

Article

Three-Dimensional Biofilm Electrode Reactors with Polyurethane Sponge Carrier for Highly Efficient Treatment of Pharmaceuticals Wastewater Containing Tetrahydrofuran

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Abstract: Three-dimensional biofilm electrode reactors (3D-BERs) exhibit efficacy in the removal of refractory wastewater of pharmaceuticals due to the resistance of pharmaceutical wastewater to biodegradation. In this paper, a new 3D-BER with a polyurethane sponge carrier was applied to the treatment of pharmaceutical wastewater containing tetrahydrofuran (THF) with an objective of exploring the removal efficiency, degradation pathway and main functions of microorganisms of 3D-BERs for wastewater containing THF. The results indicate that when the voltage is 10 V, the highest COD_{Cr} removal efficiency is (95.9 ± 1.6)%. Compared to the control group, the removal rate was increased by 21.97 ± 4.69%. The main intermediates of THF, γ-butyrolactone and 4-hydroxybutyric acid, were detected, respectively, by Gas Chromatography–Mass Spectrometry (GC–MS), indicating that 3D-BERs contribute to the degradation of THF with electro-oxidation as well as microbial synergism. Microorganisms, such as *Proteobacteria* with extracellular electron transfer capacity, *Bacteroidetes* capable of degrading complex carbon sources and parthenogenic anaerobic bacteria *Firmicutes*, were found to be enriched by high-throughput sequencing analysis in 3D-BERs, which were conducive to the degradation of refractory pollutants. At the genus level, *Chryseobacterium*, *Brevundimonas*, *Erysipelothrix*, and *Desulfovibrio* were the main functional genera, whose degradation of THF intermediates was found by functional prediction, mainly through chemoheterotrophy, aerobic chemoheterotrophy, etc. It is to be hoped that this study will provide a solution to the practical treatment of pharmaceutical wastewater containing THF via this new 3D-BER system with a polyurethane sponge carrier.

Keywords: three-dimensional biofilm electrode reactors; particle electrode; pharmaceutical wastewater; tetrahydrofuran; bioelectrochemical effects



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1. Introduction

In recent decades, the development of pharmaceutical production has led to a large amount of waste emission, which seriously affects the ecological environment [1]. Several pharmaceutical pollutants, such as antibiotics, dexamethasone and sulfadiazine, have been found in wastewater. Among the pharmaceutical wastewaters, THF is one of the most consistent pollutants. However, the average treating ratio of pharmaceutical wastewater containing THF is less than 30%, and it has become a serious source of water pollution [2]. Pharmaceutical wastewater causes serious detriment to both humans and to the environment if it is discharged before proper treatment [3,4]. Therefore, there is an urgent need to develop a suitable technology for pharmaceutical wastewater treatment [5].

Owing to the resistance of pharmaceutical wastewater to biodegradation, conventional biological technologies inefficiently destroy the structures of pharmaceuticals [6]. Economical and effective technologies have always been the concern and focus in pharmaceutical wastewater treatment [7]. In this context, the biological technology-assisted advanced

oxidation reactions have found a niche to dominate in the near future. Electrochemical technology has obvious superiority in treating refractory wastewater due to its wide adaptability in contaminant decomposition as well as simple equipment demand, small area occupation, and easy operation [8,9].

In recent years, electrochemical technology, especially 3D electrochemical technology, has become the focus of study because of its easy operation, small footprint and short hydraulic retention time (HRT) [10]. However, the electrochemical process requires considerable energy input. Hence, it is a key to effectively reducing energy consumption for the future development of three-dimensional electrode reactors (3D-ERs). To address this problem, microorganisms are cultured on particle electrodes, and bipolar 3D-BERs have emerged. In addition, the response of different microorganisms to current intensity varies; therefore, functional microorganisms can be screened out by regulating current density. Under higher current density, the pollutants can be decomposed into easy components under the action of electricity, and further biodegraded by the biofilm attached on the particle electrode. Under lower current density, the activity of the biofilm is improved by electrical stimulation, thereby enhancing the biodegradation of the pollutants. Bipolar 3D-BERs can also exert versatile targets by altering the operation conditions, such as electrolyzing water to produce hydrogen to reduce nitrate and sulfate [11].

In fact, 3D-BERs integrate biofilm technology with (3D-ERs), and take the advantage from both treatment processes. Several mechanisms account for pollutants degradation in 3D-BERs, including adsorption, biodegradation, electro-adsorption, electro-chemical oxidation and electro-biodegradation [12]. The accumulation of organisms and microorganisms induces biofouling on the electrode surface due to the presence of particle electrodes and the synergy of electricity and microorganisms. Among them, electro-biodegradation, the synergy of electricity and microorganisms, is critical for the removal of refractory pollutants. The mechanisms mainly include two aspects: one is the enhanced bio-degradation due to the stimulation of the electric field on microbial metabolism and the acclimation of the microbial community [13]; the other is the positive contribution of the intermediates generated from the electrochemical process to biodegradation.

With the synergy of electricity and microorganisms, 3D-BERs also exhibit efficacy in the removal of refractory wastewater [14]. Recently, 3D-BERs have been developed for refractory wastewater treatment by taking advantage of both electrochemical and biological technologies [12]. Because of their lower operation costs than those of 3D-ERs, 3D-BERs are considered mainly for the pretreatment of pharmaceutical wastewater [15]. 3D-BERs were constructed to treat Rhodamine B (RhB) [14], and the results indicated that the application of voltage promoted the degradation of RhB. Three processes, including electro-adsorption, electro-chemical oxidation and electro-biodegradation, were identified to contribute to RhB degradation. Biodegradation of ibuprofen has been recently demonstrated in a 3D-BER. The optimal conditions were identified at a current density of 12.73 A/m^2 and an HRT of 3.5 h [11]. Recently, 3D-BERs have been developed for the treatment of wastewater containing antibiotics, and its potential for the removal of sulfamethoxazole (SMX) and tetracycline (TC) was evaluated; 88.9–93.5% of an SMX removal rate and 89.3–95.6% of a TC removal rate were obtained, respectively [9].

