

Bioelectrochemical enhancement of waste valorization: synergistic power generation, metal stabilization, and resistome dynamics in electro-stimulated composting system

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Abstract

The integration of microbial electrochemical systems with organic solid wastes treatment offers a groundbreaking strategy for sustainable resource recovery. Nevertheless, the underlying biological mechanism remains unclear, limiting its practical application. Here, a two-phase microbial desalination cell (TPMDC) was constructed using dewatered sludge (DS) and kitchen waste (KW) or corn straw as co-composting substrate. We investigated the mediating role of substrate-driven microbial succession in energy harvesting, metal stabilization, and the antibiotics resistance gene (ARG) dynamics using metagenomics. Results demonstrated that TPMDC DS+KW achieved a maximum power density of $1.910 \pm 0.009 \text{ W/m}^3$, peak voltage of $0.893 \pm 0.005 \text{ V}$, and highest organic matter removal rate of 31.13%. Notably, we demonstrated substrate-dependent metal stabilization that simultaneously enhanced Zn bioavailability (agriculturally beneficial) and immobilizes > 90% toxic Cu/Cr. Metagenomic analysis identified 74 ARGs types (relative abundance: 2.44 - 3.08%) and *Mesorhizobium* could be considered as a keystone ARG suppressor due to its significant negative correlations with multiple ARG subtypes. Additionally, we established a mechanistical link between electro-stimulation and upregulated TCA cycle genes (EC:6.2.1.1/6.2.1.16), directly coupling microbial metabolism to electron flux. These findings establish a robust scientific foundation for optimizing

bio-electrochemical co-composting systems and advance circular economy implementation.

Keywords: waste valorization; microbial electrochemical system; resistome dynamics; heavy metal speciation; substrate-dependent electrogenesis

1 Introduction

The 21st century has witnessed a dramatic surge in global sewage sludge waste production, with daily wastewater generation now surpassing 1 billion m³ worldwide^[1]. Sewage sludge, an organic-rich waste stream, constitutes a substantial reservoir of critical resource, including carbon, nitrogen and phosphorus. It is estimated that global average daily sewage emissions contain 2.5×10^5 tons of carbon^[2], 1.0×10^4 tons of nitrogen, and 3.5×10^3 tons of phosphorus^[3]. However, sewage sludge also acts as a significant reservoir for heavy metals and antibiotics^[4]. Critically, heavy metals can be transferred through bioaccumulation along food chains^[5], while residual antibiotics may promote antimicrobial resistance. All of them may trigger a series of ecological and public health impacts^[6]. The above issues could cause potential threats to ecosystem stability and human health^[5, 7]. Thus, innovative technologies that aligned with circular economy principles are urgently required to enable efficient organic waste treatment while simultaneously recovering valuable resources.

Composting has long been recognized as a cost-effective method for organics decomposition, metals stabilization^[8], and ARG suppression^[9]. Microbial electrochemical system (MES)-assisted composting leverages exoelectrogenic bacteria to bio-oxidize organic matter, enabling simultaneous organic waste treatment and bioelectricity generation. This integrated approach represents a competitive composting technology^[10].

Current research indicates that MES can accelerate sludge degradation^[11], shorten the composting duration^[12], promote antibiotic degradation^[13], reduce ARGs spread^[14], and decrease heavy metals bioavailability^[15].

As a specialized MES, microbial desalination cells (MDCs) stand out by integrating waste treatment, desalination, and energy recovery^[16]. Goren et al.^[17] utilized MDC to treat industrial wastewater, achieving 91.5% chemical oxygen demand (COD) removal and 55.5% boron elimination, while generating 9.04 mW/m³ of bioelectricity. Soroosh Danaee et al.^[18] established a photosynthetic microbial desalination cell (PMDC) and achieved 50% COD removal and 94% total nitrogen elimination from human faecal sludge, generating a maximum power density of 362.5 mW/m². Meng et al.^[19] demonstrates that MDC enhances dewatered sludge suitability for land application by boosting humification and nutrient availability while reducing heavy metal(loid) bioavailability. Compared with mono-sewage sludge compost, the synergistic treatment of multiple organic solid waste is more economical and effective^[20]. Hu et al.^[21] obtains an efficient organic waste stabilization using dewatered sludge and kitchen waste as co-composting substrates in a TPMDC. Additionally, the co-digestion of sludge and food waste increases methane production and promotes phosphorus mobilization^[22, 23]. The incorporation of corn straw enhances compost quality and safety through microbiome-mediated nitrogen retention and improved metal immobilization^[24, 25].

Despite these advances, fundamental bottlenecks and mechanistic ambiguities persist in applying MDC to treat complex organic solid wastes. Critical limitations include low power density and progressive anodic salinization in solid-based MDCs^[26, 27]. Additionally, it remains uncertain whether MDC-assisted co-composting of multiple organic solid waste further enhances heavy metals detoxification efficiency^[27]. The influences of carbon fraction diversity (e.g., labile vs. recalcitrant organics) on electron transfer pathways and resistome dynamics in substrate-dependent MES also remain elusive. Critically under-characterized are the functional interconnections among substrate composition, electroactive microbiome dynamics, and ARG dissemination when mixed organic solid wastes serve as anode substrates.

To address these knowledge gaps, a laboratory-scale two-phase microbial desalination cell (TPMDC) was designed to treat different composting substrates: (i) mono-dewatered sludge (DS), (ii) dewatered sludge with corn straw (DS+CS), and (iii) dewatered sludge with kitchen waste (DS+KW). Our study systematically evaluated: (1) bioelectricity generation efficiency across the three systems; (2) integrated detoxification performance through metal (Zn/Cu/Cr) detoxification and ARGs dynamics; (3) host-dependent ARGs dissemination; and (4) microbial metabolic network. Our findings will provide mechanistic insights into multi-substrate MDC optimization while establishing a dual-function platform

for simultaneous waste valorization and contaminant control.

2. Materials and methods

2.1 Composting materials and procedure

The study employed three primary composting substrates: (1) DS: Sourced from a municipal wastewater treatment plant (WWTP) in Harbin, China; (2) CS ($8.4 \pm 0.64\%$ moisture): Collected from conventional farmland in Harbin. It was mechanically crushed to 2-mm particles to ensure substrate homogeneity and preconditioned with secondary sludge supernatant from the same WWTP to enhance biodegradability; (3) KW: Sampled from a university cafeteria in Harbin. It contained starch-rich (45 - 50%, w/w; rice/dumplings/potatoes), proteinaceous (15 - 20%, cooked meat), and lignocellulosic fractions (25 - 30%, greens/mushrooms), mirroring municipal organic waste composition. The critical parameters were characterized by high moisture ($90.42 \pm 0.98\%$), organic content ($81.14 \pm 1.09\%$), and balanced C/N ratio (24.33 ± 0.23). Strategic substrate mixing protocols were implemented to form two experimental systems: DS+CS (1:1.2, w/w) and DS+KW (1:0.8, w/w). The C/N ratio and moisture content were optimized to 25 and 74%, respectively, establishing ideal composting conditions.

