnMBR. Additionally, a 16.20 % reduction in methane production was observed by Yang et al. (2022) when oil content reached 20.00 % of total solids (TS).

Biochar addition not only enhanced biogas production, but also significantly mitigated the inhibitory effect of oleate-Na, facilitating methane production during the anaerobic digestion of food wastewater (Fig. 1). In addition to its notable production at the initiation of anaerobic digestion, there were two peaks of biogas production within day 6 – 8 and 14 – 21, respectively. The rapid breakdown of readily biodegradable organic matter in food wastewater could explain the first peak (Qi et al., 2021), while the delayed second peak in biogas production was due to the conversion of refractory organic matter (Zhen et al., 2022). The initial peaks of maximum daily biogas production for digesters T4–2, T4–5, and T4–8 were 1.68, 1.80, and 1.89 times higher than that from the digester T4–0 (8.44 mL), respectively (Fig. 1a). The observed difference showed that biochar addition facilitates the swift conversion of biodegradable organic matter, leading to increased biogas production (Yang et al., 2021).

Unlike other treatments, there was no second peak in biogas production for the digester T4–0, possibly due to the inhibitory effect of oleate-Na, which prevented the breakdown of refractory organic matter and caused biogas production to stagnate (Xu et al., 2018). In contrast, digesters T4–5 and T4–8 showed a second peak on day 14, reaching 90.57 and 81.12 mL/d, respectively, which was earlier than the peak observed in T4–2 on day 21 (84.17 mL/d). This outcome could be attributed to the higher biochar dosage, which accelerated refractory organic matter decomposition in wastewater (Qi et al., 2021), particularly at doses above 5 g/L.

Biochar addition, particularly at 5 g/L, improved anaerobic digestion efficiency, resulting in higher methane production from food wastewater (Fig. 1b). Compared to the T0–0 digester, the cumulative methane yield decreased by 36.61 % for the T4–0 treatment with oleate-Na addition. By contrast, the cumulative methane yield reached 70.64, 81.94, and 80.92 mL/g COD $_{removal}$ for the digester T4–2, T4–5, and T4–8, respectively, which was 2.05, 2.54, and 2.50 times higher than that of T4–0 (23.13 mL/g COD $_{removal}$). It has been reported that biochar with a porous structure could serve as a favorable matrix for the immobilization and growth of bacteria and methane-producing microorganism, accelerating organic matter transformation into methane (Wang et al., 2023). Nevertheless, the digester T4–8 exhibited slightly lower methane production than the digester T4–5, which was possibly due to the excessive addition of biochar to 8 g/L, leading to the immobilization of biomass and organic substances that hindered methanogenesis. Excessive biochar has also been shown to adsorb key intermediates, such as VFAs, reducing their availability for methanogens and limiting methane production (Shi et al., 2022; Wang et al., 2020). In addition, biochar could adsorb the produced methane to reduce its release for detection (Wang et al., 2020). It is noteworthy that biochar could enhance microbial activity for converting oil into methane, as methane production decreased by 36.61 % in digester T4–0 but increased by 147.63 %, 196.50 %, and 192.08 % in digesters T4–2, T4–5, and T4–8, respectively, in comparison to the control digester T0–0. This could be attributed to biochar's conductive properties, which facilitated electron transfer among microbial communities, particularly between methanogens and exoelectrogenic bacteria, thereby boosting methane production (Park et al., 2018). According to Yin et al. (2019), adding biochar at 1.00 g/g (dry matter sludge) during high-temperature anaerobic digestion stimulated the growth of hydrogenotrophic methanogens (e.g., *Methanothermobacter*)

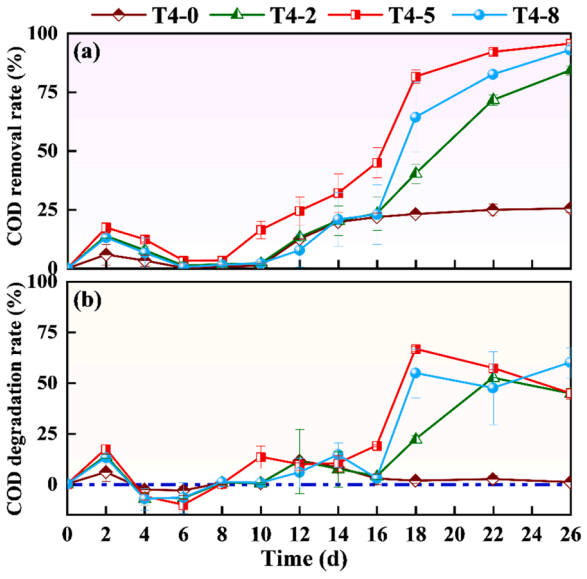

Fig. 2. Effect of biochar on the (a) COD removal rate and (b) degradation rate during anaerobic digestion of food wastewater with 4 g/L oleate-Na.

and enhanced DIET, leading to an 18.00 % increase in VS removal and a 25.00 % rise in methane production.

