Organic matter removal from landfill leachate using a biochar-enhanced microbial electrolytic cell-anaerobic digestion system at different HRT☆

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ABSTRACT

The integration of microbial electrolytic cell with anaerobic digestion (MEC-AD) system is a promising method for improving landfill leachate treatment. Biochar addition further enhances overall process efficiency. However, the impact of varying hydraulic retention times (HRT) on operational efficiency and microbial dynamics is not well understood. This study examines the effects of different HRT on the degradation efficiency and microbial composition of biochar-amended MEC-AD system. Here, optimal performance was observed at 48 h HRT, achieving 70.67% of chemical oxygen demand (COD) removal efficiency, with a notable enrichment of functional microbial strains, which represented a 67.58% increase compared with the removal efficiency at 24 h of HRT. Furthermore, the COD removal efficiency was enhanced by approximately 4% with biochar addition under 24 h of HRT. Essentially, maintaining appreciable HRT coupled with biochar addition is a plausible strategy for enhanced landfill leachate treatment. Mechanistically, this enhanced system performance, specifically efficient organic matter degradation involved the disruption of key structural components within contaminants, including heterocyclic aromatic hydrocarbons and conjugated unsaturated bonds as indicated by GC–MS analysis. Microbial community dynamics revealed that the addition of biochar and the extension of HRT both facilitate *Acidobacteria* proliferation, while the addition of biochar particularly promoted the enrichment of functional microbes like *Pseudomonas*. Essentially, both HRT regulation and biochar addition were critical in microbial community structuring. Enrichment of acidogens including *Azoarcus* and *Longilinea*, facilitates the subsequent production of acetic acid production, optimizing carbon metabolism. Finally, investigating the scalability of biochar-amended MEC-AD systems under optimized operational parameter (HRT) conditions is urgent to ensure sustainable enhanced landfill leachate treatment and resource recovery.

1. Introduction

In recent years, the production of landfill leachate in China has been increasing year by year. Specifically, the daily output of landfill leachate in China ranged from 0.53 to 0.75 million tons in 2021, and it is expected to reach 7.2×10^7 tons by 2030 (Shi et al., 2021). Landfill leachate is characterized by high organic matter content (with COD as the main reference parameter), elevated heavy metal content, high ammonia nitrogen concentration, and salinity. Notably, refractory organic compounds, such as, aromatic hydrocarbons, and aliphatic hydrocarbon organics, comprise 30–50% of landfill leachate (Aralu et al., 2024). The proportion of these recalcitrant compounds has been rising

annually (Saghi et al., 2024).

The treatment of landfill leachate, which is characterized by a low ratio of biochemical oxygen demand to chemical oxygen demand ($BOD_5/COD < 0.3$) and low biodegradability, remains a significant challenge (Yang et al., 2025; Martins dos Santos et al., 2023). Conventional methods, such as physicochemical (e.g., adsorption, membrane separation) and biological (e.g., anaerobic digestion, aerobic treatment) processes often exhibit limited effectiveness in addressing the high concentration and complex composition of refractory organic matter in leachate. To enhance the biodegradability of complex organic substrates and improve the overall treatment efficiency of anaerobic digestion systems, integration with microbial electrolytic cell (MEC-AD) has

emerged as a particularly promising approach in recent research (Li et al., 2023). The MEC-AD system leverages the advantages of both electrochemical and biological reduction methods, while also facilitating methane recovery. This synergistic integration can effectively promote the metabolism of microorganisms and the efficiency of interspecies electron transfer during anaerobic fermentation, thereby improving the treatment of refractory organic matter. For instance, under high ammonia concentration stress (5.0 g/L), the methane production rate in the MEC-AD system has been reported to be 2.0 to 2.7 times higher than that in the conventional AD system (Zhao et al., 2022). This enhanced performance highlights the potential of the MEC-AD system in overcoming the limitations of standalone anaerobic digestion for the treatment of recalcitrant landfill leachate.

Biochar, a carbon-rich residue from biomass thermal conversion processes like pyrolysis and gasification under anoxic conditions, exhibits excellent electrical conductivity and chemical stability, making it a crucial medium for electron transfer (Yu et al., 2025). In the MEC-AD system, biochar enhances anode organic matter oxidation and cathode reduction reactions, significantly boosting treatment efficiency. Its abundant redox-active functional groups facilitate direct electron exchange with metabolic intermediates, particularly quinone-like compounds. Within the MEC-AD system, uniformly distributed biochar forms a continuous conductive network, reducing solution resistance and improving extracellular electron transfer and proton migration efficiency. The addition of biochar in the MEC-AD system increased biogas production by 16.73% to 29.37% compared to the control group (Li et al., 2024). Moreover, the addition of biochar significantly reduces the number of OTUs in the reactor while facilitating the enrichment of key functional microorganisms such as *Soehngenia*, *Bacteroidota*, and *Desulfomicrobium*, thereby enhancing the treatment performance of the reactor (Zhu et al., 2024).

HRT is a critical parameter in the operation of MEC-AD systems. HRT represents the average residence time of the wastewater within the reactor, and it directly influences the adsorption, degradation, and conversion processes mediated by the prevailing microorganisms (Xiao et al., 2024). An appropriate HRT ensures optimum microbial metabolism, thereby facilitating effective pollutant removal. Furthermore, for biochar-enhanced MEC-AD system, the appropriate HRT helps enrichment of functional microorganisms on biochar surface, which in turn enhances the synergistic effect between electrochemical reactions and anaerobic digestion (Yu et al., 2025). Studies have demonstrated that in MEC-AD systems, efficient biogas production and organic matter removal can be achieved via the optimization and combination of specific parameters, including HRT, applied voltage, and organic loading rate. Notably, this can be attained even at relatively shorter HRTs (1 d), a condition that poses challenges in traditional anaerobic reactors (Dalkilic and Ugurlu, 2024). At a total HRT of 25 h (1 d for MEC and 1 h for the GAC biofilter), efficient pollutants removal was realized during grey water treatment using an integrated MEC and GAC biofilter process, highlighting the critical role of specific HRT settings for optimal organic waste treatment and energy recovery using the MEC-AD system (Dhadwal et al., 2021). From an engineering application perspective, determining the optimal HRT provides significant reference value for the large-scale deployment of biochar-enhanced MEC-AD system. Landfill leachate treatment facilities are frequently confronted with the challenge of balancing removal efficiency, operational costs, and process stability. Longer HRT typically translates to larger reactor volumes and higher construction costs (Shen et al., 2022). Optimizing the HRT of MEC-AD system guides the adjustment of influent flow during practical operation, which helps enhance the treatment capacity and economic feasibility of system. In addition, clarifying the response pattern of pollutant degradation to HRT variations enables flexible operation for addressing fluctuations in the quality and quantity of landfill leachate, thereby improving the shock resistance of system in practical engineering applications (Yu et al., 2025). However, electrode stability during HRT operations may be compromised by microbial and chemical

corrosion, leading to process inefficiencies. Current research has investigated the impacts of electrode materials and applied voltage on the efficiency of MEC-AD systems (Yang et al., 2023). Nevertheless, the role of HRT in biochar-enhanced MEC-AD systems, particularly the intrinsic correlations between HRT and the enhancement of COD removal efficiency, the regulation of organic matter degradation pathways, and the driving of microbial dynamics, remains insufficiently elucidated.