Three identical, seven-chambered symmetrical TPMDCs were designed and operated anaerobically for 90 days at room temperature. The construction and electrode materials of the TPMDCs followed previous

descriptions, with the exception of composted material^[19]. The reactor was manufactured with plexiglass. At its core was a cylindrical anaerobic anode chamber (diameter = 12 cm, height = 13 cm). Symmetrically arranged around this central chamber were cubic structures: concentration chambers (9 cm in length $\times$ 3 cm in width $\times$ 9 cm in height), desalination chambers (9 cm in length $\times$ 3 cm in width $\times$ 9 cm in height), and cathode chambers (9 cm in length $\times$ 5 cm in width $\times$ 9 cm in height). Open square windows (90 mm $\times$ 90 mm) were incorporated to connect each chamber and facilitate ionic migration. Cation-exchange membranes (CEMs, Ultrex CMI-7000, 9 cm $\times$ 9 cm) separated the anode chamber from the concentration chambers and the cathode chambers from the desalination chambers. Anion-exchange membranes (AEMs, Ultrex AMI-7001, 9 cm $\times$ 9 cm) separated the concentration chambers from the desalination chambers. The anode electrode was a 9 cm diameter carbon fiber brush (STS40 24 K, Toho Tenax). The cathode electrode was a composite of carbon brushes integrated with graphite particles (1 - 5 mm diameter, Jiuxin Carbon Goods Co., Jilin, China). The full voltage of TPMDC was continuously monitored using a multi-channel voltage collector (PISO-C813, ICP DAS, Co., Ltd, Beijing, China), with daily mean values calculated from 24-hour recordings. While the current (I) and the volumetric power density (P) of the system were calculated according to Ohm's law, as shown in Eq. (1) and Eq. (2).

$$I = U/R \quad (1)$$

$$P = UI/V \quad (2)$$

where U is the output voltage (V), I is the current (mA), R is the external resistance (Ω), V is the net anode chamber (NAC) volume of the reactor (m^3).

The composting process was systematically stratified into three distinct phases according to bioelectrochemical performance: (i) an initiation phase, (ii) a bioelectricity generation peak, and (iii) a maturation phase, as defined by real-time voltage output dynamics.

Polarization curves and power density curves were acquired by systematically changing external resistance from 10,000 Ω to 10 Ω following 12 hours circuit disconnection for during stable operation. For each adjustment, the voltage was allowed to stabilize for at least 15 min to ensure consistent conditions. The voltage at each resistance setting was recorded to construct the polarization curve. Current density and power density corresponding to each resistance value were then calculated, and a power density curve was plotted for comprehensive analysis.

2.2 Sample collection and analysis

Composite samples were collected every five days from stratified reactor layers (upper, middle, and bottom), homogenized, and immediately divided into two aliquots. One stored at 4 $^{\circ}\text{C}$ for physicochemical characterization, and the other flash-frozen at -80 $^{\circ}\text{C}$ for genetic testing.

Sampling spanned initiation, bioelectricity generation peak, and maturation phases. Organic matter (OM) content was quantified by combustion at 550 °C for 4 hours in a muffle furnace^[28]. Elemental composition of C and N was determined using an elemental analyzer (vario EL III, Elementar German). Dissolved organic carbon (DOC) was measured via a Shimadzu TOC-L analyzer (Shimadzu, TOC-L CPH/CPN, Kyoto, Japan), after extraction with deionized water (sample-water ratio, w/v, 1:10).

The total contents of heavy metals were determined by means of Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES)^[29]. Extraction of different forms used a modification of the European Community Bureau of Reference (BCR) sequential extraction procedure^[29]. Metal speciation can be categorized into the four fractions: (1) exchangeable fraction (Exc), which includes free metal ions and those weakly bound to carbonates, was extracted by shaking 0.5 g of sample at 150 rpm for 16 hours using acetic acid (20 ml, 0.11 M) as the extracting solution; (2) reducible fraction (Red), representing metals associated with amorphous Fe/Mn (oxy)hydroxides, was obtained by shaking at 150 rpm for 16 hours using hydroxylamine hydrochloride (20 ml, 0.5 M) at pH=2; (3) oxidizable fraction (Oxi), containing metals complexed with organic matter or sulfide-bound phases, was oxidized with H₂O₂ (5 ml, 8.8 M) and then extracted by shaking at 150 rpm for 16 hours using ammonium acetate

(20 ml, 1.0 M) at pH=2; (4) residual fraction (Res), corresponding to metals permanently incorporated into the crystal lattice of silicate minerals, was obtained after HNO₃ - HCl - HF - HClO₄ digestion. The total content was obtained with the same methods as Res^[30, 31]. As Zn, Cu and Cr concentrations were higher than those of other heavy metals, this study focused on their form changes. The bioavailability indices of heavy metals are defined as the sum of the proportions of Exc and Red^[32].

2.3 Metagenomic sequencing and bioinformatics analyses

Triplicate samples from critical composting phases underwent metagenomic sequencing: (i) initial phase (DS-1), (ii) bioelectricity generation peak (DS-2, DS+CS-2, and DS+KW-2), and (iii) maturation phase (DS-3, DS+CS-3, and DS+KW-3). DNA libraries were prepared by Sangon Co., Ltd. (Shanghai, China) and sequenced on an Illumina HiSeq platform. The primary steps included (1) raw sequencing reads were quality-assessed using fastp software (FastQC, Version 0.11.2), followed by adapter trimming and quality filtering via Trimmomatic^[33]. High-quality clean reads were retained for downstream analysis; (2) Filtered reads were assembled using IDBA-UD (Version 1.1.3)^[34]. The optimal assembly was selected based on contig metrics (N50, max length) and completeness; (3) protein-coding genes were predicted from contigs using Prodigal (Version 2.6.0)^[35], retaining open reading frames (ORFs) with $\geq$ 100 bp (translated to amino acid sequences); (4) predicted genes were

clustered (95% identity, 90% coverage) using CD-HIT (Version 4.6)^[36], generating a unified non-redundant gene set (NR-genes); (5) clean reads were aligned to the NR-genes using Bowtie2 (Version 2.1.0)^[37], with length-normalized counts generated via SAMtools (Version 0.1.18)^[38]. NR-genes were annotated against the National Center for Biotechnology Information (NCBI) microbial taxonomy database (<http://ncbi.nlm.nih.gov/>). ARGs annotation results were obtained by integrating gene sequences from target species with their resistance functions, determined via comparison with the Antibiotic Resistance Genes Database (ARDB, <https://ardb.cbc.umd.edu/>). The bacterial functions were predicted through the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (<http://www.kegg.jp>).

2.4. Statistical analysis

All experiments were conducted with biological triplicates. Data visualization was performed using OriginPro 2021 (OriginLab, USA). The statistical significance was assessed on SPSS 22.0 for t-tests and ANOVA. Spearman correlation analysis was conducted using R Studio 4.0. Results were considered statistically significant at $p < 0.05$.

3. Results and discussions

3.1 Effect of different compost substrate on the power generation performance of TPMDC

Totally, three TPMDCs with different substrates were operated for 90

days. All systems displayed a consistent voltage trajectory: an initial rise, a stable plateau, and a gradual decline (Fig 1a). The power generation performance of both TPMDC-DS+CS and TPMDC-DS+KW significantly outperformed the TPMDC-DS control ($p < 0.05$), and the specific advantages are as follows:

Firstly, the addition of both CS and KW could shorten the startup time by 95.24%. Specifically, TPMDC-DS required 42 days to reach a stable voltage more than 0.300 V, while TPMDC-DS+CS and TPMDC-DS+KW achieved a rapid startup within two days. These results also showed an obvious superiority over traditional MDC and sediment microbial fuel cell (SMFC). The traditional MDC had a startup period of 5d using dehydrated sludge as sole anode substrate^[19], and the SMFC installed in urban river had a 6d startup period even after being enhanced by electronic mediators^[39].

Secondly, the addition of both CS and KW could increase the peak voltage by 1.3-fold, along with the increased average voltage (Fig. 1b). Specifically, the maximum output voltage of TPMDC-DS peaked at 0.391 ± 0.022 V, while TPMDC-DS+CS and TPMDC-DS+KW achieved their peak voltage of 0.884 ± 0.019 V and 0.893 ± 0.005 V, respectively. Additionally, TPMDC-DS also had a lower average output voltage of 0.19 ± 0.002 V, compared to TPMDC-DS+CS and TPMDC-DS+KW (0.57 ± 0.011 V and 0.73 ± 0.004 V, respectively). This finding indicated that the

two conditioners could improve the output voltage of TPMDCs with the order being KW > CS. Notably, the average voltage outputs of amended systems were substantially higher than those previously reported novel MFC system for PEFB-MFC (471.68 mV)^[40] and CW-MFC (210 mV)^[41].