3.2. Biodegradation and removal of organic substances

3.2.1. COD removal and biodegradation rate

COD removal was measured to evaluate the overall biodegradation and removal of organic substances during the anaerobic digestion of food wastewater. Biochar addition significantly improved the removal of organic matter, especially in food wastewater containing oleate-Na. Compared to the T4–0 digester (25.57 %), the COD removal rates of T4–2, T4–5, and T4–8 digesters reached 84.29 %, 95.70 %, and 92.98 %, respectively, which were 3.30, 3.74, and 3.64 times higher than that of T4–0. Such notable increase was more pronounced from day 10 onward, which could be attributed to biochar addition to promote the conversion of VFAs and thus alleviate their accumulation for increased biogas production (Luo et al., 2015). Similar profiles were also observed for COD biodegradation rates for all digesters, which however, were negative within day 4 – 8. The negative COD biodegradation rate was possibly due to the rapid production but slow conversion of VFAs (Fig. 2b).

Among all digesters with biochar addition, the T4–5 digester exhibited the highest COD removal rate, which showed a marked improvement starting from day 8 (Fig. 2a). In contrast, the COD removal rates of the T4–0, T4–2, and T4–8 digesters increased since approximately day 10. Moreover, the T4–5 digester experienced the lowest COD biodegradation rate of −10.28 % within day 4 –8, which was 214.37 %, 45.20 %, and 39.67 % lower than that of T4–0, T4–2, and T4–8, respectively (Fig. 2b). Nevertheless, the COD degradation rate of T4–5 reached its maximum value of 66.71 % on day 17, which was 483.13 %, 27.24 %, and 11.20 % higher than that of T4–0, T4–2, and T4–8, respectively. These results suggest that a biochar dosage of 5 g/L was more effective in enhancing the decomposition of large molecular weight organics, such as oil, for VFAs production and further conversion to methane in comparison with other dosages. Indeed, biochar addition at 5 g/L accelerated organic conversion to rapidly reach the maximum methane yield as discussed above (Fig. 1b).

3.2.2. Nitrogen conversion and VFAs production

The addition of biochar enhanced TN removal in the treatment of food wastewater through anaerobic digestion (Fig. 3a). Although the digesters are closed systems with no external nitrogen input, TN concentrations increased in all digesters due to the breakdown of nitrogenous compounds originally present in the food wastewater, such as proteins and amino acids, which were converted into NH_4^+-N during anaerobic digestion (Fig. 3b). A slight reduction in TN concentrations was observed for all digesters within day 8 – 10, possibly due to microbial uptake of nitrogen for growth and activity (Astals et al., 2021). Such nitrogen uptake was more intensive for digesters with biochar addition as the TN concentrations in T4–2, T4–5, and T4–8 were 14.77 %, 20.43 %, and 15.54 % lower than that of T4–0 (759.48 mg/L) by the conclusion of anaerobic digestion, respectively. In addition, biochar's porous structure allowed it to effectively adsorb nitrogen substances, while its large surface area further enhanced this adsorption capacity (Zhang et al., 2020a). Notably, the T4–5 digester exhibited 6.14 % lower TN concentration than the T4–8 digester, which was consistent with biogas production as discussed above to further evidence the over dosage of biochar up to 8 g/L to limit biomass growth for organic conversion. TN was mainly contributed by NH_4^+-N, and the concentration also increased in all digesters (Fig. 3b). In the T4–0 digester, oleate-Na not only directly induced organic ammonification, but also inhibited microbial activity to reduce nitrogen assimilation for notable NH_4^+-N accumulation. By contrast, biochar addition resulted in NH_4^+-N reduction in the T4–2, T4–5, and T4–8 digesters at the end of

