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Evaluating the influence of gradient applied voltages on electro-enhanced sequence batch reactor treating aniline wastewater: System performance, microbial community and functional genes

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ABSTRACT

Aniline is an important industrial raw material. Its complex structure makes it difficult to be chemically oxidized and its intense biological toxicity also increases the difficulty of biodegradation. Therefore, electro-biological system based on biological processing have become a new direction in the field of environmental governance. Comparative evaluation of the performance differences of reactors at gradient external voltages (1.5, 3.0, 4.5 and 6.0 V) was investigated to seek the optimal applied voltage in the electro-enhanced sequence batch reactor treating aniline wastewater. Results showed that all reactors degraded aniline effectively, the aniline degradation rate of five reactors was about 100%. However, the highest removal efficiency of total nitrogen was obtained at 1.5 V, up to 88.89%. Compared with control, high applied voltage (>3.0 V) deteriorated the nitrification performance and the removal efficiency of TN was descended along with the increase of applied voltages. Besides, proper applied voltage promoted the secretion of extracellular polymeric substance. The sludge activity such as specific oxygen utilization rate (SOUR), dehydrogenase (DHA) was highly increased under the applied voltage of 1.5V, so did the Ammonia monooxygenase (AMO) and nitrate reductase (NAR). Based on the high-throughput sequencing analysis, introducing applied voltages altered the microbial diversity and community structure. Particularly, several functional genera such as autotrophic nitrifying bacteria and denitrifying bacteria were enriched in the system at 1.5V. Network analysis also found that the cooperative/competitive relationship between key functional genus became simpler under the higher voltage. Additionally, the contribution of the functional flora to key metabolic pathways (benzoate and nitrogen metabolism) and the expression of various functional genes was augmented at the 1.5V applied voltage.

1. Introduction

Aniline($C_6H_5NH_2$) is an important raw material and intermediate in the textile printing and dyeing industry and its wastewater is continuously produced in the industrial production process (Abdel-Rahman et al., 2020; Mohammed et al., 2020). However, aniline also is a recognized toxic chemical that can cause many health problems in both organisms and humans (Zhang et al., 2021a). It can enter the body through skin contact, respiratory and digestive pathways. The toxicity mechanism of aniline to organisms is mainly reflected in the destruction of the structure of hemoglobin and the loss of its oxygen-carrying capacity, and the subsequent oxidative damage to proteins, lipids and DNA and the damage to various tissues (Tao et al., 2022). Therefore, due to its strong toxicity, refractory characteristics and easy to accumulate in the water environment, aniline has been listed as a priority pollutant by national environmental authorities, which is one of the key monitoring and governance objects (Jiang et al., 2016). As a high-efficiency and economical wastewater treatment technology, biological methods have been applied to the field of aniline wastewater because of its function of organic matter degradation and simultaneous nitrogen removal. For

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example, moving bed biofilm reactors, aerobic granular sludge and bio-augmented sequencing batch reactors have been developed to treat aniline, which showed high removal efficiency (Dvořák et al., 2014; Jiang et al., 2017; Zhang et al., 2021b). Although these studies provided unique insights into the biological degradation of aniline wastewater, they only focused on the removal of aniline and ignored the subsequent nitrogen removal process. By reason of the special structure of aniline, ammonia nitrogen will be released during its degradation. The treatment process of aniline wastewater should not only effectively achieve aniline degradation, but also have excellent nitrogen removal performance. In biological nitrogen removal process, ammonia nitrogen needed to undergo aerobic nitrification and anaerobic denitrification to be converted into N_2 to escape (Yan et al., 2022). Therefore, in addition to the degradation of aniline, special attention should be paid to whether the subsequent nitrification (oxidation of ammonia nitrogen to nitrate) and denitrification (reduction of nitrate to N_2) can be effectively carried out. As eutrophication has become the focus of environmental problems, the study of nitrogen metabolism in aniline degradation system has gradually become a hot topic in recent years (Peng et al., 2022). Nevertheless, the contradiction between aniline degrading bacteria and autotrophic nitrifying bacteria is hard to reconcile, such as dissolved oxygen competition. This makes nitrification become a stumbling block in the nitrogen removal process of the aniline biodegradation system (Fan et al., 2021).

In recent years, bio-electrochemical technology which combines an electric field and biological process, is attracting more and more attention because of its excellent performance on enhancing the degradation of refractory organic matter and nitrogen removal (Zhang and Angelidaki, 2014). Compared with the traditional biological wastewater treatment process, bio-electrochemical technology can stimulate the growth of microorganisms, increase metabolic activity and promote the transfer of electrons under the action of electric field (Lin et al., 2019). At present, this technology has been used to remove various pollutants, such as azo dyes, aromatic hydrocarbons (Cardenas-Robles et al., 2013). In our previous study, the electro-enhanced sequence batch reactor (E-SBR) was established to degrade aniline, demonstrating the improvement of pollutant removal rate (Feng et al., 2022). Whereas, the operation conditions of E-SBR have not been optimized. As the critical index in the electro-biological system treating aniline wastewater, the applied voltage might be a double-edged sword. Microorganisms attached to the electrode or suspended in solution as the core of the system, which is directly or indirectly affected by the electric field, thereby affecting the degradation of pollutants (Hu et al., 2022). It is accepted that appropriate electrical stimulation has a positive effect on microorganisms, which can change the permeability of cell membrane, improve microbial activity and promote microbial metabolism. On the contrary, high applied voltage would inhibit the activity of microorganisms, even led to death. Therefore, it is important to explore the optimal applied voltage in the electro-enhanced sequence batch reactor treating aniline wastewater.