Electrodes (particle electrode, anode and cathode), which are important units of 3D-BERs, play critical roles in pollutant degradation, microbial attachment, electrons transfer, etc. [16]. Filled particles are the important units of 3D-BERs. When the external potential is applied, they are polarized to form a large number of micro-electrodes, and the area of the main electrode is enlarged, namely, the particle electrode in 3D-BERs. Particle electrodes can improve the performance of 3D-BERs, not only because of their participation in the electrochemical reaction, but also due to their function of microbial carriers for colonizing organisms to promote associated microbial growth and reproduction. Currently, granular activated carbon (GAC) has a porous structure and a large specific surface area, which is beneficial for mass transfer and microbial attachment [17,18]. A 3D-BER packed with GAC particle electrodes was developed to treat reactive brilliant red X-3B

(RBRX-3B) dying wastewater [19]. Under the conditions of HRT 24 h, 90% of the average decolorization efficiency and 80% of the COD_{Cr} removal efficiency were obtained when the initial concentration of RBRX-3B was 1000 mg/L. In recent years, some other particle electrodes such as zeolite, lithium slag, steel slag, ceramist and sulfonated cation-exchange resin have been applied in 3D-BERs to treat wastewater [11]. Different particle electrodes resulted in a distinct interaction with microorganisms in terms of biocompatibility, electrical conductivity, microbial activity and specific metabolic functions.

The particle electrode in 3D-BERs is an important place for pollutants degradation. Therefore, its materials need to have advantages such as good conductivity, high corrosion resistance, nontoxicity and good biocompatibility. GAC is most widely used due to its good conductivity. However, bioreactors filled with GAC can be easily blocked with the increased biomass. The biofilm on the particle electrode is usually considered to have weak conductivity, but a growing number of microorganisms have been shown to have electroactivity, which can be attached to a biofilm formation [20]. The microorganisms with electroactivity are called electroactive microorganisms (EAMs), which have the ability to carry out the flow and exchange of electrons between intracellular and extracellular redoxactive electron donors and acceptors. In addition, EAMs have efficient extracellular electron transfer (EET) capabilities [21]. EAMs can be used for pollutant degradation and environmental remediation via their EET capabilities, which have been enhanced by modifying EAMs using synthetic biology and material engineering strategies. For example, the synthesis of electron shuttles can be enhanced through synthetic biology to improve the EET, and boosting biofilm formation can be improved by material engineering to increase the conductivity of EAMs [22].

The polyurethane sponge carrier with a medium pore can not only increase the biodiversity of microorganisms in 3D-BERs, but also ensure an excellent biofilm structure and provide excellent treatment performance. However, weak electrical conductivity was the shortcoming of polyurethane sponges as particle electrodes. In 3D-BERs, the electric field was conducted to improve EET capabilities and boost biofilm formation, which produces a growing number of EAMs, and significantly improves conductivity for the polyurethane sponge carrier, which, therefore, could be filled as particle electrodes in 3D-BERs because of their biofilm conductivity.

In this study, a polyurethane sponge carrier is adopted to build a new particle electrode in 3D-BERs, which aims to develop the biomass and activity for the particle electrode by boosting EAMs, and which is used in the advanced treatment of pharmaceutical wastewater containing THF. The treatment efficiency for pharmaceutical wastewater in the 3D-BER system was explored, and the degradation process of THF was identified. Moreover, the function and microbial community of the new 3D-BERs was investigated. The results can provide a solution to the practical treatment of pharmaceutical wastewater containing THF via these novel 3D-BERs filled by new particle electrodes with a polyurethane sponge carrier.

2. Materials and Methods

2.1. Experimental Setup

The 3D-BERs are shown in Figure 1. The reactor is a rectangular high-density polyethylene tank (200 mm × 150 mm × 300 mm) with an adequate working volume of 5 L. The anode plate is a Ti-IrO₂ electrode (300 mm × 200 mm) and the cathode plate is a pure titanium mesh placed on both sides of the reactor with a thickness of 2 mm, and the two electrodes are connected to the positive and negative DC-regulated power supply. A combined suspension ball polyurethane sponge carrier of Φ60 mm and Φ90 mm (filling rate of 50%) is filled between the electrode plates, and the filling rate of the polyurethane sponge carrier in each individual suspension ball is 30–50% to form a 3D biofilm electrode by loading the biofilm. The polyurethane sponge carrier is a medium-pore carrier (20 mm). Suitable micropores can maintain not only an appropriate number of microorganisms, but also a good biofilm structure and activity. The high surface area provides a huge breeding

ground for EAMs. During enrichment, electrical acclimation helps to form EAMs with an EET, which plays the same role as conductive particles, thus increasing conductivity and forming a new type of 3D-BER, which is beneficial to the degradation of refractory pollutants [20]. Six aeration heads were uniformly arranged at the bottom of the reactor to regulate dissolved oxygen and provide agitation. An unpowered control group was set up during the experiment, and all experimental materials and conditions were the same as the 3D-BERs. A DC regulated power supply (MS303D-30V10A) was manufactured by Maisheng Power Supply (Dongguan, China), and the air pump (S-30B) was purchased from Sailor Lipa (Ningbo, China).

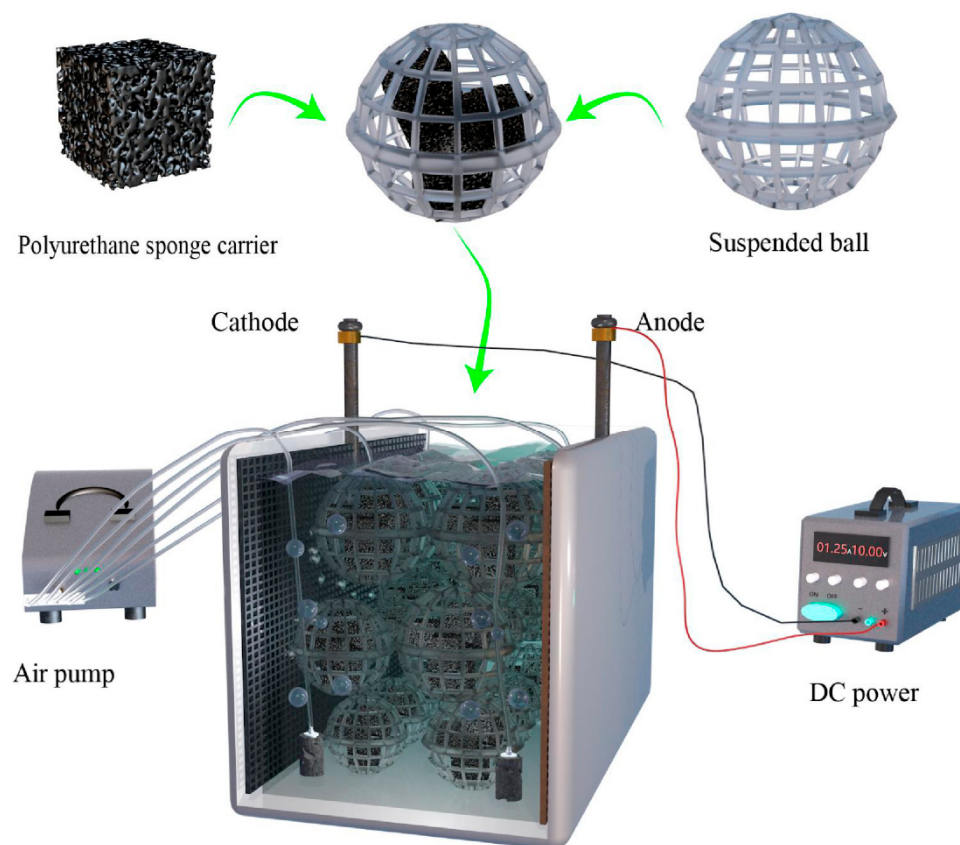


Figure 1. Schematic diagram of 3D-BERs.