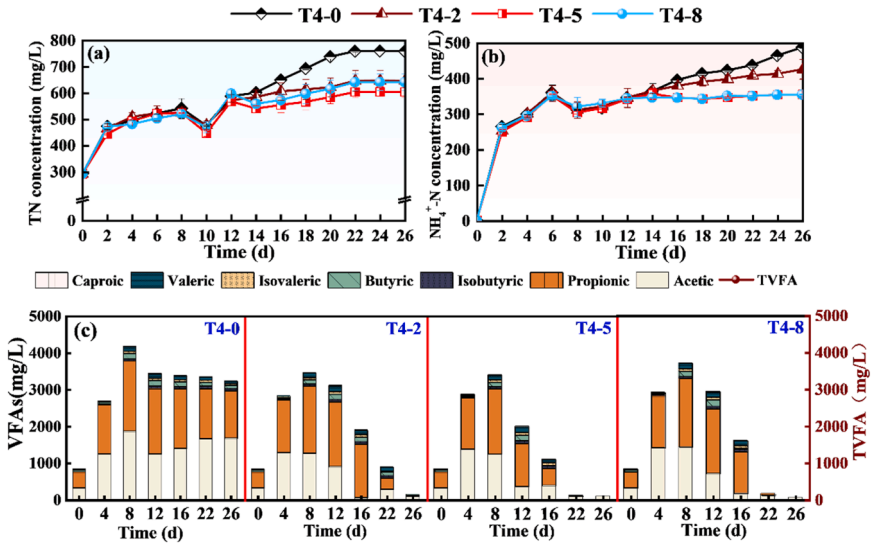

Fig. 3. Effect of biochar on (a) TN, (b) NH_4^+–N, and (c) Total volatile fatty acids (TVFA) concentration and fractions of VFAs during anaerobic digestion of food wastewater with 4 g/L oleate-Na.

anaerobic digestion by 12.60 %, 27.15 %, and 27.15 %, respectively.

The addition of biochar facilitated VFAs conversion toward biogas production (Fig. 3c). Regardless of biochar addition, all digesters experienced an increase within the first 8 days, followed by a decrease in VFAs concentration. This profile was expected to follow organic decomposition and methanation in anaerobic digestion. Research has found that VFAs, when present at levels of 1000–3000 mg/L, can inhibit methanogenic bacterial activity (Cavaleiro et al., 2008). Without biochar addition, VFAs concentration increased to above 3000 mg/L from day 8 onward and then slightly decreased, indicating insignificant methanogenesis for their consumption. By contrast, VFAs concentrations decreased notably since day 8 for all digesters with biochar dosage, particularly at 5 g/L. As discussed above, biochar could enhance DIET between bacteria and methanogenic consortia to facilitate the utilization of VFAs (Lee et al., 2022b). Furthermore, the T4–5 digester exhibited the fastest and highest VFAs degradation rate (347.77 mg/L·d) within day 8 – 12 in comparison with T4–2 and T4–8 at 303.35 and 331.70 mg/L·d between day 12 and 16, respectively. This result further evidenced the dosage of biochar at 5 g/L favored organic conversion to methane production.

Further compositional analysis revealed that acetic acid and propionic acid were the dominant VFAs across all digesters (Fig. 3c). Unlike acetic acid, propionic acid was thermodynamically unfavorable to degrade ($\Delta G = +76$ kJ/mol), thus reducing methane output in anaerobic environments (Lee et al., 2022b). Nevertheless, biochar sped up propionic acid conversion, particularly at dosages above 5 g/L. This enhancement is likely due to biochar's content of alkaline earth metals and organic functional groups, which improved its buffering capacity and facilitated electron transfer among microorganisms (Masebinu et al., 2019b; Pan et al., 2019a).

3.3. Biomass characteristics

Biochar addition promoted the sludge growth and metabolism, particularly for food wastewater with oleate-Na. Compared to T4–0, biochar addition increased MLSS and MLVSS concentrations in all digesters. Specifically, the MLSS concentrations in the T4–2, T4–5, and T4–8 digesters increased by 24.93 %, 78.09 %, and 66.78 %, while the MLVSS concentrations increased by 8.41 %, 59.55 %, and 43.98 %, respectively. These increases in biomass concentrations were attributed to the biochar's adsorption capacity for organic matter, which facilitated the formation of dense aggregates and multi-layer structures in the sludge (Li et al., 2022). However, increasing biochar addition to 8 g/L lowered the MLSS and MLVSS concentrations by 6.35 % and 9.78 %, respectively, in comparison to the T4–5 digester, which could be attributed to the excessive dosage of biochar to absorb organic substances and thus reduce their utilization by sludge for biomass growth (Cai et al., 2016). Compared to T4–0, the MLVSS/MLSS ratio in the T4–2, T4–5, and T4–8 digesters decreased by 13.20 %, 10.31 %, and 13.59 % respectively. This decrease could be related to biochar addition to directly increase MLSS concentration over biomass growth as indicated by the MLVSS content (Masebinu et al., 2019b).