In this work, different gradient applied voltage was exerted in sequence batch reactor treating aniline wastewater. The purpose was to: 1) compare the aniline degradation and nitrogen removal performance under different applied voltages; 2) evaluate the effect of applied voltage on aniline degradation bio-system from the perspective of enzyme activity and microbial community structure; 3) reveal the enhancement mechanism of system performance under weak electrical stimulation combined with metagenomics.

2. Materials and methods

2.1. Synthetic wastewater and inoculated sludge

In order to ensure the stability of influent quality, artificial simulated aniline wastewater was used in this experiment. Its composition was as follows: 400 mg/L aniline as the sole carbon and nitrogen source, 13 mg/L $NaH_2PO_4·2H_2O$ and 50.4 mg/L $Na_2HPO_4·12H_2O$ as phosphate source and buffer solution, 0.03 mg/L $MgSO_4·7H_2O$, 0.15 mg/L $ZnSO_4·7H_2O$, 0.18 mg/L NaCl and 0.024 mg/L Fe $(NO_3)_3·9H_2O$. The seed sludge was inoculated from aerobic pool of Longwangzui Wastewater Treatment Plant in Wuhan City, Hubei Province, China. Activated sludge generally consisted of four parts: a microbial community with metabolic functions, residues of microbial endogenous respiration and self-oxidation, refractory organic matter carried by raw sewage and inorganic substance adsorbed by sludge floc. The size of activated sludge floc was generally between 0.02–0.2 mm, with an indefinite shape and large specific surface area. The purification function of activated sludge mainly depended on the microorganisms inhabiting the activated sludge. The mixed liquor suspended solids (MLSS) concentration of the five reactors was controlled at about 3500 mg/L.

2.2. Reactor set-up

Five groups of sequencing batch reactor (SBR) with a cylindrical structure made of Perspex were used in the experiment. The effective volume of the reactors was 1 L and the size was 20 cm (height) × 10 cm (diameter). The applied voltage of the five reactors was Y1-0 V, Y2-1.5 V, Y3-3.0 V, Y4-4.5 V and Y5-6.0 V, respectively. Carbon brush was used as the electrode material, and its effective area was 108 cm^2 (3 cm × 3 cm × 12 cm). The electrode distance was 5 cm and the pretreatment method of electrode was referred to the previous study (Qin et al., 2022). Cathode and anode were connected with the DC regulated power supply (PS-305DF, China). The operating cycle was 12 h and the volume exchange rate was 50%. The reactor operated with the mode of O/A and the complete operational cycle included 5 min feeding - 6 h aerobic phase - 4.5 h Anoxic phase - 80 min setting - 5 min discharge.

2.3. Analysis method

Aniline, COD, NH_4^+-N, NO_3^--N, NO_2^--N and total nitrogen (TN) were measured everyday according to the standard method (APHA, 2005). The thermal extraction method was used to separate extracellular polymeric substances (EPS) from sludge. The content of protein (PN) and polysaccharide (PS) was determined by Folin-phenol method and colorimetry of anthrone-sulfuric acid. Besides, dehydrogenase (DHA) and specific oxygen utilization rate (SOUR) were measured by the same method as previously reported (see supplementary data) (Li et al., 2014; Wang et al., 2016). The activity of ammonia monooxygenase (AMO) and nitrate reductase (NAR) was measured by ELISA Kit. The detailed steps were in accordance with the literature (Li et al., 2021). Each determination was conducted in triplicate and average values were presented at graphs.

2.4. High-throughput sequencing

Sludge samples were collected for 16s rRNA sequencing and metagenomic sequencing on the last day of operation. All the sequencing was done by Shanghai Majorbio Bio-pharm Technology Co., Ltd, China. The sequencing process and data analysis followed previous studies (Peng et al., 2022; Zhang et al., 2021b).

3. Results and discussion

3.1. Comparison of aniline degradation and nitrogen removal performance

The aniline degradation and synchronous nitrogen removal performance of SBR under different applied voltage (0–6.0 V) was presented in Fig. 1. For aniline degradation, Y1 could almost fully degrade aniline within 5 days, and the time required for Y2 and Y3 was the same as that for Y1. However, Y4 and Y5 needed 8 days to adapt to the environment of aniline, so as to achieve the removal rate of about 100%. Since the