Thirdly, the addition of CS and KW could increase the maximum power density by 8.02 and 9.05 times, respectively (Fig. 1d). Specifically, the maximum power density of TPMDC-DS was only $0.190 \pm 0.003 \text{ W/m}^3$, compared to $1.910 \pm 0.009 \text{ W/m}^3$ for TPMDC-DS+KW and $1.714 \pm 0.006 \text{ W/m}^3$ for TPMDC-DS+CS (Fig. 1d). This result surpassed previous reports on bio-base MESs. For instance, the maximum power density of plant MFC and soil MFC were 0.37 W/m^3 and 0.68 W/m^3 , respectively^[42, 43]. More specifically, compared to conventional sludge-based MDCs (e.g., 0.19 W/m^3 in Meng et al^[19]), the TPMDC-DS+KW system achieved a record-high 1.91 W/m^3 power density, representing a 10-fold enhancement. This resolves the long-standing bottleneck of low energy output in solid-state bioelectrochemical systems^[15, 27, 44] by strategically utilizing labile organics in kitchen waste to augment electron flux.

Fourthly, the addition of both CS and KW could prolong the period of stable operation. Specifically, TPMDC-DS only showed a brief stabilization period of 19 days, while those of TPMDC-DS+CS and TPMDC-DS+KW were 43 days and 61 days, respectively. Meanwhile, TPMDC-DS+KW exhibited a narrower voltage fluctuation (fluctuation

range narrowed by 21.14%) and had 1.81 times more days with voltages above 0.700 V compared to the TPMDC-DS+CS system. These results implied that the addition of corn straw and kitchen waste accelerated exoelectrogen stabilization through enhanced bioavailable carbon provision, while the degree of hydrolysis of corn straw was relatively insufficient during the whole operation. More specifically, TPMDC-DS+CS entered the stable period on the 16th day, which was earlier than that of TPMDC-DS+KW on the 25th day. These differences were probably due to inconstant electrons number released from different organics oxidation^[39, 45, 46]. This is specifically manifested in the rapid consumption of soluble organic matter in the original sludge of TPMDC-DS+CS and the suboptimal utilization of this lignocellulose. Therefore, there was a temporary increase in voltage, followed by an early decrease. In contrast, the TPMDC-DS+KW system exhibited prolonged operational stability due to the addition of easily hydrolysable kitchen waste. These findings indicate that labile organic fractions in kitchen waste synergistically enhance sludge degradation, promoting sustained bioelectrogenesis through improved substrate bioavailability for electroactive microbial communities^[47].

Fifthly, the addition of both CS and KW could reduce the resistance within the reactor. As shown in Fig. 1c, modifying the anodic substrate with conditioning agents reduced polarization curve slopes relative to the DS

control ($p < 0.05$). The internal resistance decreases followed this order: TPMDC-DS (245.5 Ω) > TPMDC-DS+CS (111.6 Ω) > TPMDC-DS+KW (104.7 Ω). However, both TPMDC-DS+CS and TPMDC-DS+KW systems exhibited similar magnitude reductions in ohmic resistance, underscoring the consistent efficacy of these additives in enhancing interfacial charge transfer. Furthermore, this suggested the additives may also affect the TPMDCs performance through other pathways, such as affecting the microbial community^[48] and the availability of organic matter^[49] at the anode.

Collectively, the co-composting integrated corn straw and kitchen waste with dewatered sludge yielded statistically significant enhancement in bioelectricity generation of TPMDCs ($p < 0.05$). Notably, kitchen waste addition demonstrated significantly superior bioelectricity generation performance compared to corn straw supplementation ($p < 0.05$).

3.2 Changes of organic matter in TPMDCs anodic substrate

TPMDC-assisted composting represents a microbially-driven biodegradation process, wherein organic matter (OM) is progressively decomposed by microbial communities^[50]. Different additives can also affect the key physicochemical factors of TPMDC substrates. Fig. 2a demonstrated a time-dependent decline in OM in all TPMDC anodes during the operational cycle, indicating the conversion of organic chemical energy to electrical energy. Notably, at the end of composting, the OM

content in the TPMDC-DS+CS and TPMDC-DS+KW anodic substrates decreased from $54.77 \pm 1.35\%$ to $39.91 \pm 0.97\%$, and from $54.93 \pm 1.53\%$ to $37.83 \pm 1.22\%$, respectively. The corresponding removal rates of 27.13% and 31.13%, which were 3.07 times and 3.52 times higher than the 8.84% removal rate observed in the TPMDC-DS system. The modified anodic substrate increased the utilization efficiency of OM in TPMDC-DS+CS/DS+KW, greatly promoting the energy metabolism of microorganisms. Furthermore, the rapid degradation of OM mainly occurred during the transition from the initial electricity generation phase to the peak voltage output period. This could be attributed to the rapid consumption of easily degradable OM due to the massive reproduction of microorganisms. Then, the degradation rate of OM tended to stabilize because of the balance between high microbial activity and abundant organic substrate^[11]. Compared to a bioelectrochemically-assisted anaerobic composting process (AnC_{BE}) with dewatered sludge as the anode^[11] and a microbial fuel cell (MFC) for macrophyte litter degradation^[51], the OM removal rate in TPMDC was higher than in the other two reactors (19.5% and 20.8%, respectively). This may be attributed to the unique characteristics of the TPMDC systems. When amended with kitchen waste or corn straw, they demonstrated a superior capability in stabilizing key physicochemical parameters (e.g., EC, pH)^[19], thereby enhancing microbial metabolic activity during the composting process.

The dissolved organic carbon (DOC) is an important indicator for describing the dynamic changes of the microbial utilization and organics stabilization. Unlike the variation in OM, the DOC concentration in the TPMDCs anodic substrate demonstrated distinct fluctuations, characterized by an initial increase followed by a subsequent decline (Fig. 2b). The performance of TPMDC systems exhibited strong substrate dependence. TPMDC-DS+KW showed the highest DOC concentration, peaking at 145.76 ± 3.01 mg/kg dry sludge, while TPMDC-DS+CS and TPMDC-DS reached maximum DOC levels of 82.34 ± 3.13 and 54.09 ± 1.58 mg/kg dry sludge, respectively. This kinetic progression confirmed the canonical two-stage organic matter transformation: hydrolytic liberation precedes microbial assimilation. Microbial decomposition of organic carbon initially releases water - soluble organic acids, increasing DOC. In the later phase, reduced carbon decomposition and increased microbial DOC consumption cause the DOC content to decline^[15, 52]. Notably, the kitchen waste amendment significantly enhanced hydrolysis, leading to a 2.7-fold increase in the highest DOC concentration compared to TPMDC-DS, which fundamentally enabled the bio-electrogenic performance. While corn straw supplementation showed a 1.5-fold improvement over TPMDC-DS, its hydrolytic efficiency remained suboptimal when compared to kitchen waste. These results reveal feedstock-specific degradation pathways, where the applied

electrochemical potential simultaneously stimulates microbial metabolism and extracellular electron transfer - synergistically accelerating DOC mineralization through a self-reinforcing cycle. Such electrostimulation effects on organic matter turnover are well-established in bioelectrochemical systems^[40], though our work newly demonstrates their substrate-dependent manifestation in complex waste matrices.

3.3 Transformation of heavy metal form in TPMDCs

The heavy metal contents in anodic substrate will be concentrated or diluted during the composting process. Fig. 3a illustrates the total concentrations of Zn, Cu and Cr in initial phase (Day 0) and terminated phase (Day 90). Throughout composting period, Zn contents exhibited an increased tendency due to the decomposition of OM^[15]. Notably, the accumulation of Zn, Cu, and Cr in TPMDC-DS+KW surpassed that in the other reactors (TPMDC-DS+CS and TPMDC-DS) by a factor 1.2 - 1.5. This phenomenon could be due to enhanced organic matter degradation caused by kitchen waste addition, leading to metal ions concentration within the reactor, as evidenced by mass calculations.