Biochar addition significantly decreased SMP concentration (Fig. 4b). Compared to T4–0, SMP concentration in T4–0, T4–2, T4–5, and T4–8 digesters decreased by 33.46 %, 83.20 %, and 80.20 %, respectively. This decrease could be attributed to biochar addition to reduce inhibition (e.g. VFAs accumulation) on microbes to reduce SMP production and to promote the activity of bacteria and methanogens for SMP degradation (Sun et al., 2022). Such reduction was mainly contributed by the decrease in polysaccharide

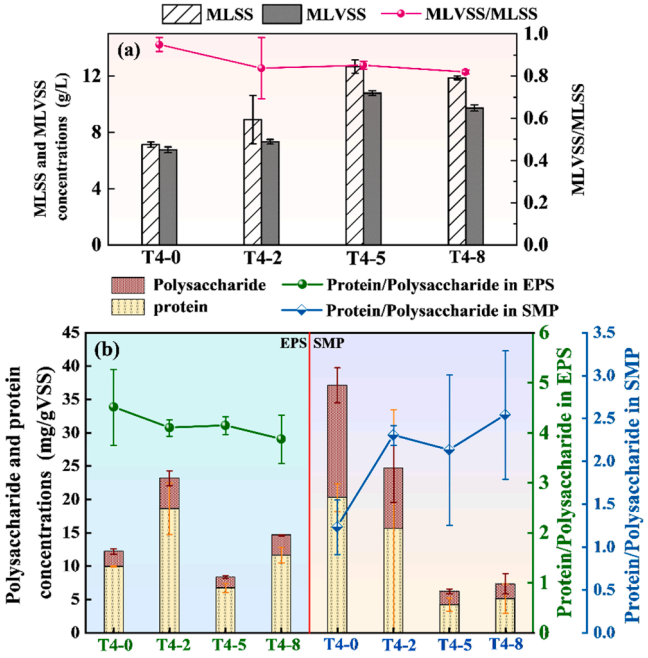

Fig. 4. Effect of biochar on (a) MLSS and MLVSS concentrations and MLVSS/MLSS ratio, and (b) protein and polysaccharide concentrations, as well as protein/polysaccharide ratios in SMP and EPS, during anaerobic digestion of food wastewater with 4 g/L oleate-Na.

content, as biochar addition increased the ratio of protein/polysaccharide in SMP by 87 %, 73 %, and 106 % in the T4–2, T4–5, and T4–8 digesters, respectively, which could be attributed to the accelerated cell hydrolysis caused by biochar addition (Xu et al., 2017). Unlike SMP, biochar addition caused a fluctuation in EPS content. Compared to the T4–0 treatment, biochar dosage at 2 and 8 g/L increased EPS content, which however, was reduced by 31.38 % at 5 g/L, possibly due to the accelerated degradation of VFAs (Fig. 3c) to alleviate microbial stress and thus diminish EPS secretion (Astals et al., 2021).

3.4. Microbial community and methanogenic pathway

3.4.1. Microbial community structure

At both the phylum and genus levels, biochar addition altered the structure of the microbial community. However, Thermotogae, Firmicutes, Proteobacteria, and Bacteroidetes remained the four predominant bacterial phyla with a total abundance exceeding 90.00 % (Fig. 5a). These phyla were crucial to the processes of hydrolysis, acidification, and acetylation, which convert organic matter into biogas during anaerobic digestion (Pan et al., 2019b).

A notable rise in the relative abundance of Thermotogae was observed following biochar addition (Fig. 5a). Thermotogae abundance in digesters T4–2, T4–5, and T4–8 increased from nearly undetectable to 36.10 %, 17.09 %, and 34.02 %, respectively, compared to T4–0. This notable increase could be related to the genus *AUTHM297* (belonging to family Petrotogaceae, phylum Thermotogae) (Ahmad et al., 2020; Zhang et al., 2022), with its abundance increasing from nearly undetectable in digester T4–0–41.12 %, 30.05 %, and 42.24 % in T4–2, T4–5, and T4–8, respectively. Due to the absence of cultured representatives, the putative functions of *AUTHM297* remain unknown. Both Ahmad et al. (2020) and Zhang et al. (2022) observed that *AUTHM297* was enriched in the later phase of a UASB reactor treating selenium- and sulfate-laden synthetic mine water mixed with wood and hay, where a very low concentration of organic acids was recorded. The enrichment could stem from biochar's role in reducing VFA accumulation by promoting DIET among microorganisms, thus facilitating the conversion of VFAs into methane (Fig. 3c) (Khan et al., 2021). It has also been proposed that *AUTHM297* harbors a specialized metabolic pathway, allowing it to thrive in conditions where organic matter is extensively degraded (Mirjafari and Baldwin, 2016). Although the specific interaction involving *AUTHM297* and methanogens has not been investigated, a study has suggested that Thermotogae members are capable of growing on acetate when *Methanobacteriaceae* methanogens are present (Balk et al., 2002). Therefore, biochar addition might have enriched the genus *AUTHM297* by accelerating organic matter decomposition and enhancing VFA conversion to methane.