Therefore, it is crucial to investigate the effects of varying HRT on both treatment efficiency and microbial structure in a biochar-enhanced MEC-AD system. To address these gaps, the specific objectives of this study are to: (i) evaluate the influence of different HRT on COD removal efficiencies and microbial composition, (ii) profile the microbial community dynamics across varying HRTs and biochar amendment, (iii) elucidate the mechanisms by which biochar addition and HRT modulation enhance electron transfer, biofilm composition and metabolic efficiency in the MEC-AD system, and (iv) provide insights into practical strategies for optimizing landfill leachate treatment, thereby advancing the applicability of MEC-AD systems in mitigating the challenges posed by complex refractory organic contaminants. This integrative approach aims to bridge the gap between microbial ecology and operational parameter optimization, ultimately providing a framework for future enhancements in landfill leachate management through biochar-assisted MEC-AD technologies.

2. Materials and methods

2.1. Landfill leachate source

The landfill leachate was sourced from a mature landfill site situated in Suzhou City, Jiangsu Province, China. The water quality characteristics of leachate were as follows: COD of 1850–1950 mg/L, BOD_5 of 200–230 mg/L, NH_4^+-N of 820–850 mg/L, pH of 8.0–8.7, electrical conductivity of 8.0–9.0 mS/cm, and salinity of 4500–5600 mg/L. To enhance the growth of anaerobic microorganisms and facilitate the observation of experimental results, the inoculum for MEC-AD system was primarily prepared from landfill leachate and sodium acetate, with the leachate being diluted to reduce its complexity. Prior to operation, the influent was deoxygenated by continuous nitrogen (N_2) purging for 30 mins. Since landfill leachate contains abundant refractory organic compounds, its composition is highly complex. To improve microbial acclimatization, a mixed substrate way of diluted landfill leachate and sodium acetate was employed in this study, which not only enables a direct reflection of water quality variations but also promotes the proliferation of anaerobic microorganisms.

Subject to the substrate type, the MEC-AD system was operated in two stages. Initially, a sodium acetate solution at a concentration of 1.282 g/L was prepared to simulate a landfill leachate treatment environment for a 10 d sludge acclimation period. In the subsequent formal operation stage, landfill leachate, after 2.5-fold dilution with raw water to maintain system stability, was fed into the reactor, with sodium acetate dosed at a concentration of 0.641 g/L. This operational phase lasted for 37 d, bringing the total operation duration to 47 d.

2.2. MEC-AD system construction

In this study, five identical set of single-chamber MEC-AD systems, designated R1 through R5, were constructed. The study employed R1 (HRT = 24 h) as a control without biochar addition. Reactors R2-R5 were operated at 12, 24, 36, and 48 h of HRT, respectively, each receiving 1.5 g of coconut shell-derived biochar. Coconut shell is a prevalent biological waste known for its high carbon content and low ash content, making it an ideal candidate for biochar production. The biochar was produced through the pyrolysis of coconut shells at 700 ℃. For the coconut shell biochar employed in this study, nitrogen adsorption–desorption analysis revealed a BET specific surface area of 24.33 m^2/g and a total pore volume of 0.0501 cm^3/g, indicative of its

moderate porous structure. Applying a 0.6 V potential has been shown to enhance microbial activity in 1.0–5.0 g/L biochar amended setups (Dalkilic and Ugurlu, 2024). The reactor, as depicted in Fig. 1, features a cylindrical design with an inner diameter of 8 cm and a height of 10 cm, providing an effective working volume of 502 mL (Huang and Lee, 2021). Two electrode ports, each with a 10 mm diameter, are positioned at the top for electrode insertion (Zhu et al., 2024). The cathode of the reactor is connected via an electrode port to a stainless steel mesh electrode (SSM-100 μm), while the anode employs a carbon fiber brush. The system employs a continuous flow influent, with the inlet located at the bottom and the outlet at the top, arranged diagonally. To ensure uniform mixing within the reaction system, an integrated magnetic stirring device was installed at the base.

Both the cathode and anode materials were pretreated separately before the experiment. The stainless-steel electrodes were immersed in a 1 mol/L H_2SO_4 solution for 4 h to remove the oxide layer, followed by ultrasonic cleaning with deionized water for later use (Xie et al., 2023). The carbon fiber brush was degreased by soaking it in acetone for 12 h and then activated through calcination in a muffle furnace at 450 °C for 30 min to activate the surface, before being assembled into a three-dimensional anode structure. The electrochemical system employed a three-electrode configuration, with a saturated calomel electrode (SCE, Model-217) serving as the reference electrode. A constant voltage of 1.2 V was applied between the anode and cathode using a DC regulated power supply (Model VC3003, Shengli Instrument Company, China). An external 10 Ω sampling resistor was used to monitor the current through a data acquisition instrument (KEITHLEY2700 multi-channel data measurement and monitoring software), which documents the electrode potential every 10 min and recorded the data for the positive and negative electrodes.

### 2.3. Analytical methods

2.3.1. Wastewater quality
Water samples were pre-treated through a 0.45 μm filter membrane prior to detection and analysis. COD and 5 d biochemical oxygen demand (BOD_5) were routinely determined using a DR3900 spectrophotometer (HACH, USA) and BOD Trak II analyzer (HACH, USA), respectively. The COD of influent and effluent samples were measured at different HRT. For instance, in the R5 reactor, with a 48h HRT, water samples were collected every 48 h. The DR3900 spectrophotometer was used to measure COD, with all samples analyzed in triplicate to minimize error. The average value of the total COD effluent from each reactor was calculated, and the stability of the reactor treatment efficiency is evaluated using analysis of variance (ANOVA). The process is divided into two stages. In Stage I (sludge acclimation period), the influent COD concentration is basically maintained at 455–500 mg/L, and the COD removal rate of all reactors showed significant upward trends. In Stage II (formal operation period), the influent COD concentration was maintained between 900–1000 mg/L.

2.3.2. Electrochemical performance
Cyclic voltammetry (CV) is a widely employed technique in electrochemical research for evaluating the electrocatalytic performance of electrode materials. This method records the current–potential curve during the redox reaction on the electrode surface by cycling the applied potential linearly between two set values. The resulting voltammogram provides insights into the kinetic characteristics of the electrocatalytic reaction, the number of active sites, and the reversibility of the electrochemical process.