2.2. Wastewater Source

The raw pharmaceutical wastewater adopted in this study was collected from the wastewater treatment station of a pharmaceutical production company in Lanzhou, China. The station's wastewater was mainly produced from the wastewater of the anti-tumor pharmaceutical workshop, the wastewater of the traditional Chinese medicine extraction workshop, and the enterprise's internal domestic sewage. The main pollutants in the mixed pharmaceutical wastewater were THF, ethyl acetate, trichloromethane, and dichloromethane, etc. The raw wastewater had a poor biodegradability as a consequence of its complexity and toxicity [23]. After mixing it for electro-Fenton pretreatment (electro-Fenton operation parameters: HRT = 240 min, $\text{H}_2\text{O}_2 = 50 \text{ mmol/L}$, Fe^{2+} injection amount determined as 10 mmol/L , initial pH = 3, an optimal current density of 15 mA/cm^2), the effluent was then adjusted to a pH between 7.5 and 9.0 to obtain a 3D-BERs influent. The obtained experimental water quality parameters of the pharmaceutical wastewater are shown in Table 1.

Table 1. Source characteristics of pharmaceutical wastewater.

Parameter	pH	Temperature (°C)	NH ₄ ⁺ -N(mg/L)	COD _{Cr} (mg/L)	THF (mg/L)
Raw wastewater	5.5~7.0	25 ± 5	9 ± 1	5750 ± 750	304 ± 39
Electro-Fenton effluent	3.0~4.0	20 ± 5	8 ± 1	4190 ± 585	195 ± 7

2.3. Experimental Procedure

The reactor-inoculated sludge was taken from the secondary sedimentation tank of a municipal wastewater treatment plant in Lanzhou City, Gansu Province, with the sludge pH = 6.9, the water content of 98.95%, and the suspended solids mass concentration of 5,000 mg/L. After precipitation, the inoculum to the 3D-BERs activated sludge was 7 g/L, and after adding it, the membrane hanging began with a starting voltage of 0 V. The COD_{Cr} of the electro-Fenton effluent was diluted to about 1000 mg/L, the dissolved oxygen was controlled within 3–4.5 mg/L, and after continuous aeration, when the COD_{Cr} changed little and remained stable, water exchange was started while gradually increasing the influent concentration according to a gradient, and the effluent COD_{Cr} removal rate was stabilized at 60 ± 4% for one consecutive week after 21 d, marking the completion of the membrane-hanging startup [24]. The reactor voltage was increased from 0 V to 14 V, and a charge of 2 V was started on the 21st d. After holding for 5 days, it was increased to 4 V. This operation was continued, with each voltage increase of 2 V, until the voltage was increased to 14 V. After one week of a continuous 14 V operation, we entered the experimental phase. Under a specific voltage, the COD_{Cr} changed slightly and remained essentially stable (for about 5 days), indicating that biological adaptation had entered the next stage. Meanwhile, the control group was started in the same way as above, without powering up during the startup and formal experiment phases.

During the sequencing batch experiment, the dissolved oxygen in the reactor was maintained within 5–8 mg/L, the pole-plate spacing was set to 150 mm, the voltage was maintained at 10 V for 39 days, and the HRT of the reactor was operated for 24 h (including 0.2 h for inlet water, 23 h for aeration, 0.5 h for precipitation, and 0.3 h for discharge water). The inlet and discharge water samples were taken after daily monitoring of the water pH, temperature and dissolved oxygen. The water samples were taken in pre-cleaned 500 mL brown glass bottles and stored in a refrigerator to avoid light at a temperature of 4 °C. COD_{Cr} and GC-MS of water samples were detected within 48 h.

Measuring instrument: pH meter (PHS-3C, Leici, Shanghai); Glass rod thermometer (Chenhua, Shanghai); DO instrument (HQ30d, HACH, USA). COD_{Cr} determination (6B-12 digestion instrument /6B-201 rapid analyzer, Shengaozhua, Jiangsu); GC-MS (7890A-5977A, Agilent, Santa Clara, CA, USA).

The intermediate compound analysis of the pharmaceutical wastewater sample was carried out using GC-MS (7890A-5977A, Agilent, USA) by extracting wastewater samples (100 mL) with dichloromethane. The GC was equipped with a DB-WAX ms capillary column (L × D × T = 30 m × 0.25 mm × 0.25 µm). The chromatographic conditions included the following temperature programs: the column temperature was initially held at 30 °C for 6 min; this was subsequently increased to 60 °C at 4 °C/min, then increased to 110 °C at 10 °C/min and kept constant for 5 min; it was then increased to 250 °C at 20 °C/min; the final temperature was held for 2 min. A voltage of 1.2 kV was used, with electron impact ionization at 70 eV for molecular fragmentation. The mass spectra of the peaks were compared with the GC-MS database with a standard library for compound identification.

During the biological stabilization, the measured biomass of the polyurethane sponge carrier was 25–30 mg/cm³; at the same time, the packed biofilm near the anode (AB), the packed biofilm near the cathode (CB), and the control group activated sludge inoculated in the reactor were taken (AS), and the microbial community structure was measured. All samples of COD_{Cr}, GC-MS and high-throughput sequencing were tested in triplicate.

3. Results and Discussion

3.1. COD_{Cr} Degradation

When the pretreatment electro-Fenton effluent was adjusted to pH 7.5–9.0 and was added to the 3D-BERs, as shown in Figure 2a the removal rate showed a decrease after an increase in the voltage from 0 V to 14 V; the best removal efficiency appeared at 10 V, and gradually decreased after 10 V. When the voltage of the 3D-BERs varied from 0 V to 10 V, the overall COD_{Cr} removal efficiency showed an increasing trend, and the average COD_{Cr} removal rate increased from 70.95% at 0 V to 95.91% at 10 V. This is due to the fact that the electric field can stimulate the growth and metabolism of microorganisms in the biological community and increase the biological enzyme activity, thus improving the degradation ability of microorganisms in the wastewater.