In the T4–2, T4–5, and T4–8 digesters, the relative abundances of Bacteroidetes increased by 14.50 %, 39.48 %, and 11.53 %, respectively, when contrasted with the T4–0 digester. Bacteroidetes have been reported to convert amino acids from protein hydrolysis into acetate through symbiotic degradation of biopolymers (Johnson et al., 2017). Genus-level analysis further revealed that *Petrimonas* and *Macellibacteroides* were the key contributors to the increased abundance of the phylum Bacteroidetes. The genus *Petrimonas*, as a syntrophic acid-producing bacterium, was reported to convert oleate-Na into acetate while producing hydrogen (Grabowski et al., 2005). Guo et al. (2019) and Zhang et al. (2017) indicated that the fermentative genus *Macellibacteroides* acts to decompose organic substances into smaller molecules such as acetate, which supports the formation of methane. Therefore, biochar addition may contribute to enhanced COD degradation through the enrichment of the phylum Bacteroidetes.

In contrast to the increase in Bacteroidetes, a significant reduction in the relative abundance of the phylum Firmicutes was observed

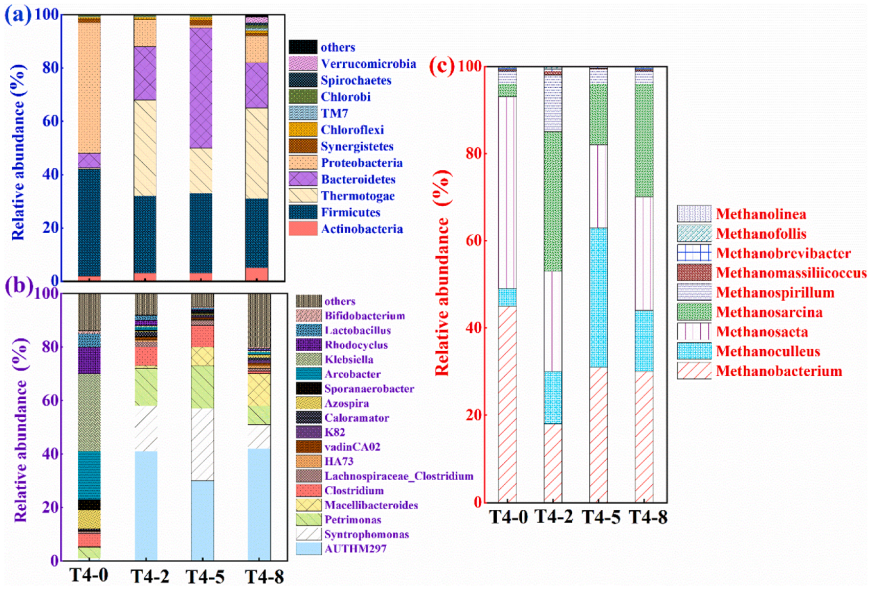

Fig. 5. Effect of biochar on the relative abundance of bacteria at the (a) phylum level, (b) genus level, and (c) archaea at the genus level during anaerobic digestion of food wastewater with 4 g/L oleate-Na.

(Fig. 5a). A similar reduction was observed in the phylum Proteobacteria, where biochar addition decreased its relative abundance by 79.59 %, 97.96 %, and 81.02 % in the digesters T4–2, T4–5, and T4–8, respectively, compared to T4–0. Further analysis at the genus level showed a notable reduction to the genera *Rhodocyclus* and *Klebsiella*. It has been reported that both *Rhodocyclus* and *Klebsiella* were able to tolerate high concentrations of oleate-Na by consuming VFAs (Ahmad et al., 2020; Fonseca et al., 2017). Thus, the significant reduction in their relative abundance might have resulted from biochar's role in facilitating VFA conversion into methane, thereby decreasing carbon sources available for their proliferation (Rafieenia et al., 2019).

Organic matter was ultimately broken down through anaerobic treatment processes, with methanogens playing a key role in this energy conversion. The primary archaeal genera were not notably affected by biochar. *Methanobacterium*, *Methanosaeta*, *Methanoculleus*, *Methanosarcina*, and *Methanospirillum* were key archaea with the total relative abundance of over 90.00 % (Fig. 5c). Among these, the genus *Methanobacterium* and *Methanoculleus* were typical hydrogenotrophic methanogens that utilized H_2 to reduce CO_2 and generate methane (Guo et al., 2020a). However, the relative abundances of these genera exhibited distinctly different trends in response to biochar addition. *Methanobacterium* relative abundances decreased by 57.34 %, 27.37 %, and 30.83 % in digesters T4–2, T4–5, and T4–8, respectively, compared to T4–0, while the relative abundances of *Methanoculleus* increased by 1.94, 6.81, and 2.44 times, respectively (Fig. 5c). This phenomenon could be attributed to biochar as a conductive medium to facilitate DIET among microorganisms. *Methanoculleus* exhibited a stronger adaptability and was more effective to utilize hydrogen, thereby enriched in anaerobic digestion in comparison with *Methanobacterium* (Feng et al., 2023).