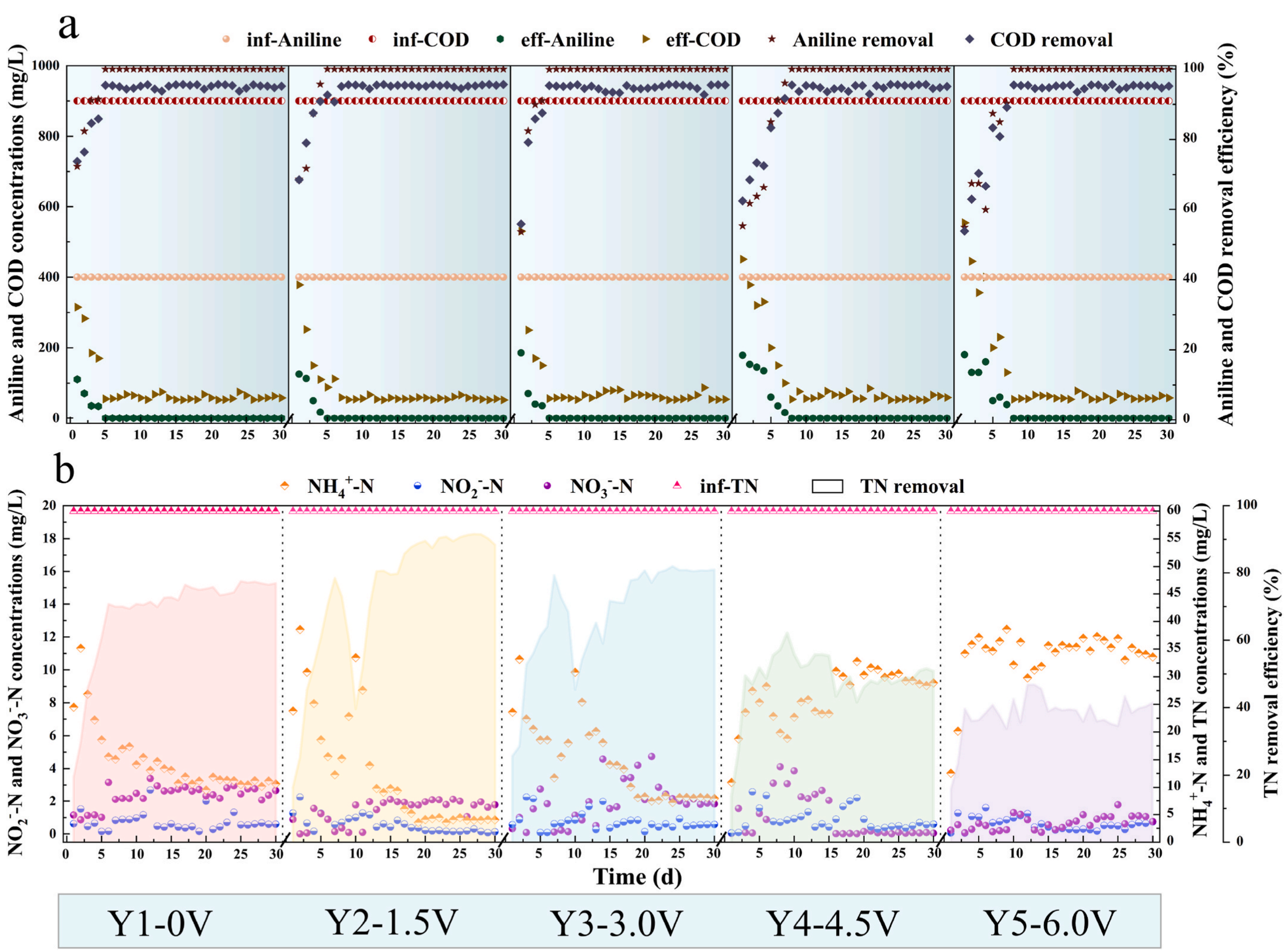

Fig. 1. The decontamination performance under the different applied voltages. (a) aniline and COD; (b) nitrogen.

biodegradation process was carried out by microorganisms with certain functions under suitable environment, when microorganisms were in an environment unsuitable for their growth, its metabolic performance might be inhibited (Foesel et al., 2011). In this study, the biodegradation processes of aniline degradation needed to be driven by various enzymes that secreted by bacteria, whose activities might be impacted by high voltage. Obviously, too high applied voltage inhibited the ability of aniline degradation during the initial operation of system. However, complete aniline degradation could be achieved and remained stable after long-term domestication. This indicated that the aniline degradation performance of the system did not differ significantly with the applied voltage. Meanwhile, the COD degradation trend of the five reactors was consistent with that of aniline, and the effluent concentration of COD was basically stable at 55 $\pm$ 10 mg/L.

Different from aniline degradation, the nitrogen removal performance of each system varied with the applied voltage. As can be seen from Fig. 1b, the average TN removal efficiency of Y1 gradually reached 75.53% in the last 15 days of operation. At this time, the effluent concentration of NH_4^+-N and NO_3^--N in Y1 was stable at 10.92 and 2.58 mg/L respectively. As the main TN species in the effluent was NH_4^+-N, the bottleneck of nitrogen removal in this sequencing batch reactor treating 400 mg/L aniline was nitrification. Under the applied voltages of 1.5 V and 3.0 V, the effluent concentration of NH_4^+-N in Y2 and Y3 were 4.61 and 8.87 mg/L, respectively. Y2 and Y3 were superior to Y1 in the nitrification efficiency, and the order of the three was Y2>Y3>Y1. Applying appropriate lower voltage ($\leq$3.0 V) could notably enhance the microbial activity, thereby obtaining the higher removal efficiency of ammonia nitrogen. Whereas, the higher applied voltage (>3.0 V) inhibited the NH_4^+-N removal, and the NH_4^+-N effluent concentration of Y4 and Y5 were 30.07 and 35.26 mg/L respectively. Apparently, the high applied voltage severely inhibited the activity of nitrifying bacteria in the system, resulting in the stagnation of nitrification. In addition, the NO_2^--N concentration under different applied voltages was trace amounts, except for the occasional fluctuation. With the enhancement of nitrification performance, Y2 and Y3 have lower NO_3^--N effluent concentration than Y1 (Y2-1.83 mg/L and Y3-2.34 mg/L). For one thing, Y2 and Y3 had the excellent nitrification effect, which produced much more NO_3^--N as the reaction substrate, bringing better denitrification performance. For another, H_2 might be generated by electrode electrolysis, which can be used by denitrifying bacteria as the electron donor for autotrophic denitrification (Li et al., 2009). Owing to the lower effluent concentration of NH_4^+-N and NO_3^--N, the TN removal rate of Y2 was nearly 10% higher than Y3, up to 88.89%. It was illustrated that the applied voltage of 1.5 V improved the nitrification and denitrification performance of aniline biodegradation reactor more significantly than 3.0 V. However, the NO_3^--N effluent concentration of Y4 and Y5 was 0.06 and 0.87 mg/L, while TN removal efficiency were only 47.8% and 38.7%. The low effluent concentration of NO_3^--N in the two reactors implied that their poor TN removal ability might not be on account of the weak denitrification performance. On the contrary, NH_4^+-N took up more than 96% of TN effluent in Y4 and Y5. This showed that further increase of applied voltage did not bring the outstanding performance of nitrogen removal. It can be seen that the applied voltage had an important effect on the aniline degradation and nitrogen removal performance of the system. Therefore, these indexes of average removal rate of aniline, COD, NH_4^+-N and TN were selected for regression analysis of applied voltage (see the supplementary data). Obviously, the applied voltage was negatively correlated with these indexes, and the trend was more significant under the higher voltage, indicating that high voltage would deteriorate the decontamination performance of the system. Meanwhile, the results of R^2 showed that COD (0.8865) >Aniline (0.8476) >NH_4^+-N (0.7949) >TN (0.7825). Therefore, it is considered that the average removal rates of aniline and COD were the most affected by the changes of applied voltage, followed by NH_4^+-N and TN. To sum up, the appropriate applied voltage improved the degradation efficacy of pollutants, whereas, an inhibition effect was exerted at high voltage. It has been reported that the interaction between the electrode and microorganism accelerated the electron transfer rate under appropriate applied voltage, thus potentially increasing the microbial degradation rate (Zhang et al., 2018). However, excessive voltage might destroy cell membrane and slow down the microbial growth and metabolism (Ding et al., 2018). Therefore, the applied voltage of 1.5 V in this study was the best choice to break through the bottleneck of nitrogen removal in conventional aniline degradation SBR.