The electrocatalytic performance of the anodic biofilm was evaluated using a CHI760E electrochemical workstation (CH Instruments, Inc., China) and cyclic voltammetry (CV) (Yin et al., 2019). A standard three-electrode system was employed, with the anode loaded with biofilm serving as the working electrode, a carbon brush as the counter electrode, and a saturated calomel electrode as the reference electrode. The potential was scanned from −1.0 V to + 1.2 V at a rate of 10 mV/s. The anodic biofilm is the primary site for electrocatalytic reactions, and the microbial community on its surface, as well as the extracellular electron transfer process, directly influence the efficiency of these
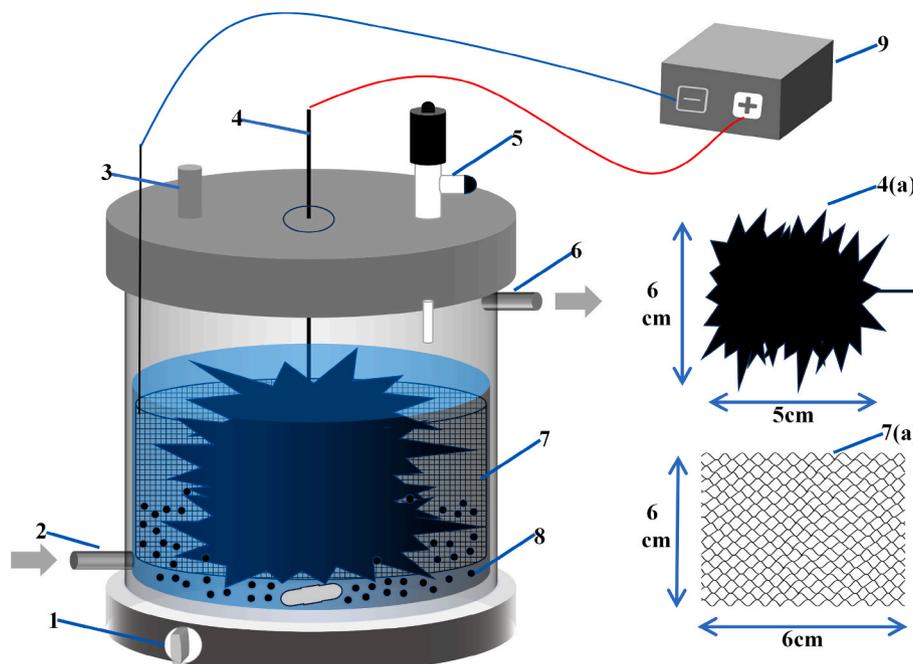
1-Magnetic stirrer, 2-Influent, 3-Gas collection port, 4-Anode carbon brush, 4(a)-Carbon brush structure, 5-Reference electrode, 6-Effluent, 7-Cathode stainless steel mesh, 7(a)-Expanded structure of cathode stainless steel mesh, 8-Biochar particles, 9-Power supply

Fig. 1. Schematic diagram of MEC-AD System.

electrochemical reactions. The applied potential scan range allows for the oxidation of organic substrates and the redox conversion of electron carriers within the biofilm. The scan rate of 10 mV/s was selected to avoid polarization due to excessively fast rate or background current interference from an excessively slow rate, enabling the capture of the current response states of the biofilm at different potentials.

2.3.3. Full spectrum

Ultraviolet–visible (UV–Vis) spectroscopy was conducted using a Shimadzu UV-3600 spectrophotometer, covering a wavelength range of 200–600 nm. Samples were diluted fivefold to ensure absorbance values remained within the instrument's linear range and to minimize turbidity interference. Once the system stabilized, the effluent spectrum from the reactor containing biochar was measured to assess the degradation of refractory organic compounds (Dai et al., 2025).

2.3.4. Gas chromatography

In order to further explain the transformation of organic pollutants in the refractory organic wastewater after treatment, GC–MS (GC/MS:7890A-5975C GC/MS, Agilent Technologies, USA) was used to measure the types and relative contents of refractory organic pollutants in the effluent of each system.

For the pretreatment process prior to GC–MS analysis, a 100 mL water sample was initially extracted using liquid–liquid extraction (Nijenhuis et al., 2025). This extraction process was repeated three times. It is important to note that the volume of each individual extraction was the same (5 mL). Subsequently, the pH of the remaining water sample was adjusted to above 11 by adding NaOH. The liquid–liquid extraction process was then repeated three more times with 15 mL of dichloromethane each time. To enhance the extraction efficiency, an appropriate amount of NaOH was added during the extraction process to neutralize the water sample. Once all extraction phases were combined, the residual water was filtered using 30 g of anhydrous sodium sulfate. Next, the organic phase was concentrated to 5 mL utilizing a rotary evaporator. Subsequently, the sample was further concentrated to 1 mL by purging it with high-purity nitrogen. The final concentrated sample was then ready for analysis. For the GC–MS analysis, 0.4 mL of the concentrated sample was taken and analyzed using a GC–MS instrument equipped with an HP-5MS chromatographic column measuring 30 mm in length, 25 mm in diameter, and 25 mm in thickness. A carrier gas flow rate of 1 mL/min was maintained throughout the analysis. Here, the column oven temperature was initially set at 50 °C for 5 min, followed by a linear temperature increase to 280 °C at a rate of 5 °C/min. To identify the structural components accurately, the obtained data was compared with the NIST 16 mass spectrometry library database for comprehensive matching.

2.3.5. Microbial community dynamic

After stable operation of the MEC-AD systems (at 47th day), a total of 10 sludge samples of cathode biofilm and anode biofilm were collected from all the five reactors. The cathode samples were labeled as R1-1, R1-2, R1-3, R1-4, R1-5 respectively; the anode samples were labeled as R2-1, R2-2, R2-3, R2-4, R2-5 respectively. The prefixes "1-" and "2-" denote the cathode and anode, respectively, while the suffix corresponds to the previous reactor numbering. All samples were immediately stored in an ultra-low temperature refrigerator at −70 °C until DNA extraction. DNA was extracted using FastDNA®Spin Kit (MP Biomedicals, USA) and quality verified by 1% agarose gel electrophoresis, DNA concentration and purity determined using NanoDrop2000 (Thermo Scientific, USA). Amplification of the V3 −V4 variable regions of the 16S rRNA gene employed two primers: a universal primer 338F (5′-ACTCCTACGG-GAGGCAGCAG-3′) and another commonly used primer 806R (5′-GGACTACHVGGGTWTCTAAT-3′), PCR reaction system (25 µL) containing 5 × FastPfu Buffer 4 µL, 2.5 mM dNTPs 2, 5 primers 0.8 µL each, FastPfu DNA polymerase 0.4 µL and 10 ng DNA template, amplification program run on ABI GeneAmp®9700 PCR instrument: 95 °C pre-

denaturation for 3 min followed by 27 cycles (denaturation at 95 °C, annealing at 55 °C, extension at 72 °C for 30 s each), followed by extension at 72 °C for 10 min (Liu et al., 2016). PCR products were detected by 2% agarose gel electrophoresis, then gel cut and purified, quantified by QuantiFluor™-ST (Promega, USA), and finally PE300 double-ended sequencing was completed on Illumina MiSeq platform (Illumina, USA) (Huang et al., 2021). The obtained data were analyzed bioinformatically by QIIME 2 (Chen et al., 2018).