In addition to the total concentration of heavy metals, the toxicity and bioavailability of metals strongly impacted by the chemical forms^[53]. The metal bioavailability decreases in the order of Exc > Red > Oxi > Res. Generally, the heavy metals in the Exc and Red fractions are considered more mobile and bioavailable, whereas those in the Oxi and Res fractions

are regarded as stable and non-bioavailable^[31, 54]. Thus, the metallic biotoxicity depended on the distribution regarding their chemical forms was discussed in this study (Fig. 3b - 3d).

The dynamic changes in Zn fractions (%) within composting process were demonstrated in Fig. 3b. In initial substrates, speciation analysis revealed higher bioavailability indices of Zn across all substrates, followed the order: TPMDS-DS+CS (61.42%) > TPMDC-DS (60.51%) > TPMDC-DS+KW (50.37%). This substantial bioavailable Zn (representing > 50% of total Zn) indicates significant environmental mobility potential^[55]. After composting, the bioavailability indices of Zn, decreased by 20.55% and 15.22% in TPMDC-DS and TPMDC-DS+CS, respectively. Whereas in TPMDC-DS+KW, bioavailability indices of Zn increased by 15.64%. This redistribution of Zn fractions in TPMDC-DS+KW was characterized by decrease of 36.03% in the Oxi-Zn fraction, accompanied by increases of 5.54%, 20.25%, and 12.82% in the Res-Zn, Red-Zn, and Exc-Zn fractions, respectively. The observed shift in Zn forms may be reasonably ascribed to the amendment of kitchen waste. This practice altered local redox potentials and modified the complexation behavior of Zn. These findings indicated that short-term operation was less efficient for Zn stabilization in TPMDC-DS+KW. However, increased Zn bioavailability does not compromise the safety of using compost products on land, as plants and animals have developed greater resistance to Zn^[15]. Meanwhile, Zn is

commonly recognized as a trace element that is beneficial to health.

During composting, the fractionation of Cu (Fig. 3c) exhibited a distinct pattern from that of Zn. Regardless of TPMDC-DS or TPMDC-DS+CS/KW, Cu mainly contained Oxi and Res, and the percentage was greater than 51.52% and 26.67%, respectively. During composting, stable-fraction (Oxi-Cu + Res-Cu) content increased by 2.32% in TPMDC-DS, 3.1% in TPMDC-DS+CS, and 2.93% in TPMDC-DS+KW. This fraction redistribution demonstrated TPMDCs converted labile fractions (Exc-Cu and Red-Cu) to stable oxidative forms in the composting process, consistent with the well-documented chelation of Cu^{2+} by labile organic compounds into humic complexes^[15, 44]. Fig. 3d illustrated the dynamic changes in Cr fractions during composting. The addition of both CS and KW was promoted the increase in Res-Cr fractions as well as the decrease in Exc-Cr and Red-Cr. Especially, Cr was primarily in Res-fraction and this fraction was increased by 17.68% in TPMDC-DS, 5.24% in TPMDC-DS+CS, and 14.70% in TPMDC-DS+KW, indicating that composting reduces Cr mobility in the final product, which is consistent with the finding from Cui et al.^[56].

While prior studies confirmed MDCs enhance general metal stabilization^[19, 21], we uniquely demonstrate substrate-specific speciation control: KW amendment simultaneously enhanced Zn bioavailability (16% increase, agriculturally beneficial) and immobilized > 90% toxic Cu/Cr -

achieving contradictory metal fates unattainable in homogenous waste systems^[15, 44]. These findings exhibited that TPMDC-DS+KW had a superior metal stabilization performance, demonstrating its viability as an innovative and sustainable strategy for organic waste detoxification.

3.4 Characteristics of resistome and microbiome in the TPMDCs

Fertilizers derived from organic waste can disseminate ARGs to farmland soil and water bodies, causing severe environmental issues and food-chain contamination with detrimental effects on human health^[57]. Hence, we performed metagenomic sequencing and functional annotation for TPMDC anode microorganisms to closely delineate the antibiotic resistome and reveal the mechanisms governing the fate of ARGs during composting. Totally, 74 types of ARGs were identified across all TPMDCs, with a relative abundance range of 2.44 - 3.08%. Among the identified ARGs, macrolide, bacitracin and vancomycin were the three most predominant types, collectively accounting for 46.80% of the total ARGs. Their coverage ranges were 0.58 - 0.69%, 0.29 - 0.33%, and 0.24 - 0.37%, respectively (Fig. 4a). These findings were consistent with the research conducted by Han et al.^[58] and He et al.^[59]. In terms of diversity, a comprehensive analysis of the TPMDCs revealed a total of 322 unique ARG subtypes. Each sample contained 281 (DS-1), 289 (DS-2), 271 (DS-3), 304 (DS+CS-2), 310 (DS+CS-3), 294 (DS+KW-2), 306 (DS+KW-3) ARG subtypes. Of them, 20 ARG subtypes were found to be prevalent in

TPMDCs, including 5 subtypes of vancomycin resistance genes (*VanRG*, *VanRE*, *VanRB*, *VanRC*, *VanHB*), 3 tetracycline genes (*tetPB*, *tetW*, *otrA*), 3 VT genes (*VanSD*, *VanRA*, *VanSA*), 3 LSM genes (*CarA*, *OleB*, *TlrC*), *BcrA* (bacitracin), *MexF* (CF), *CeoB* (chloramphenicol), *MacB* (macrolide), *pbp1a* (penicillin), *MexW* (unknown) (Fig. 4b). Collectively, these 20 subtypes accounted for approximately 65.80% of the total ARGs.

With regard to the microbiome, the Shannon and Simpson indices (Table 1) indicated that TPMDC-DS+CS and TPMDC-DS+KW systems exhibited higher community diversity and richness than TPMDC-DS system. This suggested that sustained electrochemical stimulation increased the diversity and richness of microbial community in anodic substrates. Metagenome sequencing of seven samples from the TPMDCs detected 105 phyla. At the phylum level, the five most dominant bacterial phyla were *Proteobacteria*, *Planctomycetes*, *Actinobacteria*, *Bacteroidetes*, and *Firmicutes*, which collectively constituted 78.99% to 91.40% of the total microbial community abundance (Fig. 5a). This was consistent with previous research on organic solid waste composting that identified these phyla as predominant^[60, 61]. Among them, *Proteobacteria* showed a notably greater relative abundance in contrast to the other phyla in the TPMDCs, making up 41.85 - 74.25% of the total abundance. These findings align with prior studies, identifying *Proteobacteria* as the dominant electrogenic microorganisms, which enhance electron transfer by participating in redox

reactions^[62]. Additionally, *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, *Verrucomicrobia*, *Chloroflexi*, and *Acidobacteria*, were all abundant within the three TPMDC reactors. These phyla comprise key functional groups essential for sludge composting, including taxa that drive organic hydrolysis, immobilize metal, and attenuate ARG^[63]. For instance, *Firmicutes* and *Actinobacteria* serve as the principal carriers and disseminators of ARGs, a function attributed to their role as major antibiotic producers^[64].