3.2. Transformation of pollutants in typical cycle

In order to further explore the removal and transformation process of pollutants under different applied voltage, the time profiles of aniline and nitrogen concentration in five reactors during one cycle were showed in Fig. 2. First of all, the aniline degradation rate of the five reactors were 26.3, 33.1, 30.6, 19.4 and 10 mg/(g·MLSS·h) respectively, that of the five reactors presented an order of Y2>Y3>Y1>Y4>Y5. Although there was no difference in the aniline effluent concentration of five reactors mentioned at section 3.1, the periodic data confirmed that the degradation rate of aniline can be effectively increased by nearly 20% at the low voltage of 1.5 V compared with the control, while the applied voltage higher than 4.5 V seriously inhibited the degradation rate of aniline. Similarly, the COD degradation of the five reactors was synchronized with the degradation trend of aniline, which could also be found in our previous studies (Peng et al., 2021). Undoubtedly, the byproducts of aniline degradation could also be quickly absorbed and metabolized by microorganisms in the system.

Along with the degradation of aniline, NH_4^+-N was simultaneously released. Theoretically, 400 mg/L aniline can release 60 mg/L NH_4^+-N after complete degradation, but all reactors only released 18–25 mg/L ammonia nitrogen after complete degradation of aniline. There were two explanations for this phenomenon: 1) It was found that there was a certain ammonia nitrogen removal in the process of aniline degradation, which might be caused by heterotrophic nitrification-aerobic denitrification (HN-ADB) (referred to a class of bacteria that can perform heterotrophic nitrification and aerobic denitrification in the presence of oxygen) according to our previous study (Zhang et al., 2022). 2) Heterotrophic bacteria consumed ammonia nitrogen through assimilation while degrading aniline. The average removal rates of NH_4^+-N in the five reactors within the 6h of aerobic phase were calculated as Y1-4.04 mg/L·h, Y2-4.61 mg/L·h, Y3-4.37 mg/L·h, Y4-2.37 mg/L·h, and Y5-2.19 mg/L·h, respectively. This result was consistent with the analysis in section 3.1. Low applied voltage could significantly enhance the removal rate of NH_4^+-N, while higher than 4.5 V applied voltage was opposite. It was worth noting that, as the main pathway of NH_4^+-N removal in aniline degradation reactor, autotrophic nitrification would only appear in the aerobic phase after complete degradation of aniline (Fan et al., 2021). However, it could be found that the remaining time for nitrification in Y4 and Y5 after aniline degradation was short (only 2 h) and the autotrophic nitrification rate was low. These results indicated that the weak NH_4^+-N removal performance of them was probably caused by the comprehensive inhibition of aniline degradation and autotrophic nitrification under high applied voltage, especially in Y5. Due to different degree of nitrification occurred in the reactor except for Y5, the peak values of nitrate and nitrite appeared. When entering the anoxic phase, the total amount of nitrate and nitrite through