3. Results and discussion

3.1. COD removal efficiency at different HRT

The COD removal efficiency is an important indicator reflecting microbial activity, and its variation reflects the metabolic characteristics of microorganisms in the reactor under different operating conditions.

The COD removal efficiency of each reactor exhibited a significant positive correlation with the HRT. R5 at an HRT of 48 h exhibited a significantly higher removal efficiency than the other reactors ($p < 0.05$) (Fig. 2 A). HRT is a key parameter in MEC-AD systems, which affects organic matter degradation rate and methane production, thus directly affecting the removal efficiency of refractory organic matter in wastewater (Appels et al., 2008). An appropriate HRT is a prerequisite for maintaining stable operation of the system; relatively short HRTs may lead to ineffective microbial oxidation of pollutants, resulting in substandard effluent quality; excessively long HRTs may cause excessive growth of microorganisms in the system, resulting in sludge bulking and other problems. COD removal efficiencies increased in tandem with increasing HRTs (Fig. 2 B). At 48 h HRT, the reactor consistently maintained the highest removal efficiency, indicating that an appropriate HRT is key for optimizing metabolic efficiency (Nelabhotla et al., 2020). But its operation effect is not stable enough. Carbon source has a direct effect on the biomass accumulation as well as the synthesis of microbial enzyme systems. Notably, at an HRT of 24 h, the removal rate of R3 with biochar addition was significantly higher than that of R1 without biochar addition, indicating that biochar addition is beneficial for improving treatment efficiency. An HRT of 48 h not only enhanced functionality in MEC-AD systems but also facilitated adsorption and electron transfer, effectively mitigating the adverse effects associated with high organic loading. Conversely, when the biochar-free MEC − AD system was subjected to a voltage increase from 0.5 V to 1.0 V, it exhibited irreversible deterioration characterized by VFA accumulation, pH drop, reduction in removal efficiency (Xu et al., 2019). This suggests that the stability of the system without biochar addition is inferior to that of the biochar-added system and highlights the remarkable interplay between particularly biochar and HRT.

3.2. Change in cyclic voltammetry curve

The cyclic voltammetry data revealed that the anode and cathode potentials in each reactor exhibited consistent stability, ranging from 0.043 mV to 0.603 mV and 4.26 mV to 5.51 mV, respectively. These values approached the optimal potential (vs. SCE) for sodium acetate oxidation, which typically lies within the range of 400–500 mV (Fig. 3).

The enclosed area within the closed CV curve, serves as a representation of the biofilm's charging and discharging characteristics, offering insights into its electron transfer proficiency. The stability of electrocatalytic reactions mediated by the biofilm varies across reactors, likely attributed to distinctions in biofilm composition and electron transfer pathways on the anodic biofilms of R1-R5. Notably, R2 with HRT of 12 h exhibited the most balanced oxidation and reduction peaks among all reactors, characterized by a minimal peak potential difference (Δ E), indicating favorable reaction reversibility. This observation suggests that prolonged HRT maybe inhibit the biofilm's electrochemical activity (Leicester et al., 2020).

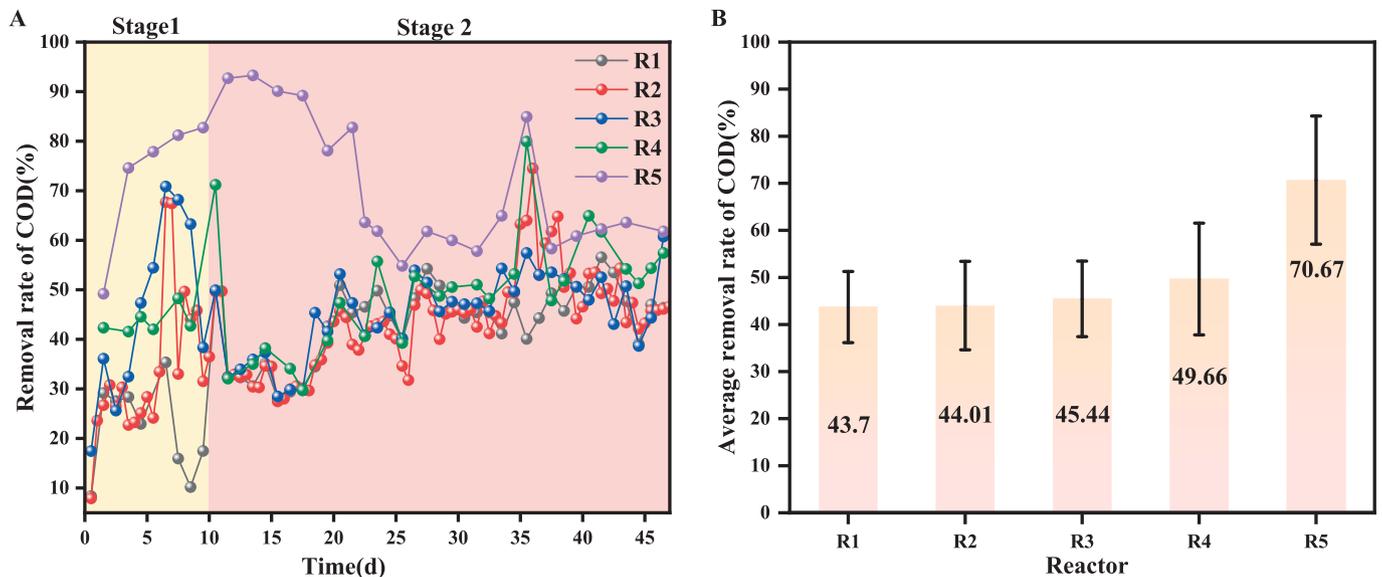The oxidation or reduction peak current magnitude in the curve