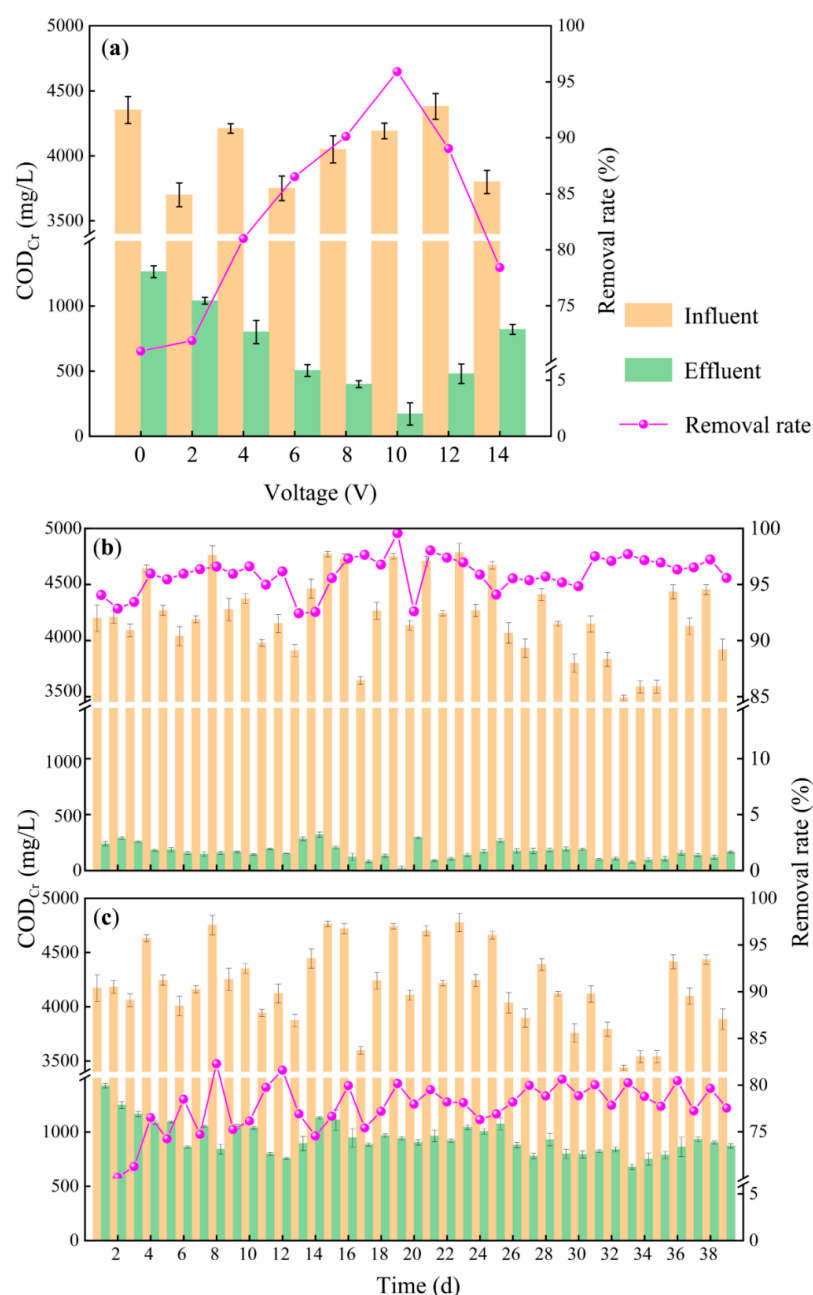


Figure 2. COD_{Cr} removal rate of pharmaceutical wastewater in 3D-BERs (a) under different voltages; (b) under a voltage of 10 V; and (c) under a control group (no power).

When the voltage of the 3D-BERs continued to increase from 10 V, the COD_{Cr} removal rate showed a decreasing trend, and the average removal efficiency decreased from 95.91% at 10 V to 78.42% at 14 V. This is due to the fact that too high a voltage induces microbial metabolic dysregulation, which leads to the limitation of the microbial COD_{Cr} degradation; and if the voltage is further increased, it will lead to microbial inactivation [18]. Therefore, it was operated steadily for 39 days at the best voltage of 10 V and HRT = 24 h. The measured changes in COD_{Cr} concentrations of influent and effluent during the operation period and the removal rate change curves are shown in Figure 2b. Dissolved oxygen was maintained within 5.07–7.92 mg/L during the whole phase of the 3D-BERs operation; the water temperature fluctuated between 15.1 and 25.0 °C, and the influent COD_{Cr} fluctuated within 3438.30–4775.70 mg/L due to different effluent discharges from pharmaceutical plants. The effluent COD_{Cr} after treatment by the 3D-BERs ranged within 20.18–331.09 mg/L and was steadily lower than 350 mg/L, with an average removal rate of $95.89 \pm 1.63\%$. Although the variation in the influent COD_{Cr} and temperature was relatively large, the effluent COD_{Cr} was relatively stable, which indicates that 3D-BERs have strong resistance to organic matter and temperature loading on pharmaceutical wastewater and can be operated stably for a long time.

Under the same condition of dissolved oxygen, temperature, HRT, and influent concentration, the results of the control group are shown in Figure 2c. We found that the COD_{Cr} removal rate was $77.44 \pm 4.85\%$ in the control group without electricity, which was significantly lower than that of the 3D-BERs at 10 V. This suggests that the removal of organic pollutants by the 3D-BERs results from the synergistic action of multiple methods: biofilter adsorption, microbial degradation, and electric field oxidation [25]. The degradation pathway of organic pollutants may be either the decomposition into easily degradable components under the action of electricity, which are further biodegraded by the biofilm attached to the filler, or that the electrical stimulation increases the activity of the biofilm, thus enhancing the biodegradation of pollutants [11]. By constructing 3D-BERs to treat ibuprofen wastewater, the experimental results for 30 consecutive days show that the removal rates of ibuprofen and COD_{Cr} by the 3D-BER are stable within 87.38–90.19% and 84.58–89.28%, which is significantly better than that of a single-aeration bioreactor [26]. Based on the above effects, in terms of an environmental feasibility assessment, it is proved that 3D-BERs can effectively degrade the pharmaceutical wastewater containing tetrahydrofuran, which is $21.97 \pm 4.69\%$ higher than that of the CG. Although the power consumption is increased, the power consumption under the 10 V voltage is 10 kWh/m³, which is acceptable in terms of energy consumption for refractory wastewater treatment.