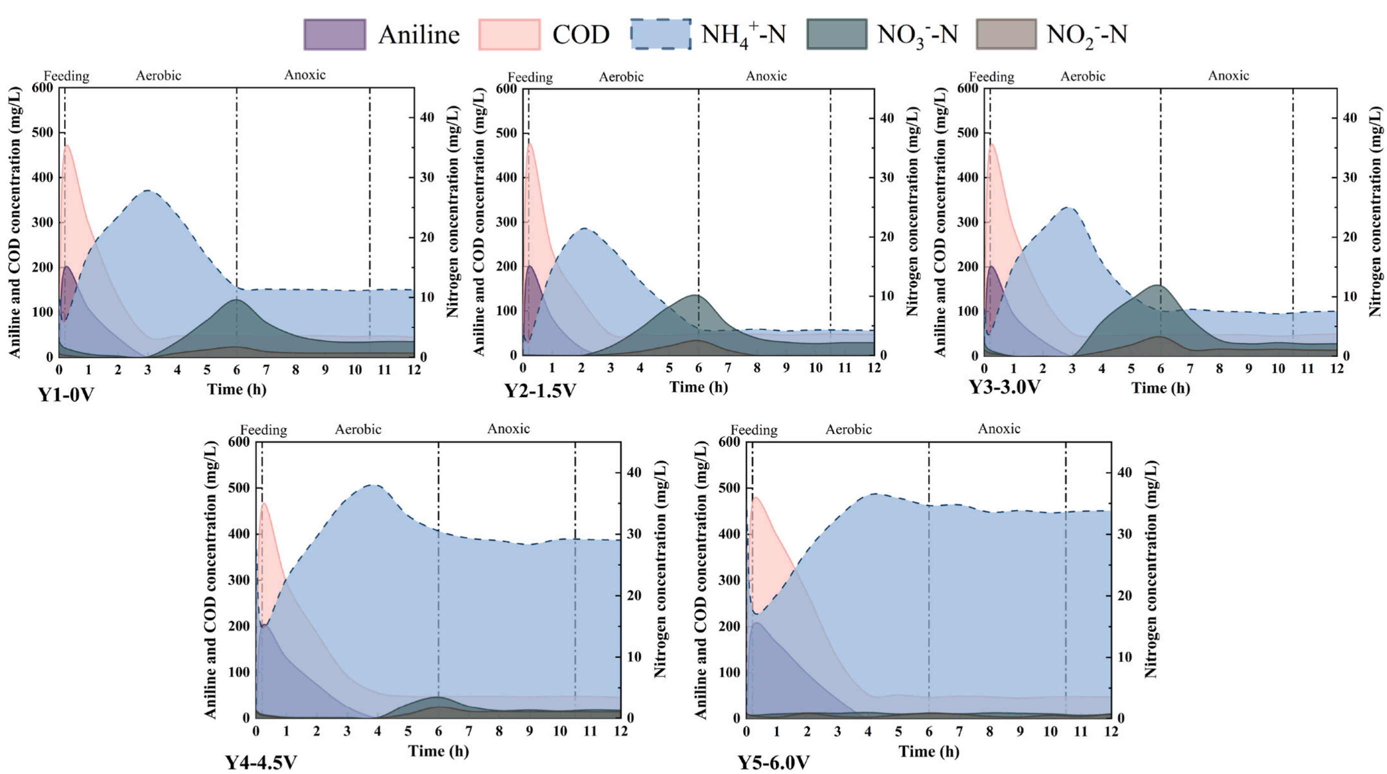

Fig. 2. Variation of aniline and nitrogen concentrations in a typical cycle.

denitrification in the five reactors reached Y1-7.99 mg/L, Y2-10.46 mg/L, Y3-12.02 mg/L, Y4-2.98 mg/L and Y5-0.28 mg/L, respectively. Y2 and Y3, stimulated by 1.5 V and 3.0 V applied voltage, had the better denitrification performance than Y1. Similarly, high applied voltage also worse the denitrification performance. Notably, the denitrification effect of Y3 in the anoxic phase was better than that of Y2, which was probably because Y2 consumed much carbon sources in the process of aerobic denitrification and assimilation in the aerobic phase, resulting in its carbon source reserve inferior to Y3 in the process of denitrification. Therefore, the key to the best nitrogen removal in Y2 might lie in the promotion of ammonia nitrogen removal by heterotrophic bacteria under 1.5 V applied voltage. Nevertheless, the improvement of autotrophic nitrification under 3.0 V applied voltage was more significant than 1.5 V, but the overall denitrification effect was still inferior to 1.5 V.

3.3. *Property of activated sludge*

3.3.1. *The analysis of EPS*

Extracellular polymeric substances (EPS) was macromolecule polymer secreted by microorganisms in response to external pressure (Siddharth et al., 2021). The change of EPS content during the operation could reflect the response of activated sludge to different applied voltage stimulation (Fig. 3a). The EPS content of the control group (Y1) gradually increased from 75.09 mg/g MLSS to 121.17 mg/g MLSS during operation. As the pollutant with high biological toxicity, aniline and its metabolites would induce free radical formation, which can lead to oxidative stress and cytotoxicity. Therefore, when the microorganisms in the activated sludge were stimulated by aniline, more extracellular polymeric substance would be secreted to enhance toxicity resistance. Similarly, the EPS content of Y2 and Y3 also increased to 150.51 and 298.93 mg/g MLSS, while that of Y4 and Y5 decreased continuously to 82.22 and 70.14 mg/g MLSS during operation. Appropriate voltage could stimulate the increase of EPS secreted by microorganisms. However, the EPS content would reduce with the applied voltage exceeding 4.5 V. This phenomenon might be caused by the dissolution of EPS on account of high applied voltage (Cao et al., 2018). When microorganisms lost the protective effect of EPS, exposed microorganisms were more likely to be stimulated by external electric field, leading to reduced activity or even death by cleavage. In addition, Protein (PN) was the main component of EPS. The main components of protein were extracellular enzymes, most of which had electrical activity or acted as electron shuttles (Qin et al., 2022). In this study, the increase of protein observed at lower applied voltage might lead to more extracellular enzymes involved in extracellular electron transfer. Besides, proteins played an important role in the cell adhesion and aggregation, helping to maintain the stability of sludge structure (Cai et al., 2022).