Fig. 2. COD removal efficiencies under different HRT within MEC-AD systems (A); Average COD removal efficiencies of reactors (B) (COD: Chemical Oxygen Demand).
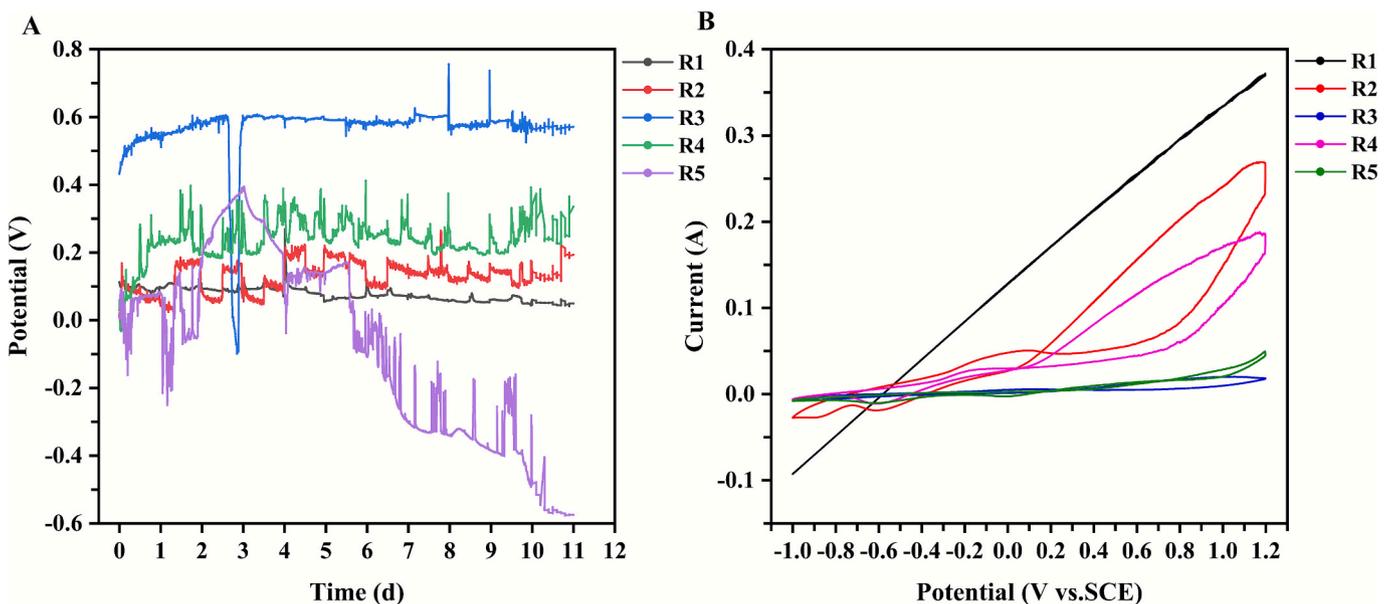

Fig. 3. Adaptability of MEC-AD systems under different HRT (A) and Cyclic Voltammetry curves of electrodes (B).

serves as an indicator of the electrocatalytic activity strength. The curve for R1(without biochar) exhibits a nearly "linear" shape without distinct redox peaks, possibly attributed to the markedly low electrocatalytic activity of the anodic biofilm in R1. This could be a result of impeded electron transfer between the biofilm and the electrode, leading to a feeble electrochemical response and the absence of significant electrocatalytic reactions (Hari et al., 2017). Conversely, peaks are observed in R2, R3, R4, and R5, suggesting that the introduction of biochar enhances microbial growth and boosts the catalytic performance of the biofilm within the MEC-AD system.

The intersection of closed curves in cyclic voltammetry (CV) may suggest the occurrence of multiple electron transfer processes within the system (Hari et al., 2017). Competition for substrates or electron transfer routes between distinct bacterial species can lead to shifts in the predominant bacterial species during different scan cycles, consequently influencing the electrode reaction current. Moreover, the electrode

current may be indirectly influenced by extracellular polymeric substances (EPS) produced by microorganisms, whereby a reduction in charge transfer resistance (RCT) is accompanied by an increase in current density (Zhou et al., 2018).

3.3. Full spectrum analysis of effluent at different HRT

Landfill leachate is mainly composed of conjugated bonds and PAHs. The absorption wavelengths below 250 nm, 250–290 nm and 290–350 nm are correlated with conjugated bonds, polycyclic aromatic hydrocarbons (PAHs) and carbonyl groups, respectively. As shown in Fig. 4, after MEC-AD treatment, the absorption intensity at wavelengths below 240 nm increased with HRT, while the absorption intensity at wavelengths from 240 nm to 280 nm decreased, indicating the increase of conjugated unsaturated bonds and the decrease of PAHs. In addition, it was found that when voltage stimulation was applied, the system
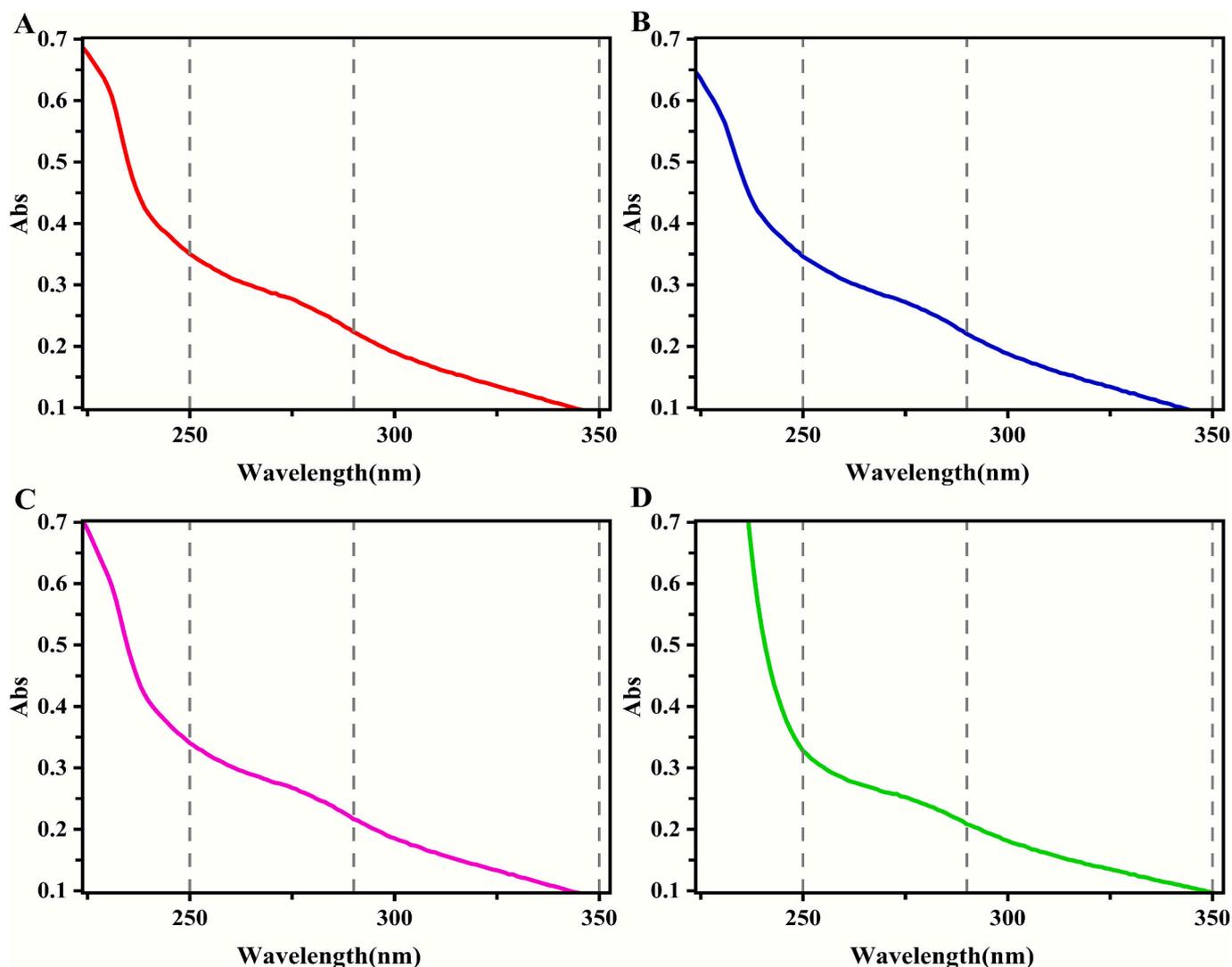
Fig. 4. UV–Vis Analysis of experimental effluent samples. R2 with HRT of 12 h (A); R3 with HRT of 24 h (B); R4 with HRT of 36 h (C); R5 with HRT of 48 h (D).