Methanosaeta efficiently utilizes acetate as the sole substrate for acetoclastic methanogenesis (Guo et al., 2020b). Compared to its relative abundance of 44.11 % in the digester T4–0, the relative abundances of *Methanosaeta* in the digesters T4–2, T4–5, and T4–8 decreased by 47.27 %, 56.81 %, and 40.91 %, respectively (Fig. 5c). This phenomenon was possibly due to biochar addition to reduce the concentrations of VFAs, including acetate, during digestion (Fig. 3c), to restrain the growth and metabolic activity of *Methanosaeta*. By contrast, *Methanosarcina*, a versatile methanogen, could use various substrates, such as acetate, CO_2, H_2, CO, and methylamine for growth, and thus enriched in response to biochar addition. Compared to the digester T4–0, the relative abundances of *Methanosarcina* increased by 9.67, 3.66, and 7.61 times in the digesters T4–2, T4–5, and T4–8, respectively (Fig. 5c). Wang et al. (2023) reported similar findings, attributing the increased methane production to a 26.90–40.80 % rise in *Methanosarcina* abundance observed in the context of swine manure undergoing anaerobic digestion with ammonia stress.

3.4.2. *Methanogenesis pathway prediction*

The KEGG database was used to predict three methane metabolism pathways, including hydrogenotrophic, acetoclastic, and methylotrophic methanogenesis, in response to biochar addition. Moderate biochar addition significantly promoted the activity of methanogenic microorganisms (Fig. 6). Compared to T4–0, the total abundance of methanogenesis-related enzymes in the digesters T4–2 and T4–5 increased by 76.27 % and 83.41 %, respectively, while decreased by 5.65 % in the digester T4–8. This phenomenon was linked to the porous nature and substantial specific surface of biochar, which created numerous sites for microbial growth and promoted enzyme synthesis by facilitating DIET among microbial communities (Lee et al., 2022b). However, excessive biochar

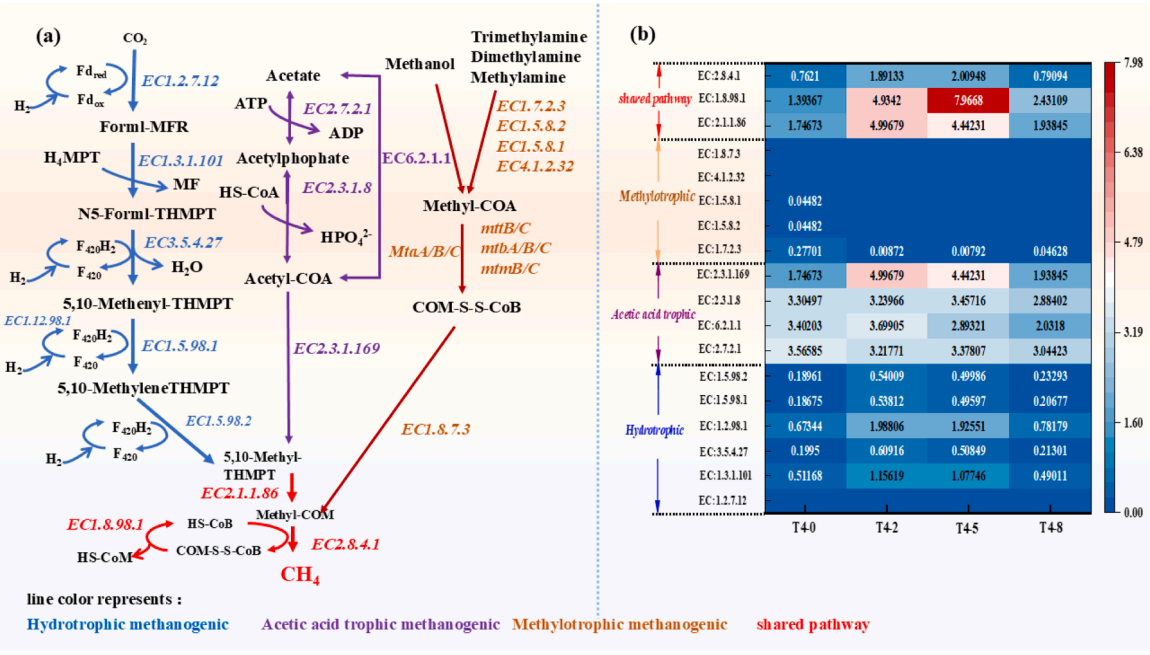

Fig. 6. Effect of biochar on (a) key methanogenic metabolic pathways and (b) the relative abundance of key enzymes in anaerobic digestion of food wastewater with 4 g/L oleate-Na.

addition intensified the adsorption of intermediates, reducing their availability for microbial activity and enzyme production (Masebinu et al., 2019b).