To sum up, the treatment of pharmaceutical wastewater by using 3D-BERs, including electro-oxidation, biodegradation, and other synergistic effects, solves the problems that cannot be solved by conventional biological processes, which is the advantage and the promising future of 3D-BERs.

3.2. Variations in the Organic Components

3.2.1. Identification of Intermediates Using GC-MS

The optimum conditions for electro-Fenton pretreatment of pharmaceutical wastewater are shown in the GC–MS chromatograms. As shown in Figure 3a,b, volatile organic compounds (VOCs) and semi-volatile organic compounds (SVOCs) can be detected. There are VOCs in the influent of 3D-BERs, namely, THF, ethyl acetate, dichloromethane, and trichloromethane. In addition, the peak time is 2.71 min, 3.08 min, 3.80 min, 6.55 min, respectively. Furthermore, there are SVOCs, namely, γ -butyrolactone and diisobutyl phthalate, and the peak time is 6.50 min and 25.09 min, respectively. The above components indicate that pharmaceutical wastewater contains a large number of aromatic proteins and organic esters. At the same time, γ -butyrolactone (THF-degraded intermediates) was detected in the influent water, indicating that it was accumulated during the electro-Fenton oxidation process.

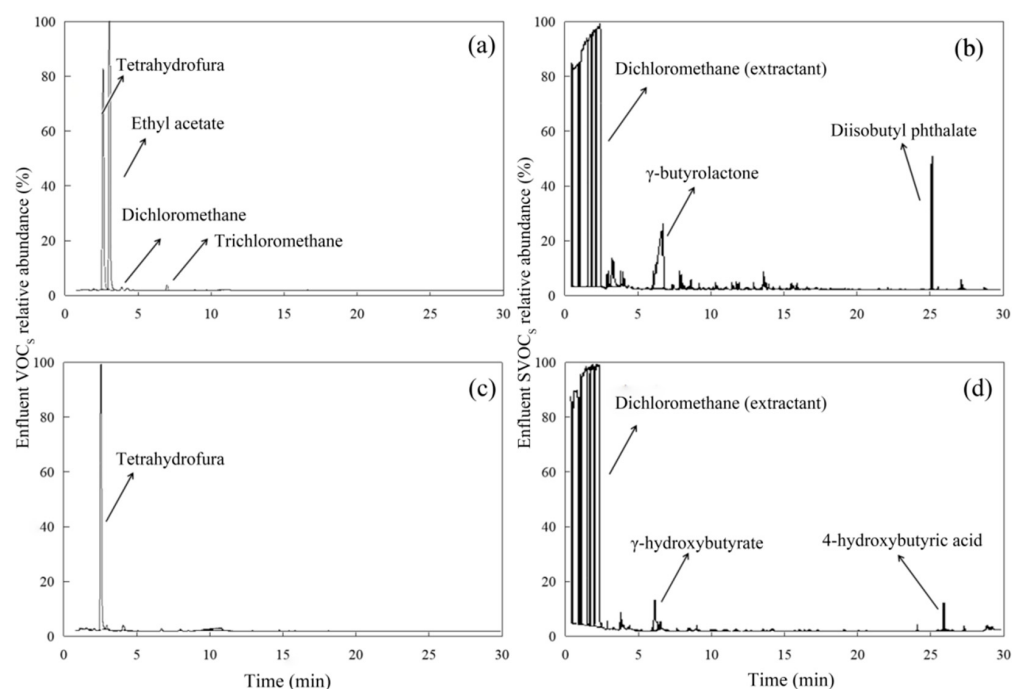


Figure 3. GC-MS spectra of pharmaceutical wastewater degradation process. (a) Electro-Fenton effluent of VOCs; and (b) SVOCs; (c) 3D-BERs effluent of VOCs; and (d) SVOCs.

After the operation of the 31st day, the effluent of the 3D-BERs was detected by GC-MS. According to Figure 3c,d, VOCs THF was detected in the effluent of the 3D-BERs at the peak time of 2.71 min. The peak time of the SVOCs γ -hydroxybutyrate and 4-hydroxybutyric acid was at 6.16 min and 26.28 min, respectively. THF, which has the largest proportion of raw water, is a typical refractory organic matter. According to the area percentage in the map and the chemical structure of the organic matter, γ -butyrolactone and 4-hydroxybutyric acid are the main intermediate products in the degradation process of THF. Among them, the characteristic peaks of γ -butyrolactone and diisobutyl phthalate in raw water have disappeared, which can be explained as the two substances in the 3D-BERs that have been completely mineralized and converted into small molecular substances that can be more easily metabolized by microorganisms. The above situation proves that benzene ring-containing aromatic organic compounds may undergo chain scission and aromatic ring hydrolysis, resulting in many substituents such as carbonyl, hydroxyl, and carboxyl groups, which are degraded by microorganisms.

In order to confirm the removal effect of the 3D-BERs on THF in pharmaceutical wastewater, the influent and the effluent were further subjected to GC-MS quantitative analysis. The THF concentrations in the influent and effluent water are 201847 $\mu\text{g/L}$ and 4744 $\mu\text{g/L}$, respectively. The removal rate of THF was as high as 97.65%, and the COD_{Cr} removal rate of the corresponding wastewater reached 98.14%.

3.2.2. Analysis of Degradation Process of THF in 3D-BERs

The isolation of tetrahydrofuran-degrading bacteria was reported, and the metabolic mechanism was also explained in detail. The refractory biodegradability of THF is mainly reflected in the C-O (360 kJ/mol) high-energy bond and low biodegradability in its cyclic ether structure [27]. In the research on the degradation of THF by *Rhodococcus* sp. Strain 219, it was speculated that the degradation pathway of THF is that THF undergoes hydroxylation of the ortho-position carbon atom of the oxygen atom to generate 2-hydroxytetrahydrofuran, which is then oxidized to γ -butyrolactone, and then ring-opened to generate γ -hydroxybutyrate, which is finally further oxidized to succinate, entering the tricarboxylic acid cycle and being thoroughly mineralized [28]. Combined with experiments, it is speculated that the main metabolic pathway of THF is shown in

Figure 4. Its metabolic pathway is more detailed: γ -hydroxybutyrate is oxidized to succinate semialdehyde and then oxidized to succinate, which is then degraded to glycolic acid, and finally mineralized into CO_2 and H_2O .