exhibited enhanced enzyme activity, promoted microbial metabolism, increased microbial community abundance, and thus promoted PAHs degradation (Chen et al., 2019). The change of absorbance showed that the structure of heterocyclic aromatic hydrocarbons and conjugated unsaturated bonds tended to be destroyed after MEC-AD treatment.

By measuring the UV full spectrum images of reactors R2-R5 (with biochar addition), it is not difficult to find that there is a peak value in the curve, but there is a difference in the slope of the curve. By measuring the UV full-spectrum profiles of reactors R2-R5 (with biochar addition), it is not difficult to find that all curves exhibit a characteristic peak value, while the slopes of the curves show slight differences with partial variations. This indicates that the core pollutant degradation mechanisms remained consistent, i.e., the adjustment of HRT did not substantially alter the degradation pathways or product types of
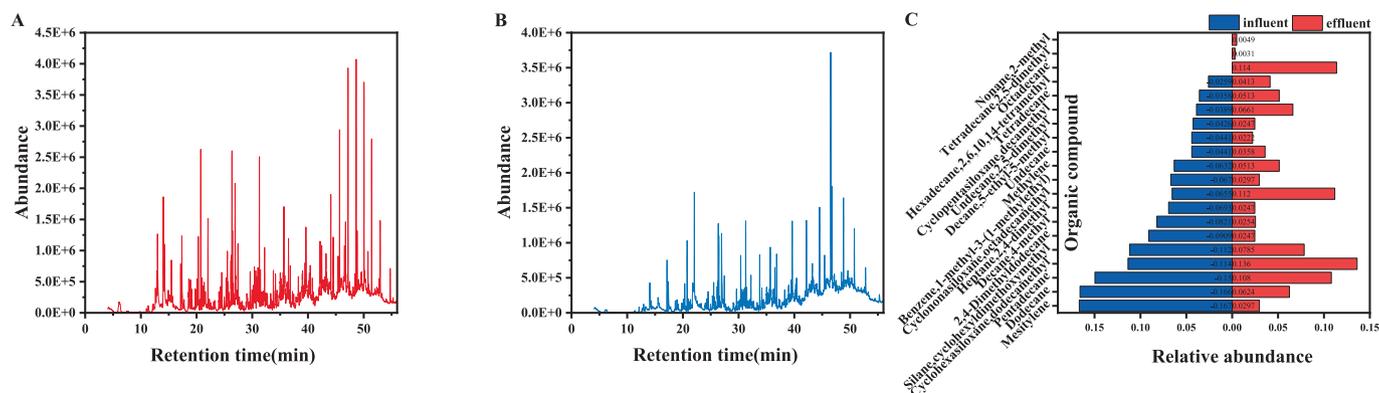

Fig. 5. Chromatogram of influent (A); Chromatogram of effluent (B); Population Pyramids of influent and effluent (C).

pollutants. In general, a longer HRT results in improved degradation of organic matter, hence better effluent quality.

3.4. Gas chromatographic analysis of effluent at different HRT

About 20 kinds of main organic compounds were detected in the influent and reactor effluent with HRT of 36 h, as shown in Fig. 5 A, B. Alkanes and their derivatives, aromatic hydrocarbons, silicon compounds and halogenated hydrocarbons were the main components in the landfill leachate stock solution, among which were Mesitylene (16.67%), Cyclohexasiloxane, dodecamethyl (11.36%), Pentadecane (14.96%), Dodecane (16.59%), and Dimethoxysilane (11.19%). After

treatment in the MEC-AD system, the peak area of each reactor showed a decreasing trend, which may be due to the oxidative cracking of long chain alkanes. However, its degradation efficiency for longer-chain alkanes is unsatisfactory. For instance, the proportions of octadecane, pentadecane, and cyclononasiloxane increased by 11.4%, 2.2%, and 4.7%, respectively, indicating that these substances are more difficult to degrade. This phenomenon may be attributed to the re-synthesis of alkanes by the cathode using CO_2 and other substances, or to the desorption and release of long-chain alkanes initially adsorbed on the electrodes after reaching saturation (Ma et al., 2023; Zhang et al., 2017).