3.3.2. *Sludge activity index*

To investigate the impact of different applied voltage on various functions of activated sludge, the corresponding sludge activity indexes (specific oxygen utilization rate (SOUR), dehydrogenase (DHA), ammonia monooxygenase (AMO) and nitrate reductase (NAR)) were monitored (see supplementary data). Firstly, the SOUR of Y1 only slightly decreased and finally remained at 26.45 mg O_2/(g MLSS · h), indicating that the sludge activity remained relatively stable during operation. The specific oxygen consumption rate (SOUR) of activated sludge was one of the important parameters to characterize the biological activity of sludge. It reflected the physiological state and substrate metabolism of activated sludge from the perspective of microbial respiration rate. Therefore, SOUR not only represented the ability of microbial system to take up oxygen during aerobic degradation, but also demonstrated the activity of heterotrophic bacteria to degrade aniline. The activity of SOUR in Y2 and Y3 increased observably, reaching 33.78, 28.35 mg O_2/(g MLSS · h) on day 30, while that of Y4 and Y5 dropped to 22.08 and 19.23 mg O_2/(g MLSS · h), respectively. Stimulation of low voltage would change the permeability of cell membrane, enabling microorganisms to make better use of nutrients to maintain their own growth and metabolism, thus increasing the consumption of dissolved oxygen (Chen et al., 2006). However, high voltage could directly oxidize the intracellular substances without destroying the cell membrane,

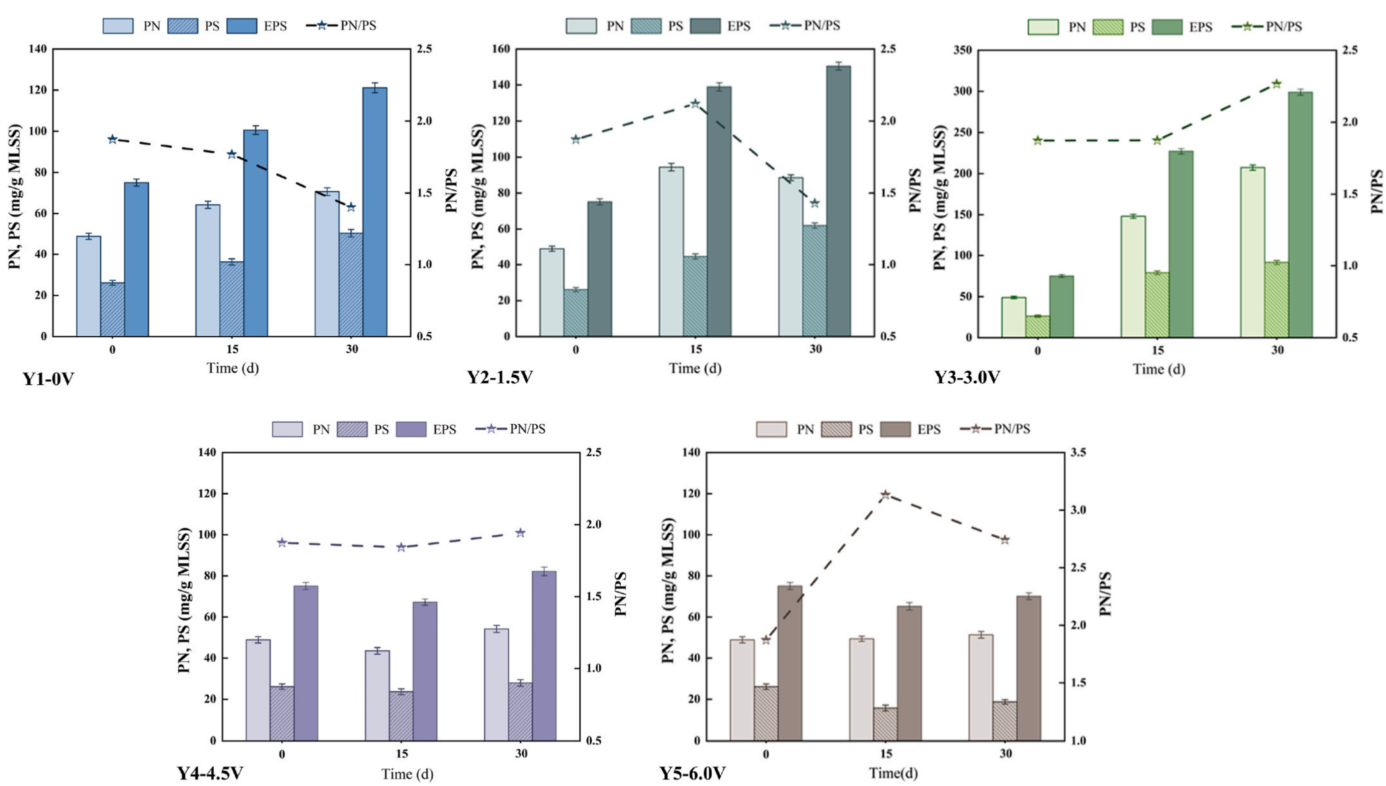

Fig. 3. The EPS content of five reactors.

reducing microbial activity and leading to the cell death (Matsunaga et al., 1992). Dehydrogenase (DHA), as an oxidoreductase, its activity was an indicator of the oxidation ability of microorganisms to degrade organic pollutants. After 30 days of continuous operation, the contents of DHA in the five systems were 38.58, 46.83, 42.82, 34.88 and 27.75 mg TF/g MLSS,h, respectively. Low applied voltage promoted the activity of DHA in Y2 and Y3 to some extent. The increase of DHA activity indicated the promotion of electron transport system in activated sludge. This indirectly enhanced the activity of key enzymes in aniline degradation, consequently accelerating aniline degradation.