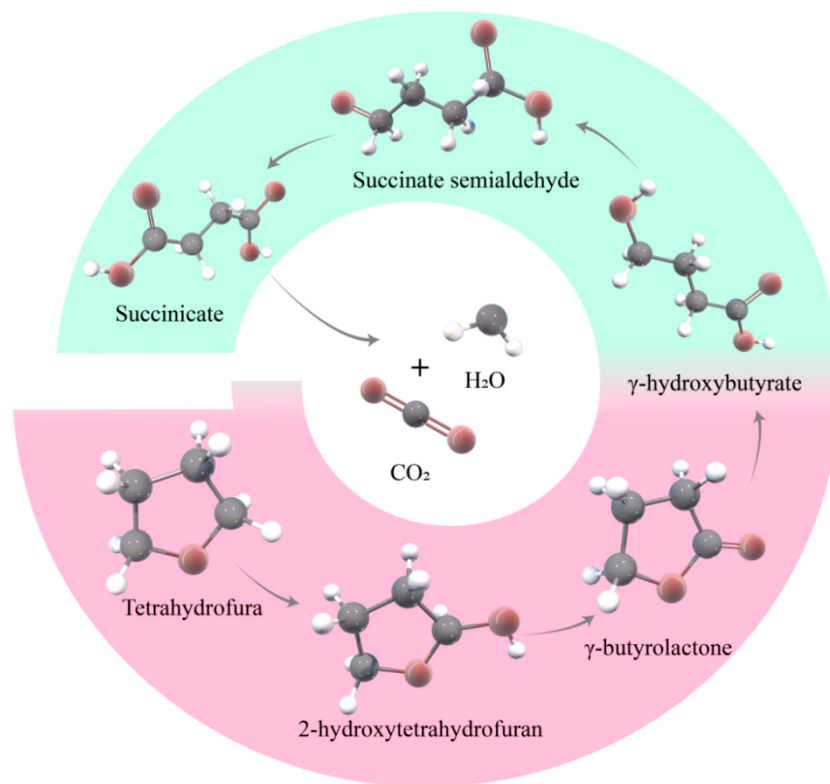


Figure 4. Degradation process of tetrahydrofuran in 3D-BERs.

In this study, γ -butyrolactone was detected in the effluent of electro-Fenton, indicating that it was accumulated in the electro-Fenton process; in contrast, γ -hydroxybutyrate was not detected, indicating that the electro-Fenton process could only oxidize THF to γ -butyrolactone and could not be further opened. Meanwhile, 2-hydroxytetrahydrofuran could not be detected, indicating that its degradation rate is greater than the generation rate. THF was primarily degraded in the 3D-BERs, γ -hydroxybutyrate was accumulated, and γ -butyrolactone was not detected, indicating that the 3D-BERs could oxidize γ -butyrolactone to γ -hydroxybutyrate gradually.

Succinate could not be detected in the effluent of the 3D-BERs, indicating that the oxidation rate of γ -hydroxybutyrate to succinate was lower than the mineralization rate of succinate. It is possible that succinate is a tricarboxylic acid when microorganisms convert complex organic matter into small molecule organic matter, and intermediates in the acid cycle can be directly used in the biosynthesis [29]. The study shows that 3D-BERs can achieve effective degradation of THF.

3.3. Microbial Community in 3D-BERs

3.3.1. Microbial Community Analysis at the Phylum Level

On the 39th day of the stable operation, three groups of sludge samples from AB, CB and CG were taken, respectively. High-throughput sequencing was employed to analyze the abundance and the diversity of microbial communities, with the biofilm of anode (AB), cathode (CB), and the control group (CG). Based on the threshold of the operational taxonomic unit (OTUs) > 0.97, the abundance and species diversity of the aerobic bioreactor were relatively high, suggesting that the microbes in the 3D-BERs grew well using pharmaceutical wastewater containing THF as substrates. The Shannon values of the sludge samples AB, CB and CG obtained from the high-throughput sequencing

report were 3.9009, 4.7747 and 4.6567, respectively. Chao1 values were 463.01, 674.98 and 887.86, respectively. The Shannon value of the CB was relatively higher than that of the CG. Nevertheless, the Chao1 and Shannon values of the AB were somewhat lower than those of the CG because anodic electro-oxidation produces some solubility that inhibited the growth of microorganisms. Organic matter resulted in an increase in the abundance of microbial communities that can perform an electron transfer.

The composition and abundance of the microbial community in 3D-BERs at the phylum level are displayed in Figure 5. *Proteobacteria* is the predominant species, with the percentages near the anode and cathode being 51.18% and 26.96%, respectively. *Proteobacteria* are Gram-negative bacteria, one of the largest bacterial phyla in the bacterial kingdom, belonging to obligate or facultative anaerobic bacteria. Extracellular electron transfer capability is widespread in the field of water treatment [30]. *Bacteroidota*, *Firmicutes*, *Desulfobacterota*, and *Actinobacteriota* were subdominant groups, accounting for 46.57% and 67.15% of the total taxa. Compared to the CG, the electric field in the 3D-BERs prompted the relative abundance and growth of *Firmicutes*, *Proteobacteria*, which are the main bacterial phyla for the biochemical system to deal with the degradation of organic matter in pharmaceutical wastewater [31]. The relative abundance of *Proteobacteria* in AB and CB samples was lower than that in the CG, which may be due to the screening effect of electrochemical action on *Proteobacteria*, leading to a decrease in their relative abundance. *Bacteroidota* are chemo-organotrophic bacteria with the ability to degrade complex carbon sources and macromolecular organic matter such as lipids and proteins [32]. Combined with GC-MS analysis, it can be seen that 3D-BERs show obvious removal effect on lipids such as γ -butyrolactone and diisobutyl phthalate, which may be the results of biofilm enrichment of *Bacteroidota* near the anode and cathode. The relative abundance of *Firmicutes* in the AB and CB was 5.79% and 34.54%, respectively. It can be seen that the electric field has an enrichment effect on *Firmicutes*. Most of them are facultative anaerobic bacteria and electricity-producing microorganisms that obtain energy for growth by oxidizing sugars or organic acids [33]. *Firmicutes* are found to be the main dominant phyla in antibiotic wastewater treatment [34]. Therefore, *Firmicutes* microorganisms are enriched near the cathode, and with succinic acid. The degradation product of THF may be used as a carbon source to realize the treatment of pharmaceutical wastewater by the 3D-BERs.