At the genus level, *Rhodanobacter* (*Proteobacteria*) was the most prevalent genus, accounting for 1.90% - 35.29%, 0.48% - 1.15%, and 0.32% - 2.01% in TPMDC-DS, TPMDC-DS+CS, and TPMDC-DS+KW, respectively. Notably, no single genus dominated any of the TPMDC systems. Instead, 7 to 14 dominant genera consistently exceeded 1% relative abundance, and their proportions shifted markedly across composting stages (Fig. 5b). In the TPMDC-DS system, *Rhodanobacter* remained the keystone genus, rising from 1.90% (DS-1) to 35.29% (DS-3). This surge likely reflects its adaptation to prolonged anaerobiosis via denitrification and its role in nitrogen transformation and ARG mobilization^[65, 66]. *Rhodanobacter* species are also documented metal-tolerant organisms, frequently enriched in heavy-metal-contaminated aquifers^[67]. *Dyella*, commonly found in metal mines and known for its resistance to multiple drugs and metals^[68], similarly increased toward the

end of composting. Conversely, *Dechloromonas*, which is typically associated with organic matter degradation^[69], decreased toward the end of composting. This suggests a reduced capacity for organic matter degradation in the TPMDC-DS with lower electrochemical activity. Co-composted systems (TPMDC-DS+CS and TPMDC-DS+KW) displayed analogous trends. Specifically, the relative abundance of *Dechloromonas* gradually decreased throughout the composting process by 4.84% in TPMDC-DS+CS and by 4.89% in TPMDC-DS+KW. This decline suggests a reduction in degradation activity^[39]. Conversely, *Pirellula*, a genus usually involved in the degradation of organic nitrogen and heterotrophic reduction of nitrate^[70], increased by 3.14% in TPMDC-DS+CS and by 3.79% in TPMDC-DS+KW by the end of the composting. *Nitrospira*, a key ammonia-oxidizing denitrifier^[71], declined by 2.47% in TPMDC-DS+CS and 2.45% in TPMDC-DS+KW, underscoring compositional shifts that collectively re-shape nitrogen cycling in co-composting reactors.

Principal coordinate analysis (PCoA) based on Bray-Curtis revealed clear samples clustering that mirrors the differences in microbial community structure. As shown in Fig. 5c, principal component 1 (PC1) and principal component 2 (PC2) explained 54.77% and 24.37% of the total variation in microbial community, respectively. Within each TPMDC system, microbial assemblages differed significantly among initiation phase (DS-1), bioelectricity generation peak (DS-2, DS+CS-2, DS+KW-2,

DS+KW-2), and maturation phase (DS-3, DS+CS-3, DS+KW-3) samples. This highlights the regulatory role of micro-electric fields in shaping microbial community structure^[13]. Minor but consistent differences were also evident between the two co-composting systems (DS+CS vs. DS+KW) at both peak and mature stages. In contrast, both co-composting systems diverged significantly from the mono-sludge composting. These findings highlighted that substrate composition is a primary driver of community assembly, which in turn governs organic-matter degradation and bioenergy recovery.

A Spearman correlation heatmap was employed to evaluate how the microbial community influences the ARG profile (Fig. 6). Given that ARGs are typically mobilized by their microbial hosts, their fate is closely linked to community turnover^[72], and correlation analysis therefore offers a powerful first screen for putative ARG carriers. Across all three TPMDCs, many genera exhibited significant relationships with a variety of ARGs. For instance, *Dyella* were positively correlated with 8 ARG subtypes, corroborating its previously reported multidrug resistance^[68]. Likewise, the genera *Rhodanobacter*, *Mesorhizobium*, and *Pirellula*, which had higher relative abundance, were linked to 7, 6, and 6 ARG subtypes, respectively. In detail, *Rhodanobacter* demonstrated significant positive correlations with *MexW*, *CarA*, *pbp1a*, *VanRC*, *CeoB*, and *OleB*, while negative relationship with *VanSD*. These results suggested that *Rhodanobacter* may

harbor multiple ARGs and potentially contribute to the dissemination of certain ARGs. Conversely, *Mesorhizobium* exhibited significant negative correlations with *VanRA*, *VanRG*, *VanSA*, *tetW*, and *otrA*, while showing a significant positive relationship with *MacB*. *Pirellula* was significantly negatively correlated with *MexW*, *CarA*, *VanRA*, *VanRG*, *tetW*, *otrA*. Notably, metagenomic resolution further identifies *Mesorhizobium* as a keystone ARG suppressor, an overlooked regulator in previous bioelectrochemical composting studies^[15, 19, 21, 27, 44]. These results highlighted that targeted suppression of specific hosts taxa could be an effective strategy for ARGs mitigation, underscoring the need to unravel the complex interplay between microbial community dynamics and ARG ecology in composting systems.

3.5 Functional analysis of microbial communities

To comprehensively investigate the biological molecular mechanisms underpinning electrochemistry-facilitated co-composting, we profiled microbial functions across reactors using the Kyoto Encyclopedia of Genes and Genomes (KEGG). As shown in Fig. 7a, metabolic pathways were the most prominent across all samples, in which carbohydrate, amino acid, cofactors, and vitamins metabolism exhibited higher relative abundances (Fig. 7b). This likely reflects the critical role of metabolism in microbial survival and growth, indicating highly active bacterial metabolism in the anodic substrate.

Under conditions of abundant organic matter, microbes preferentially up-regulate metabolic and energy pathways to manage substrate overload^[73]. Consistently, a notable enhancement of energy metabolism was observed during periods of high-power generation (TPMDC-DS-2, TPMDC-DS+CS-2, TPMDC-DS+KW-2). This indicated that bioelectricity-assisted technology effectively promoted microbial energy metabolism pathways, thereby facilitating organic matter degradation^[40]. This finding was in line with the organic matter degradation performance shown in Fig. 2a and 2b. The abundance of membrane transport and signal transduction in each system reached 2.49 - 3.52% and 2.38 - 2.66%, respectively, with higher values observed in TPMDC-DS+KW system. These results showed that microorganisms had strong interactions with the surrounding environment in terms of material transport, energy exchange, and in formation transmission in the KW-amended reactor. Furthermore, bioelectricity stimulation upregulated the expression of genes associated with bacterial cell motility, which facilitating cell aggregation and biofilm development. The accelerated biofilm formation enhanced the efficiency of organic matter degradation.

Given that glycolysis and tricarboxylic acid (TCA) cycle are crucial pathway of carbon metabolism, bridged by acetyl coenzyme A^[74], we quantified key enzymes (EC: 6.2.1.1, EC: 6.2.1.16, and EC: 6.2.1.13). Their relative abundances were markedly higher in the TPMDC-DS+CS

and TPMDC-DS+KW systems (Fig. 7c), supporting that bioelectricity stimulation upregulated the level of glycolysis-related enzyme and associated coding genes, thereby improving microbial activity. Critically, our omics-level validation demonstrated that electro-stimulation upregulated TCA cycle genes (EC:6.2.1.1/6.2.1.16), thereby coupling microbial energy metabolism to bioelectricity yield. In contrast, previous study merely inferred based on voltage correlations^[11, 15, 40, 44]. Therefore, these findings provided mechanistic insights into performance optimization of bioelectrochemical waste treatment systems while addressing critical environmental health considerations.

4. Conclusions

We find that substrate-driven microbial succession in bioelectrochemical systems mediates three functions: energy harvesting, metal stabilization, and resistome reprogramming. Labile organics in kitchen waste reinforces the dominance of hydrolytic microorganisms, enabling record power density (1.91 W/m³) through rapid DOC generation. Crucially, we demonstrate substrate-dependent metal stabilization that simultaneously enhances Zn bioavailability (agriculturally beneficial) and immobilizes >90% toxic Cu/Cr, alongside identifying *Mesorhizobium* as a keystone ARG suppressor via host-resolved metagenomics. We provide the omics-level validation that electro-stimulation is mechanistically linked to upregulated TCA cycle genes (EC:6.2.1.1/6.2.1.16), directly coupling

microbial metabolism to electron flux. This innovative platform transforms organic waste into a dual-benefit resource, advancing clean energy and circular economy.