As can be seen from Fig. 5 C, the content of most alkanes and aromatic hydrocarbons decreased significantly, with Mesitylene decreasing
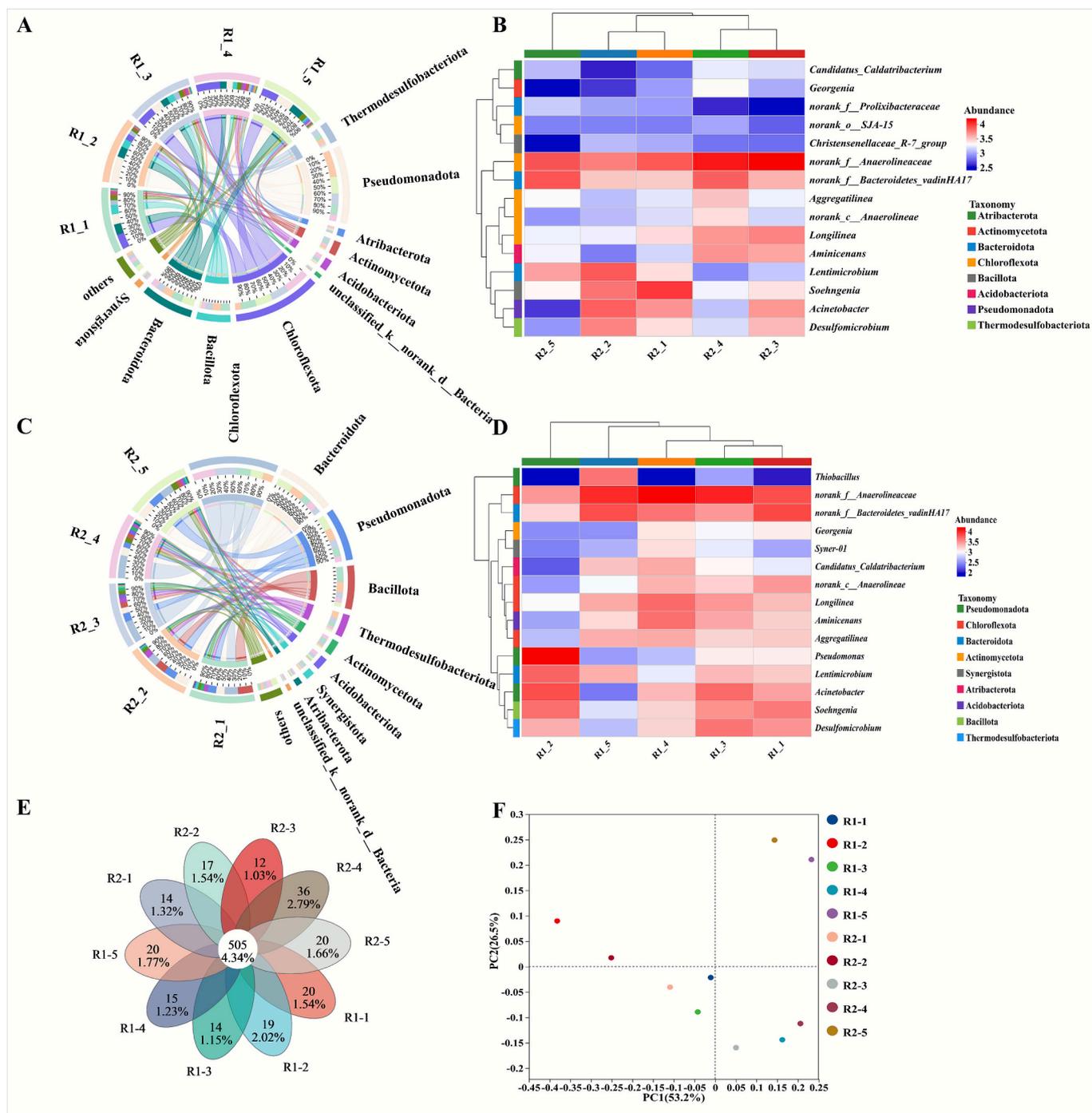

Fig. 6. Microbial Information. Phylum-level Circos plot of the biocathode (A); Genus-level community heatmap of the biocathode (B); Phylum-level Circos plot of the bioanode (C); Genus-level community heatmap of the bioanode (D); Venn diagram of species abundance (E); Genus-level PCoA plot (F).

by 77.98% and Dodecane levels decreasing by 53.46%. Silane, cyclohexyldimethyl-methyl, 2,4-Dimethyldodecane, Hexadecane,2,6, 10,14-tetramethyl decreased by about 64.71%. Branched chain alkanes were more difficult to degrade than straight chain compounds. The degradation rate of branched chain compounds might be affected by the position of substituents. The methyl substituted sites were easy to be attacked by oxidation and could be gradually degraded by microbial demethylation. Long-chain cyclic siloxanes may have partially degraded to smaller siloxanes (Peter et al., 2025). In contrast, increasing the dosage of biochar helps provide more reaction sites, thereby enhancing electron transfer efficiency and improving the degradation efficiency of the reactor.

3.5. Microbial community analysis at different HRT

Fig. 6 A and B reveal the predominant microbial phyla and genera present in each MEC-AD reactor with cathode biofilm, including *Chloroflexota* (5%), *Pseudomonadota* (18%), *Bacteroidota* (13%), *Thermodesulfobacteriota* (8%), *Actinomycetota* (12%), and *Acidobacteriota* (10%), which are all integral to fermentation and acid metabolism processes (Tian et al., 2021). Whereas the four phyla, namely *Proteobacteria*, *Bacteroidota*, *Firmicutes*, and *Chloroflexi*, are the dominant microbial communities in leachate from multiple landfill sites (Qian et al., 2024). Notably, the highly abundant taxa *Pseudomonas*, *Actinobacteria*, *Thermodesulfobacteria* and *Acidobacteria* play pivotal roles in the degradation and transformation of organic matter. Within *Proteobacteria*, *Pseudomonas* facilitates enhanced extracellular electron transfer (EET) and produces surfactants that solubilize hydrophobic organic matter, aiding in the degradation of polycyclic aromatic PAHs (Yu et al., 2018). *Acidobacteria* possess characteristics such as acid and heavy metal tolerance, and tend to accumulate in iron-rich environments (Lin et al., 2019). *Thermodesulfobacteriota* may convert sulfate to hydrogen sulfide, altering the local pH and affecting the growth and metabolism of other microorganisms, thereby indirectly impacting the overall MEC performance (Uzun et al., 2022). The electrons generated during sulfate reduction may contribute to the electricity generation process (Magalhães et al., 2024). *Chloroflexota*, known for acid production, utilize hydrolysis products to generate volatile fatty acids (VFAs). *Proteobacteria*, also acid producers, convert amino acids into VFAs, supplying crucial substrates for methane synthesis (Chen et al., 2023). This phylum is primarily responsible for hydrocarbon biodegradation (Roy et al., 2018). *Bacteroidota* decompose cellulose into cellobiose and glucose (Tian et al., 2021). Certain *Bacillus* species can directly transfer electrons from intracellular metabolic processes to the electrode surface via cytochrome *c* on the cell surface, enabling electricity generation (Deng et al., 2023). Compared to the control reactor (R1) without biochar addition, the biochar addition R3 exhibited preferential proliferation of *Acidobacteria*, with its relative abundance increasing from 2.18% to 6.38%. This indicates that biochar shapes the functional microbial community structure in the MEC-AD system. Compared with R1-1 to R1-4, the extension of HRT significantly enriched *Thermodesulfobacteria* in R1-5, which is likely to have promoted the improvement of system removal efficiency. The abundances of *Acidobacteria*, *Bacteroidota*, and *Chloroflexota* all increased first and then decreased with the extension of HRT. Specifically, their respective highest abundances were recorded at 36 h HRT in R4 with the total proportion of these three phyla in R4 exceeding 50%. Moreover, the lower operational taxonomic unit (OTU) diversity observed in R3 and R4 indicates that biochar is beneficial for selective functional microbial community enrichment.