Biochar addition did not alter the primary pathways of methane production. Acetoclastic methanogenesis dominated in all digesters, accounting for 66.59 %, 47.63 %, 42.81 %, and 58.12 % in the digesters T4–0, T4–2, T4–5, and T4–8, respectively (Fig. S3, Supplementary Data). Detailed analysis revealed a minor reduction in the proportion of enzymes involved in acetoclastic methanogenesis with biochar addition, alongside an increase in those associated with hydrogenotrophic methanogenesis. Compared to T4–0, the abundance of acetoclastic methanogenesis-related enzymes decreased by 28.48 %, 35.72 %, and 12.71 %, while that related to hydrogenotrophic methanogenesis increased by 55.60 %, 39.50 %, and 15.79 % in the digesters T4–2, T4–5, and T4–8, respectively (Fig. S3, Supplementary Data). The increased hydrogenotrophic methanogenesis could be related to biochar addition to enhance the absolute abundance of F420 hydrogenase [EC:1.12.98.1], an enzyme central to interspecies hydrogen transfer (IHT) and DIET pathways, which are significant for methane production (Wang et al., 2020; Ney et al., 2017).

Biochar addition facilitated the synthesis of common coenzymes in all three methane production pathways, including tetrahydromethanopterin S-methyltransferase [EC: 2.1.1.86], disulfide reductase [EC: 1.8.98.1], and methanogen coenzyme M reductase [EC: 2.8.4.1]. The total number of common coenzymes in the digesters T4–2, T4–5, and T4–8 increased by 202.94 %, 269.47 %, and 32.23 %, respectively, compared to the digester T4–0. Biochar addition primarily promoted methane production by increasing the abundance of disulfide reductase [EC:1.8.98.1], by 71.87 %, 101.46 %, and 40.16 % in the digesters T4–2, T4–5, and T4–8 when comparing to T4–0, respectively. This increase was possibly due to the conductive properties of biochar, which promoted DIET among microorganisms and enhanced the activity of disulfide reductase [EC:1.8.98.1], catalyzing disulfide bond reduction and transferring electrons to methanogenic enzymes for increased methane production (Ji et al., 2024).

3.4.3. Microbial response to sludge additions at biochar addition

Biochar addition showed a significant positive correlation with MLSS and MLVSS concentrations and cumulative methane production (Fig. 7a). As detailed in Section 3.3, biochar significantly increased MLSS and MLVSS concentrations by promoting microbial growth and metabolism, which in turn enhanced methane production. This effect might be attributed to biochar's role in adsorbing organic matter, which led to the development of dense aggregates and multilayer structures in the sludge, thereby enhancing sludge activity and facilitating methane production (Zhang et al., 2020). Moreover, increased sludge concentrations have been shown to enhance the anaerobic digester's resistance to detrimental factors, including the accumulation of VFAs, thus promoting microbial metabolism and contributing to stable methane production (He et al., 2023a).

Significant positive correlations were found between cumulative methane production and the relative abundances of the phyla Thermotogae and Bacteroidetes (Fig. 7a). This finding aligns with the conclusions in Section 3.4.1, indicating that biochar addition enhanced microbial synergistic metabolism by optimizing the microbial habitat. Further genus-level analysis revealed a strong positive correlation between the relative abundance of the phylum Thermotogae and the genus *AUTHM297* (Fig. 7a). Thermotogae was recognized for its ability to decompose organic matter in high-temperature environments, while the genus *AUTHM297* provided specific metabolic products or electron acceptors under these conditions, facilitating organic matter degradation and increasing the production of intermediate products that enhanced methane generation (He et al., 2023b).

Significant positive correlations were found between the phylum Bacteroidetes and the genera *Syntrophomonas* and *Petrimonas*, both of which belong to the phylum Firmicutes (Fig. 7a). This correlation could be due to the formation of a mutually beneficial metabolic network under the influence of biochar (Cui et al., 2021). The phylum Bacteroidetes is known for its ability to decompose complex organic compounds into smaller organic molecules, providing abundant substrates for the genera *Syntrophomonas* and *Petrimonas* (Johnson et al., 2017). These genera then convert these substrates into methane through synergistic metabolism (Zhang et al.,