However, this study also has some limitations: (i) Scalability gap: Lab-scale validation under controlled conditions; performance in full-scale systems with environmental variability (e.g., temperature fluctuations, waste heterogeneity). (ii) Metal scope restriction: Focus on Zn, Cu, and Cr and omits ecotoxicologically critical metals (e.g., As, Pb, Ni), restricting comprehensive compost risk profiling. (iii) Mechanisms inference: Metagenomics implies but doesn't directly prove electrostimulation links electron flux to metal/ARG dynamics. (iv) Host-ARG correlation: Statistical genus-ARG associations (e.g., *Rhodanobacter*) need culture-based host validation.

To address these limitations and advance this technology, future studies should prioritize: (i) Pilot-scale validation: Integrate TPMDCs with industrial composting to evaluate scalability, energy efficiency, and operational robustness. (ii) Expanded contaminant screening: Track co-occurring pollutants (e.g., antibiotics, POPs, unmonitored heavy metals) for holistic safety assessment. (iii) Multi-omics mechanistic resolution: Combine metatranscriptomics, metabolomics, and electrochemistry to elucidate linkages between electron transfer, metal biotransformation, and ARG regulation.

CRedit authorship contribution statement

Tengteng Hu: Conceptualization, Methodology, Data curation, Writing - Original draft. **Haoqi Pan:** Writing - Review and Editing. **Yunhan Lin:** Writing - Review and Editing. **Jin Yan:** Writing - Review and Editing. **Zhuo Gao:** Writing - Review and Editing. **Zhugen Yang:** Review and Editing, Supervision. **Fanyu Meng:** Conceptualization, Supervision, Writing - Review and Editing, Writing - Original draft, Validation, Funding acquisition.

Data Availability

Data will be made available on request.

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Figure captions

Fig. 1 Generation performance of TPMDCs (with $R_{ex}=1000\ \Omega$), (a) the voltage sequence diagram during 90d; (b) voltages; (c) polarization curves; (d) power density curves.

Fig.2 Variation of organic matter (OM) (a) and dissolved organic carbon (DOC) (b) of TPMDC anodic substrate during 90d operation (Error bars are $\pm$ SD based on triplicate sample analyses).

Fig. 3 Variation of heavy metal forms in TPMDC anodic sludge before and after the 90d operation, (a) the total contents of Zn, Cu and Cr; (b) the form distribution of Zn; (c) the form distribution of Cu; (d) the form distribution of Cr.

Fig. 4 Chord diagram showing the distribution of ARG (a) and composition of ARG subtypes in TPMDC (b).

Fig. 5 Characteristics of microbiome in the TPMDCs, (a) Chord diagram showing the distribution of microbial community at the phylum level; (b) microbial community composition at the genus level; (c) principal coordinate analysis (PCoA) showing the difference of microbial community profiles at species level among different TPMDCs.

Fig. 6 Spearman correlation analysis between genus and ARG subtypes.

Fig. 7 Functional gene profiles, at level 1 pathway (a), level 2 pathway (b) and the relative abundance of acetyl coenzyme A based on KEGG annotation (c).

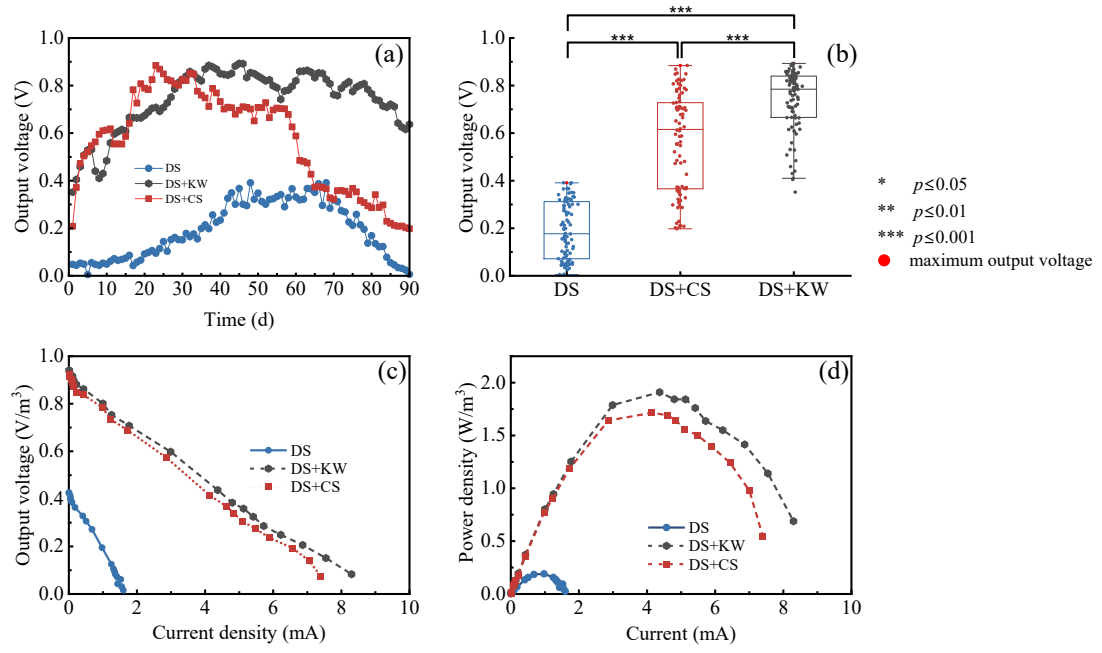


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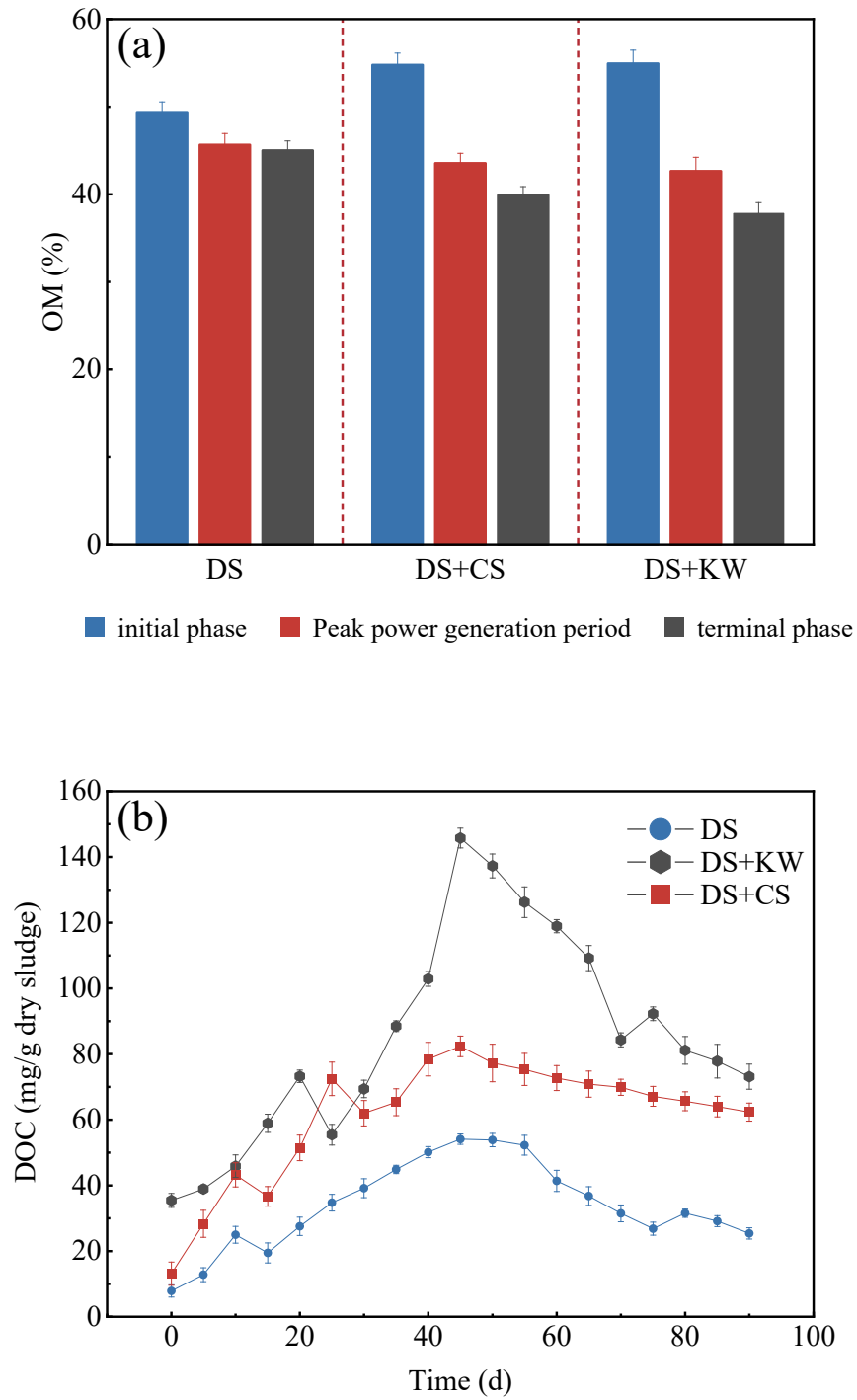