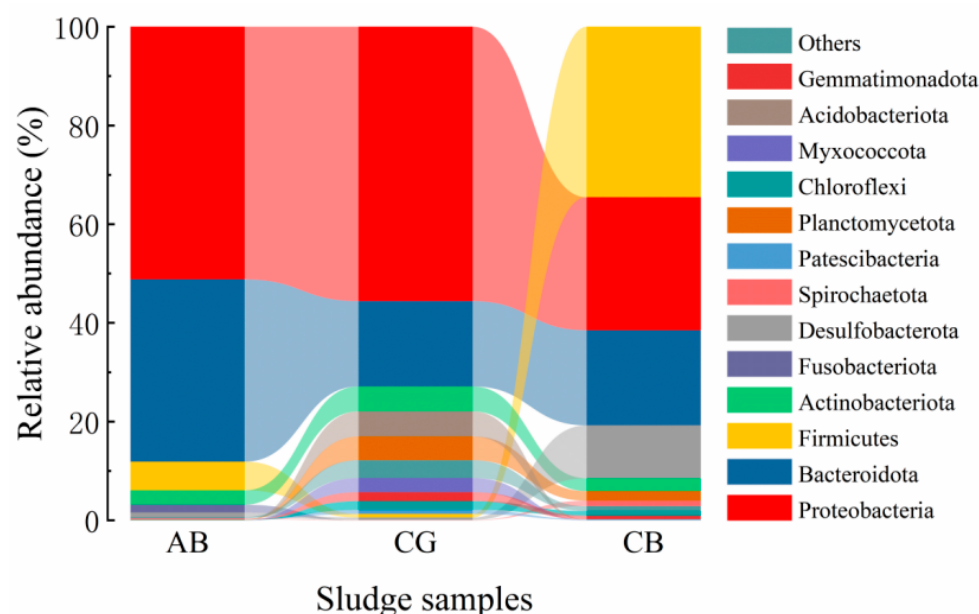


Figure 5. Microbial communities at phylum level (relative abundance over 1%).

In contrast, the relative abundance of *Planctomycetota* and *Patescibacteria* decreased in the electric field, indicating that metabolism of those bacterial populations is inhibited by

the high voltage. *Myxococcota*, *Acidobacteriota*, *Gemmatimonadota* almost disappeared under 10 V, indicating that the metabolism of those bacterial populations is significantly inhibited.

3.3.2. Analysis at the Bacterial Genus Level

The composition and relative abundance of microorganisms at the genus level further revealed the evolution of the microbial communities in the 3D-BERs. As shown in Figure 6, the top 30 microbial genera (TOP30 genera) in relative abundance were selected from the three samples, and the dominant genera obtained at the genus level were analyzed. Due to the mutual synergy of electric field stimulation and domestication of pharmaceutical wastewater, the dominant bacterial genera near the cathode and anode plates in the 3D-BERs were significantly different from the CG. There were also significant differences in the dominant bacterial genera between the cathode and anode plate samples.

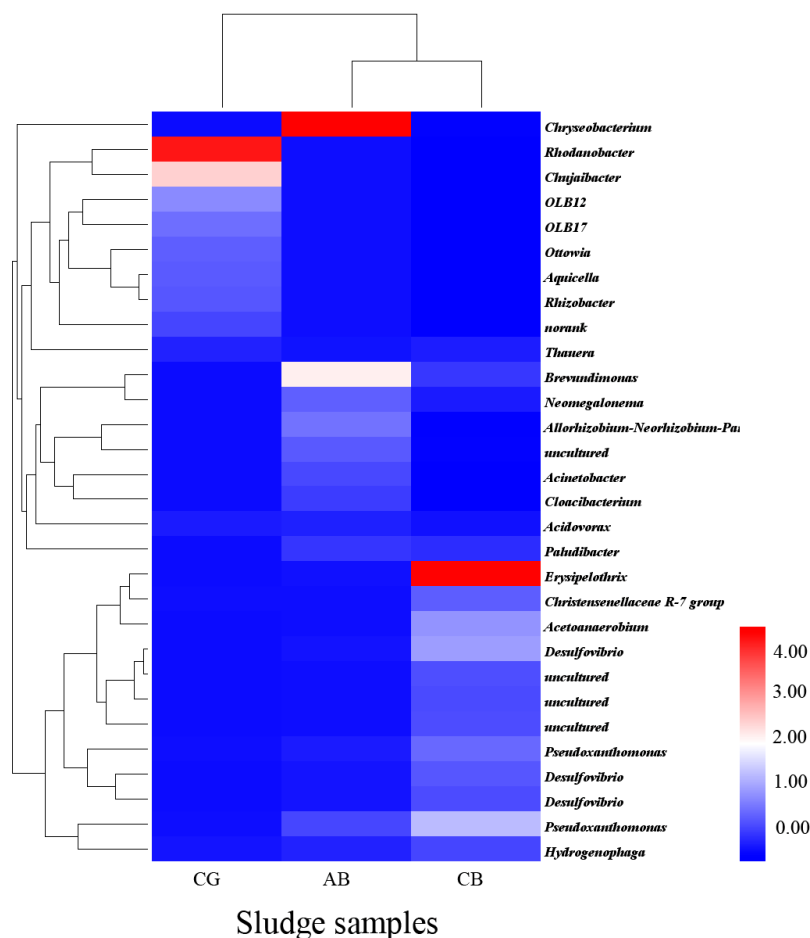


Figure 6. Microbial communities heat map analysis of 3D-BERs at genus level.

Among the genus near the anode in the 3D-BERs, *Chryseobacterium* was the dominant genus, with an average abundance of 25.94%, but only 0.51% in the cathode sample and 0.07% in the CG sample. It could utilize residual dissolved organic matter to facilitate the removal of pollutants [35]. It was found that the genus *Aureus* is the dominant bacterial genus in the anode of microbial fuel cells, with exogenous electroactivity [31]. In addition, *Chryseobacterium* has excellent tolerance to toxic pollutants and can degrade various organic substances, as well as being rich in extracellular electron transfer genes. In particular, degradation plays an essential role in the process of lignin [36], o-xylene [37], N-acyl homoserine [38], etc. A newly isolated bacterium *Arthrobacter* sp. WN18 is able to co-oxidize dioxane with THF as the primary substrate [39]. The relative abundance of *Brevundimonas* in anode samples reached 14.08%, significantly higher than that in cathode samples (2.33%)

and inoculated sludge samples (0.59%). *Brevundimonas* is also the dominant genus of the anode of microbial fuel cells and belongs to electroactive microorganisms [31]. The Group VIII carboxylesterase gene *estwx* cloned from *Brevundimonas* can convert the herbicide aryloxyphenoxy [40]. Cleavage of the ester bond of the propionate yields the corresponding acid alkyl side-chain alcohol. More importantly, *Brevundimonas* can biotransform a variety of complex substrates by hydrolysis [41].