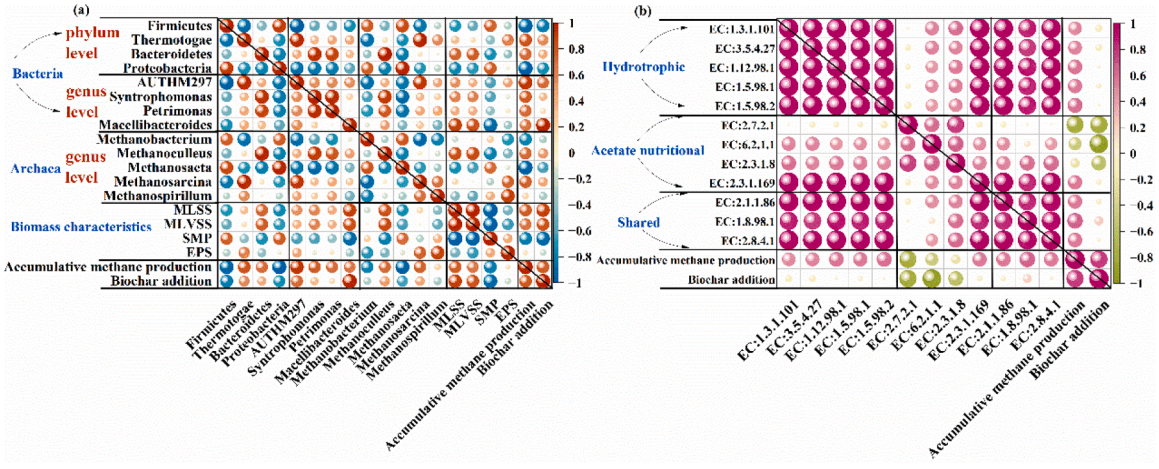

Fig. 7. Correlation analysis of (a) major microorganisms, sludge characteristics and methane production (b) methanogenic key enzymes with methane production in anaerobic digestion of food wastewater with the addition of oleate-Na at 4 g/L.

2019).

Biochar addition demonstrated a notable positive correlation with the relative abundances of the genera *Methanoculleus* and *Methanosarcina* (Fig. 7a). As detailed in Section 3.4.1, the relative abundances of these genera significantly increased with higher levels of biochar addition. *Methanoculleus* is known as a key representative of hydrogenotrophic methanogens, which primarily produce methane by utilizing hydrogen and carbon dioxide (Li et al., 2019). *Methanosarcina,* a versatile methanogen, exhibited the ability to utilize substrates such as hydrogen, acetate, and methanol to generate methane. (Zhang et al., 2020b). This suggests biochar's incorporation likely optimized the microbial metabolic environment, enhancing the efficiency of hydrogen utilization as a substrate while also activating multiple substrate conversion pathways, thereby promoting methane production.

Biochar addition showed a significant positive correlation with the abundance of hydrogenotrophic methanogenesis-related enzymes and common coenzymes across the three methane production pathways. Additionally, a notable positive correlation was observed between the abundances of these enzymes (Fig. 7b). This indicated that biochar addition likely enhanced methane production by promoting DIET among microorganisms, which in turn increased the expression of key enzymes and synergistically activated multiple methane production pathways (Lee et al., 2022a). This finding is consistent with the discussion in Section 3.4.2, which highlighted that biochar promoted the relative abundance of hydrogenotrophic methanogenesis-related enzymes and common coenzymes across the three methane production pathways.

4. Conclusion

The results indicated that high concentrations of oleate-Na significantly inhibited methane production in the anaerobic treatment of food wastewater. However, adding an optimal amount of biochar, particularly at 5 g/L, markedly enhanced the efficiency of COD removal and methane yield. Biochar not only facilitated the degradation of VFAs but also reduced system acidification, thereby improving the metabolic activity and stability of the sludge. Furthermore, biochar altered the microbial community structure, enriching *Methanoculleus* and *Methanosarcina* while promoting direct interspecies electron transfer (DIET). This process improved both the efficiency of anaerobic digestion and the optimization of methane production pathways. In this study, 5 g/L of biochar was found to be the suitable dosage for food wastewater containing 4 g/L oleate-Na. In conclusion, the addition of biochar effectively mitigated the negative impacts of high-oil wastewater on anaerobic digestion, providing a valuable strategy for improving wastewater treatment and resource recovery efficiency. Nevertheless, the suitable dosage of biochar to enhance anaerobic digestion depends highly on wastewater characteristics, thereby requiring further investigation on their interrelation for the treatment of different waste streams.

CRediT authorship contribution statement

Ashley J. Ansari: Writing – review & editing. **Na Zhang:** Validation, Resources. **Yongzhen Peng:** Writing – review & editing, Validation, Supervision. **Xiaoye Song:** Visualization, Validation, Supervision, Resources, Project administration, Funding acquisition. **Kemeng Feng:** Writing – review & editing, Writing – original draft, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.eti.2024.104000.

Data availability

Data will be made available on request.

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