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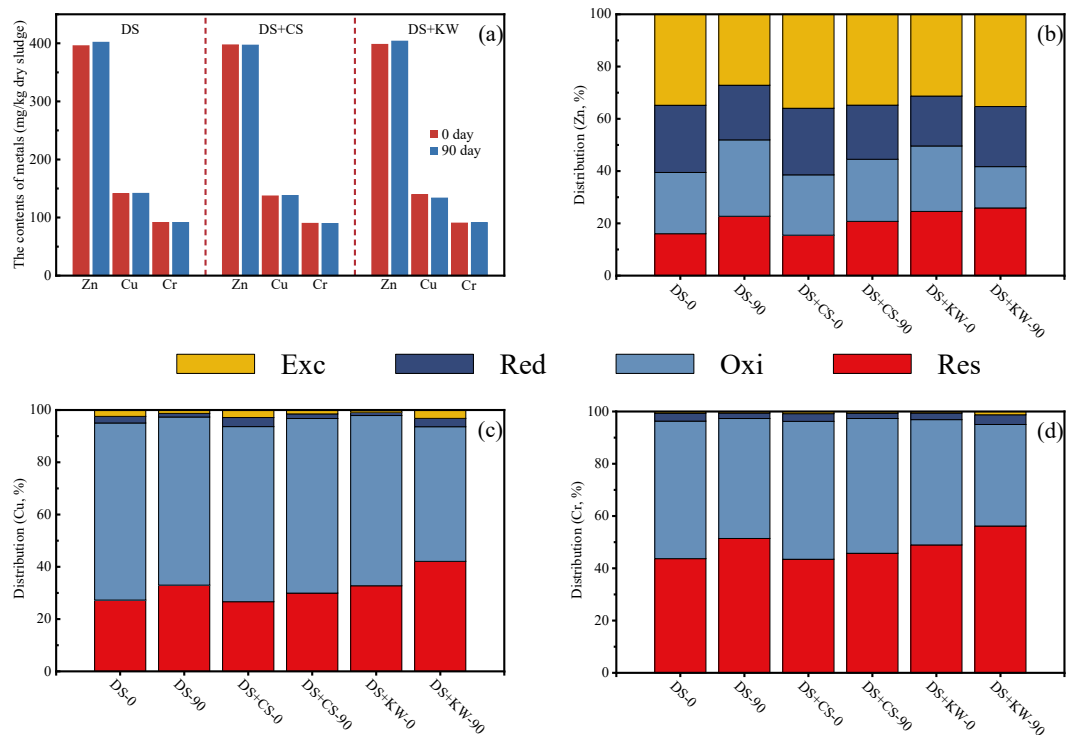


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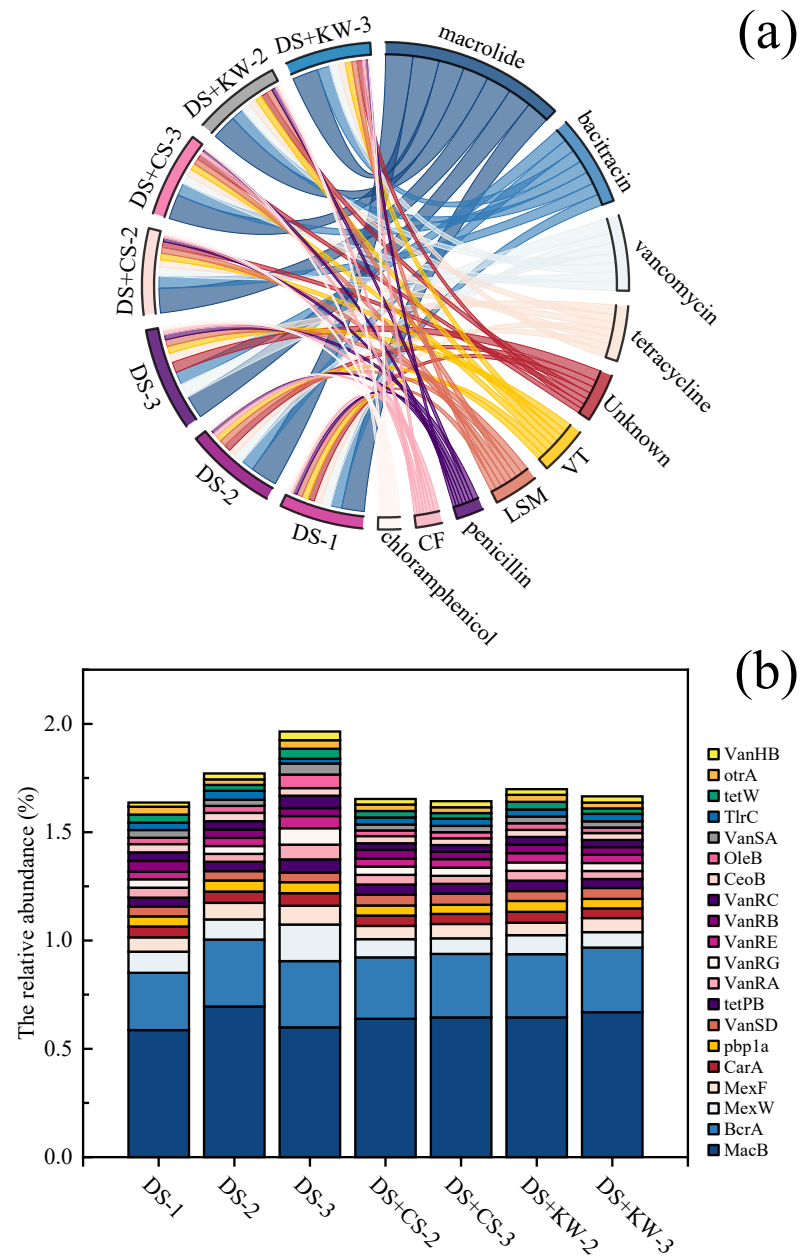