In 3D-BERs, the relative abundance of *Erysipelothrix* reached 14.57% in the genus near the cathode, which was significantly higher than that in the anode sample (0.17%), but was not detected in the control group sludge sample. *Erysipelothrix* is a Gram-positive bacillus that can use organic carbon sources for growth and reproduction and reduce nitrate to nitrogen [42]. Using microbial fuel cells to remove azide, *Erysipelothrix* was also found to be the dominant genus at the cathode [43]. In addition, in the study on the treatment of chloramphenicol wastewater [44], acid mine wastewater [45], and printing and dyeing wastewater [46] by electrobiological systems, *Erysipelothrix* was detected to be involved in the degradation process. The relative abundance of *Pseudoxanthomonas* reached 9.8%, which was 4.1% in anode samples and was rarely almost present in the CG. *Pseudoxanthomonas* showed an important role in the process of degrading cyclic organic matter [47]. This is due to the increased relative abundance of *Pseudoxanthomonas* under the effect of electrical stimulation and enhanced biological activity. At the same time, trypsin is used as a digestive enzyme to improve the degradation of organic pollutants in pharmaceutical wastewater [48]. The relative abundance of *Desulfovibrio* reached 8.2% at the cathode and 0.26% at the anode, which was absent in the seeded sludge. Studies have shown that *Desulfovibrio* can transfer electrons in both directions. It has the ability to transfer extracellular electrons at the anode, and it can use the generated hydrogen as a single energy source at the cathode, and use organic compounds such as acetic acid and carbon dioxide as electron donors and carbon dioxide [49]. In 3D-BERs, *Acinetobacter* belongs to γ -*Proteobacteria*, a γ -negative bacterium that must survive in a strict aerobic environment and has sound degradation for refractory substances, especially aromatic compounds [50]. *Paludibacter* is a strict anaerobic bacterium belonging to the phylum *Bacteroidetes*, which can ferment a variety of monosaccharides and disaccharides to produce propionic acid, acetic acid, and a small amount of butyric acid [51]. *Cloacibacterium* belongs to the phylum *Bacteroidota*, which mainly appears in water sediments. The existence of aerobic bacteria [52], facultatively anaerobic bacteria and strictly anaerobic bacteria, confirms that 3D-BERs biofilm packing forms an aerobic-facultative-anaerobic environmental change trend from the outside to the inside, providing the necessary environmental condition for the removal of refractory substances.

In summary, under the combined effect of electric field stimulation and domestication of pharmaceutical wastewater, many bacterial genera can utilize refractory biodegradable substances on the biofilm of 3D-BERs, and some bacterial genera can metabolize the electrochemical oxidation of refractory biodegradable substances and the intermediate products of electrochemical oxidation of refractory substances. At the same time, the COD_{Cr} range is 3438.30–4775.70 mg/L, and the threshold condition is greater than 0.9; we have also predicted the metabolic pathways. As shown in Figure 7, *Chryseobacterium*, *Brevundimonas* in the anode and *Erysipelothrix*, *Pseudoxanthomonas*, *Desulfovibrio*, and other dominant bacteria genera in the anode utilize chemoheterotrophy, aerobic chemoheterotrophy, fermentation, and aromatic compound degradation. Among them, *Chryseobacterium* and *Desulfovibrio* are very critical to the degradation of THF. In combination with electrode plate electro-oxidation, THF can be completely mineralized, which is a fundamental factor for the 3D-BERs to improve the efficiency of microbial degradation of pharmaceutical wastewater.

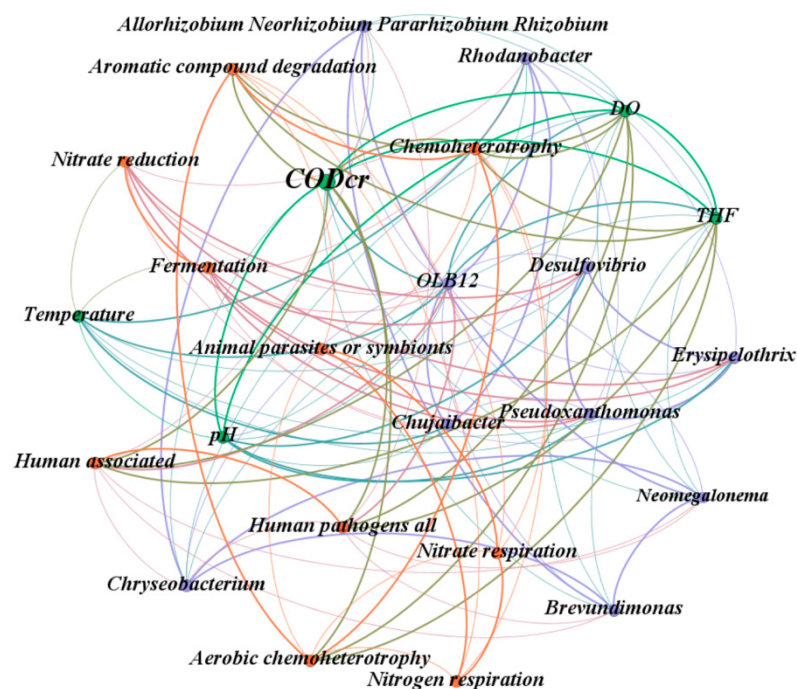


Figure 7. Network analysis diagram of microorganisms and metabolic pathways.

4. Conclusions

Our research revealed the links between electro-oxidation and microbial synergism treatment of pharmaceutical wastewater containing THF in 3D-BERs. Under the conditions of an influent pH 7.5–9.0, a voltage 10 V, an electrode spacing 150 mm, and an operating period of 24 h, 3D-BERs can effectively degrade organic pollutants in pharmaceutical wastewater. The average removal rate of COD_{Cr} is $95.89 \pm 1.63\%$ and the removal rate of THF is up to 97.65%. It has a strong impact load resistance to COD_{Cr} . The detection of the intermediate products γ -butyrolactone and γ -hydroxybutyrate suggested the degradation pathway of tetrahydrofuran in 3D-BERs. Electrochemical oxidation can break the structure of THF. Electrical acclimation enriches EAMs with EET on the surface of PU. Typical EAMs includes *Chryseobacterium* and *Desulfovibrio*. Electrochemical oxidation and electro-biodegradation work together to achieve effective degradation of THF, which is an important factor for the 3D-BERs to improve the efficiency in the microbial degradation of pharmaceutical wastewater. This study provides a reasonable analysis and a decision-making mechanism to guide and standardize the practice of comprehensive treatment of pharmaceutical wastewater in the future.

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