Fig. 6 C and D reveal the predominant microbial phyla and genera specifically within the anode biofilms of all MEC-AD reactors: *Chloroflexota*, *Bacteroidota*, *Pseudomonadota*, *Bacillota*, *Thermodesulfobacteriota*, *Actinomycetota*, and *Acidobacteriota*. In the anode biofilm, *Synergistota* and *Atribacterota* were enriched, engaging in syntrophic interactions with electroactive bacteria. Both of them have relatively strict requirements for anaerobic conditions, so the extension of HRT is conducive to their proliferation. *Synergistota* decomposes macromolecular organic matter into smaller molecules usable by electroactive bacteria, thereby indirectly boosting anode electron production efficiency and mitigating the accumulation of inhibitory metabolic products. *Atribacterota* thrives in MEC using VFA as substrates, directly oxidizing propionic acid and similar compounds to acetic acid, a prime substrate for electroactive bacteria, thus enhancing the carbon metabolic pathway. The relative abundances of *Acidobacteriota* in the anodes of reactors R1 through R5 are 2.2%, 1.5%, 5.7%, 6.4%, and 2.8%, respectively. Its relative abundance is also the highest in R4 (HRT = 36 h). *Hydrolytic bacteria* degrade organic matter on the outer layer of the anode biofilm, converting it to VFAs and subsequently oxidizing it to acetate. This acetate is further oxidized to CO_2 at the anode surface, generating electrons (Chen et al., 2023). However, in all reactors, the relative abundance of *Hydrolytic bacteria* is low, indicating that it has no obvious correlation with the extension of HRT. Studies indicate that biochar accelerates the production and degradation of VFAs, regulates organic acid production, and enhances acetate formation (Pan et al., 2023). The addition of biochar materials facilitates electron transfer in MEC. Conductive materials promote direct interspecies electron transfer (DIET) by substituting for pili in *exo*-mycorrhizal fungi (Yin et al., 2019). In contrast to R2-1 to R2-4, *Pseudomonas* and *Acidobacteria* were markedly enriched in R2-5. Notably, *Pseudomonas* exhibits stronger specificity for the degradation of refractory organic matter in the anode environment, which may also constitute a key driver underlying the enhanced removal efficiency of R5 (Qian et al., 2024).

The total number of shared microbial species between the anode and cathode biofilms was 505 (Fig. 6 E). The shared OTUs (505) accounting for 73.0% of all observed OTUs (692), suggests variability in the microbial succession within the cathode biofilm. The cathode biofilm in reactor R5 had the largest biomass, with 36 unique species. Compared to the control reactor R1 without biochar addition, OTUs in reactors R2-R5 were significantly lower, suggesting that functional microorganisms were enriched in the MEC-AD system, potentially due to the promoting effect of biochar. Within each reactor, the abundance of *Bacillotay* was significantly higher in the anode than in the cathode, while differences were observed in the abundances of *Pseudomonadota* and *Chloroflexota* between the anode and cathode. As HRT extended, the abundance of *Pseudomonadota* was first decreased and then increased, while that of *Chloroflexota* was first increased and then decreased. However, the dominance of *Pseudomonadota* was enhanced at 36 h of HRT, and the removal efficiency of MEC-AD system was significantly improved by 9.3%. These differences may be related to the metabolic characteristics of these microorganisms and their adaptations to the varying electrode environments (Bovio-Winkler et al., 2024; Dawson et al., 2023).

The main bacterial genera in the system are *norank_f_Anaerolineaceae* and *norank_f_Bacteroidetes_vadinHA17*. In reactors R1-R5, the abundance of *norank_f_Anaerolineaceae* in both anode and cathode biofilms is generally high, all exceeding 4%, with the highest proportion observed in R4, reaching 18.97%. In contrast, the abundance of *Pseudomonas* in R1 (without biochar) is significantly higher than that in the other reactors (R2-R5), where its abundance decreases. This further indicates that biochar addition promotes differential changes in the microbial community structure. From R2-R5, the acid-producing bacteria *norank_f_Anaerolineaceae* and the *norank_f_Bacteroidetes_vadinHA17* were enriched, which stimulates the synthesis of propionic acid and lactic acid by increasing enzyme activity to mitigate excessive accumulation (Guo et al., 2024). *Norank_f_Anaerolineaceae* can secrete various hydrolases, such as cellulases and proteases, to decompose macromolecular substances, providing substrates for the methanogenesis process and obtaining energy to maintain metabolism (Zheng et al., 2023). The PCoA analysis (Fig. 6 F), reveals that PC1 and PC2 account for 53.2% and 26.5% of the variance interpretation, respectively. Their combined contribution to the total data variance is approximately 80%, significantly supporting the examination of community structure disparities. Examination of the spatial distribution

patterns of samples obtained from the cathodes (R1-1 to R1-5) and anodes (R2-1 to R2-5) of each reactor indicates a dispersion of cathode and anode samples across the coordinate space. Specifically, the microbial samples from the anodes (R1-2, R1-3) were notably clustered in the negative axis region of PC1, while the cathode samples predominantly aggregated in the positive axis of PC1 and its proximate areas. These findings suggest substantial inter-group differentiation between the cathode and anode samples concerning the microbial community structure at the genus level (Feng et al., 2022). The above results indicate that biochar addition effectively promotes the enrichment of microbial communities. While the extension of HRT is conducive to the proliferation of microbial communities and improves system stability, it also weakens the correlation and connections among populations to a certain extent. Meanwhile, the extension of HRT has facilitated the further reproduction of dominant microbial communities, thereby improving the COD removal efficiency of MEC-AD system.

4. Conclusion

HRT critically influences the treatment performance of biochar-enhanced MEC-AD system. Increasing HRT boosted the degradation of organic matter and COD removal efficiency in landfill leachate. The system achieved optimal COD removal efficiency at 48 h of HRT, with the removal efficiency reaching 70.67%. This efficiency was a 67.58% increase compared with that observed under 24 h of HRT. Meanwhile, *Thermodesulfobacteria*, *Pseudomonas*, and *Acidobacteria* were further enriched, with their relative abundances increasing by 4%-7%, which enhanced the system's capacity for degrading refractory organic matter (Yu et al., 2018). Appropriate HRT ensures optimal contact between microorganisms and substrates, thus enhancing metabolic efficiency. Furthermore, the addition of biochar enhanced the COD removal efficiency by approximately 4% when the HRT was 24 h. Biochar (featuring facile preparation, low production cost, and abundant active sites) addition enriched functional bacterial species in the MEC-AD system, significantly altering the microbial community structure and enhancing the presence of relevant functional microorganisms. The findings of this study offer theoretical and data-driven support for guiding the practical engineering application of biochar-enhanced MEC-AD system in landfill leachate treatment, with a focus on different HRT.

CRediT authorship contribution statement

Luqi Yuan: Data curation, Writing – original draft, Writing – review & editing, Investigation. **Xin Liu:** Writing – original draft, Investigation. **Xiaoyan Cao:** Investigation, Writing – review & editing. **Jiahui Gao:** Investigation, Data curation. **Hang Yu:** Investigation, Data curation. **Yidi Li:** Investigation. **Chongjun Chen:** Conceptualization, Supervision, Funding acquisition.

Declaration of competing interest

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