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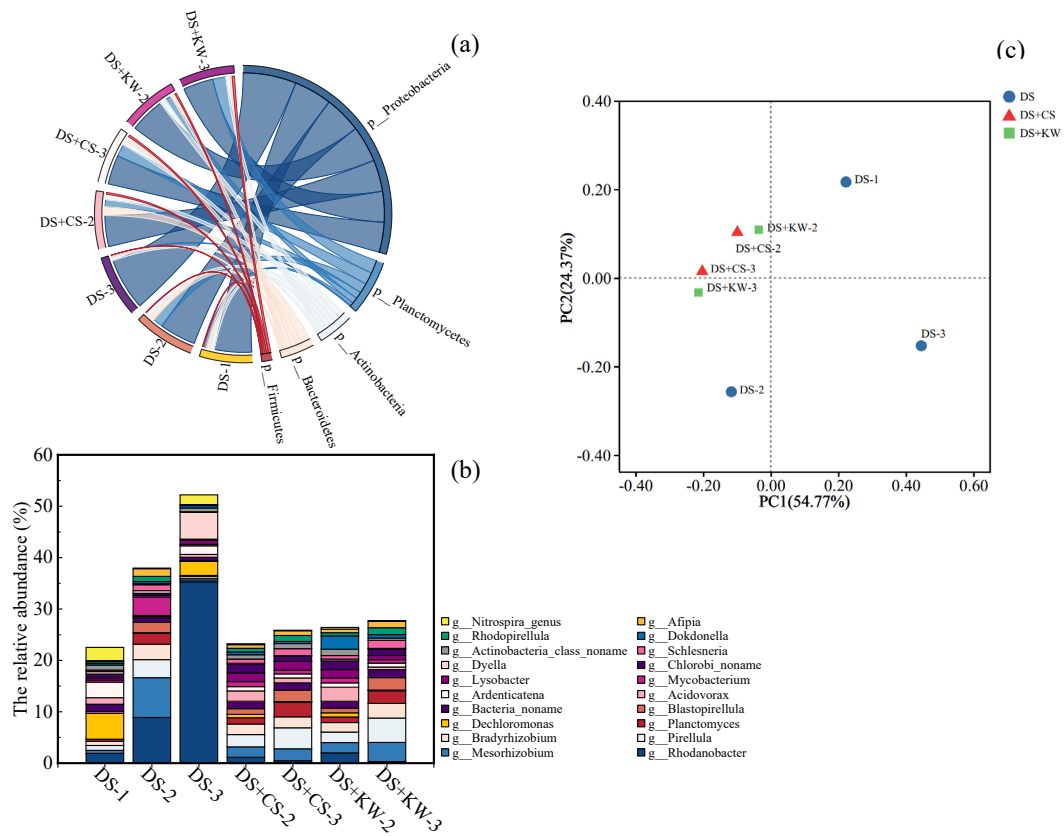


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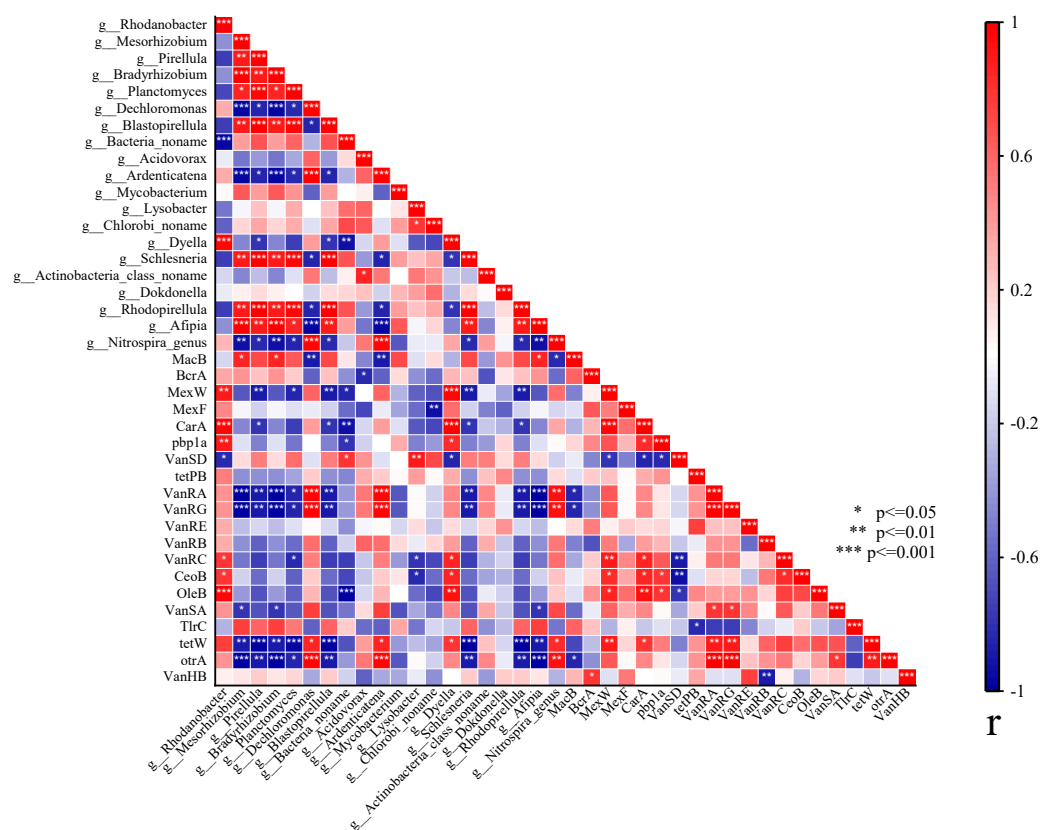


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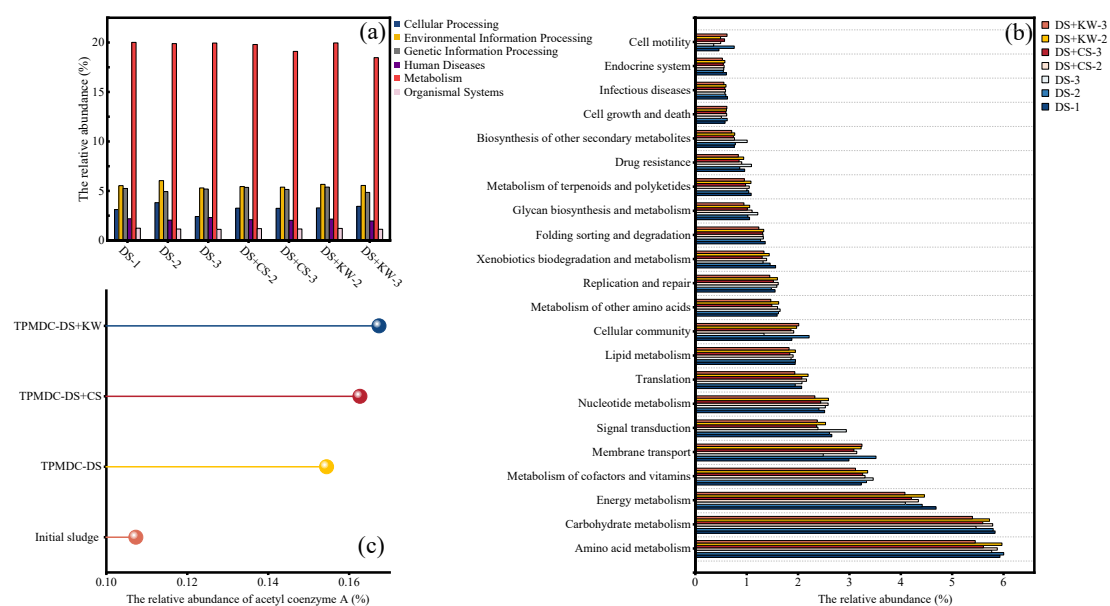


Fig. 7 Functional gene profiles, at level 1 pathway (a), level 2 pathway (b) and the relative abundance of acetyl coenzyme A based on KEGG annotation (c).

Sample	Shannon	Simpson
DS-1	13.29777	0.000004
DS-2	13.42759	0.000006
DS-3	11.54186	0.000055
DS+CS-2	13.67	0.000012
DS+CS-3	13.77676	0.000003
DS+KW-2	13.50185	0.000011
DS+KW-3	13.71688	0.000003

Table 1 The Shannon and Simpson indices in different samples.

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