At the same time, the activity of AMO and NAR also showed the same trend. Ammonia oxygenase (AMO) and nitrate reductase (NAR) were the main functional enzymes in nitrification and denitrification processes of nitrogen metabolizing functional bacteria, respectively. The content of AMO in the five systems on day 30 was 26.55, 35.95, 30.95, 16.89 and 16.88 U/L, respectively. The significant increase of AMO activity at low voltages explained why Y2 and Y3 had better NH_4^+-N removal performance. Nevertheless, too high voltage could cause metabolic disorder of microorganisms. Obviously, the nitrification performance of Y4 and Y5 became worse, and the activity of AMO decreased sharply. Similarly, the activity of NAR in the five systems on day 30 was 21.56, 27.98, 29.35, 16.77 and 16.08 U/L, respectively. The activity of denitrifying bacteria decreased in the control group (Y1) due to long-term carbon shortage. The NAR activity of Y2 and Y3 increased gradually during operation. Combined with the results of section 3.1, weak electrical stimulation could promote denitrification by increasing the activity of NAR. In contrast, the NAR activity of Y4 and Y5 decreased gradually, which confirmed that the further improvement of denitrification effect cannot be remedied by increasing the voltage stimulation. Meanwhile, high voltage could directly oxidize the intracellular substances without destroying the cell membrane, leading to the deterioration and disorder of organic (aniline) metabolism and nitrogen metabolism of microorganisms. To sum up, the change trend of sludge activity indexes under different applied voltage was roughly the same, which echoed with the macroscopic performance, showing the phenomenon of "low promotion and high inhibition".

3.4. Microbial community response

3.4.1. Shifts of microbial diversity

Sludge samples were taken for 16s RNA sequencing analysis on the last day of operation of the five reactors and the sludge sample name was consistent with the reactor. The richness and diversity of microbial community was usually evaluated by the alpha diversity. Fig. 4a displayed the Ace, Chao, Shannon and Simpson indices under different applied voltages. In general, compared with other samples, Y2 obtained the maximum Ace, Chao, Shannon and minimum Simpson values (1710, 1675, 5.51 and 0.010, respectively). Obviously, the system of 1.5 V applied voltage showed higher community richness and diversity. Combined with the best decontamination performance of Y2, it implied that microbes which were originally at a disadvantage in competition developed rapidly, thus increasing the richness and diversity of microbial communities. On the contrary, when the applied voltage was greater than 1.5 V, the richness and diversity of communities decreased with the increase of voltage. This was the result of the selection of microbial living environment condition. In addition, the same conclusion as the diversity index was drawn from the analysis of Venn diagram. The total number of OTUs of Y2 were 1366, significantly higher than that of other samples, and the unique OTUs was as high as 500. With the increase of applied voltage (≥3.0 V), the total number of OTUs gradually decreased (Y3:992, Y4:864, Y5:737). Moreover, the results of PCA also showed that the distance between each sample was relatively far, indicating that the introduction of voltage made a great difference in the microbial community structure of different systems.

3.4.2. Evolution of microbial community composition

To further illustrate the changes in microbial community structure under different applied voltages, the top ten phyla and fifty genera of the five samples was analyzed (Fig. 5). At the phylum level, *Bacteroidetes* (38.17%), *Proteobacteria* (37.69%) and *Actinobacteriota* (9.52%) was the main contributors in the control group. Thereinto, *Bacteroidetes* included a variety of bacteria with the function of degrading aromatic compounds (Liu et al., 2022), and *Proteobacteria* was the main phylum involved in organic matter degradation and nitrogen removal in wastewater treatment (Yan et al., 2021). Therefore, these two kinds of phyla could possess high abundance in aniline degradation system. Nevertheless, *Bacteroidetes*, *Proteobacteria* and *Actinobacteriota* still occupied the dominant position under different applied voltages, accounted for 74.27%, 83.39%, 84.91% and 86.05% of Y2, Y3, Y4 and Y5, respectively. With the increase of voltage, the total proportion of these three main phyla increased gradually. Moreover, the relative abundance of *Proteobacteria* and *Actinobacteriota* presented the order of Y5 > Y4 > Y1 > Y2 > Y3. This indicated that high applied voltage of 4.5 V and 6.0 V stimulated the proliferation of *Proteobacteria* and *Actinobacteriota*. Besides, *Chloroflexi* was enriched under the introduction of voltage. The relative abundance was 11.58% at the 1.5 V applied voltage, much higher than Y1.

At the genus level, the dominant genera (relative abundance >3%) included *norank_f_Saprospiraceae* (10.44%), *norank_f_NS9_marine_group* (8.24%) and *Arenimonas* (4.61%) in Y1. With the introduction of voltage, their dominant position descended in varying degrees. This was because the tolerance of different genus to applied voltage was not consistent, and the external voltage would cause great changes in microbial structure. As the voltage increased from 0 V to 3.0 V, the

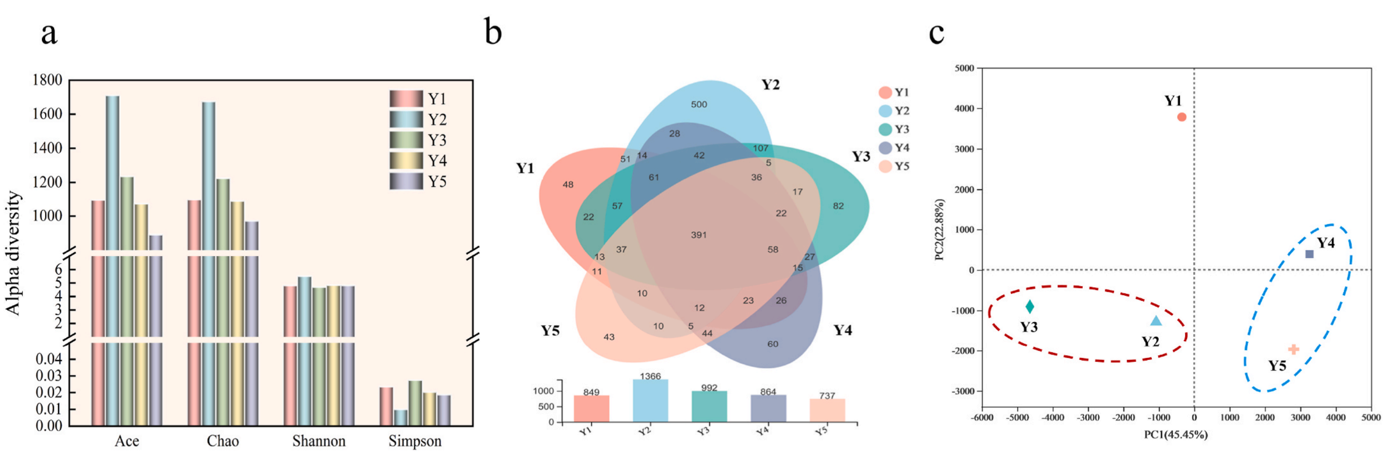

Fig. 4. The differences in microbial diversity results. (a) alpha diversity; (b) Venn diagram; (c) PCA analysis.

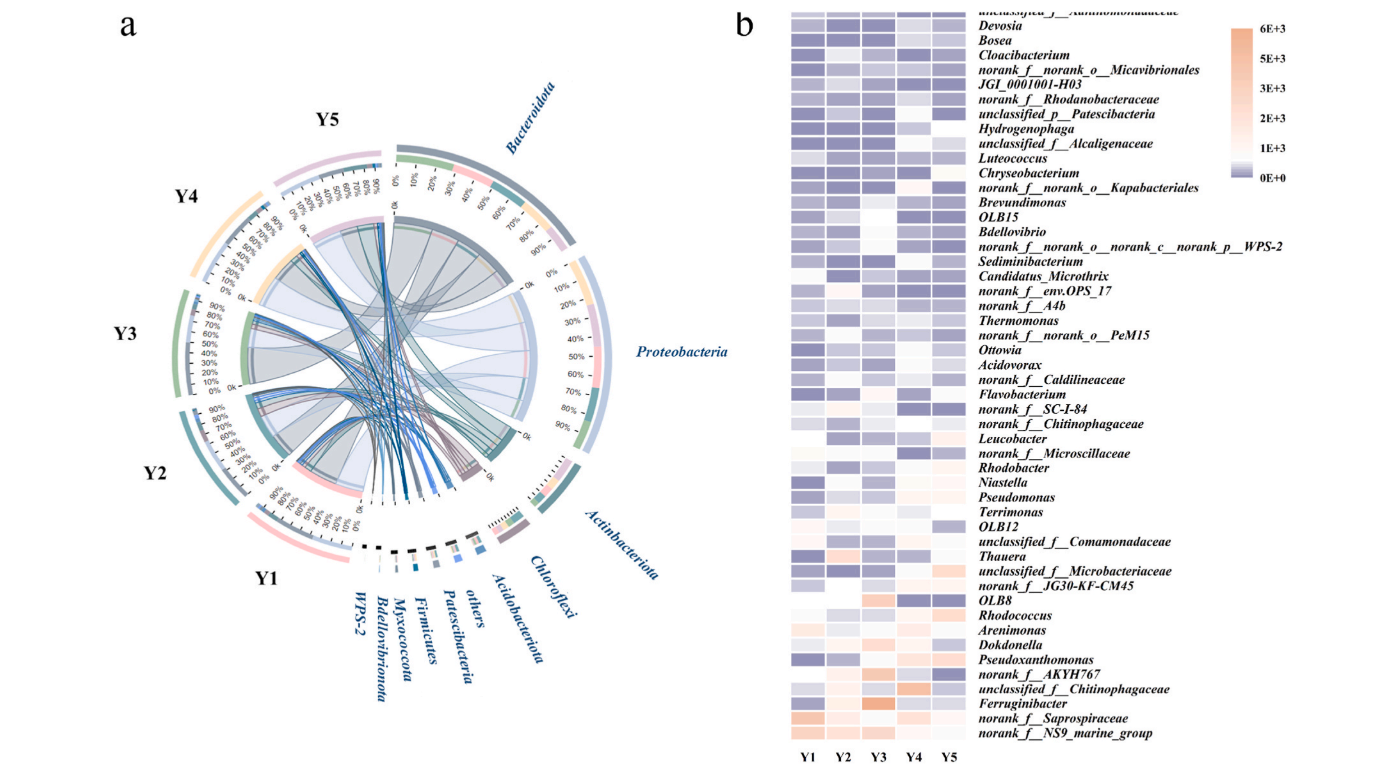

Fig. 5. The changes in microbial community structure at the phylum (a) and genus (b) level.

abundance of *Ferruginibacter* and *norank _ f _ AKYH767* gradually increased and became the main genera. It was worth noting that there was little difference in the abundance of these five genera in Y2, suggesting that compared with Y1 and Y3, these genera did not compete with other genera at the applied voltage of 1.5 V. More obvious resource sharing was conducive to the formation of higher microbial diversity. However, owing to the negative effect of higher voltage (4.5 V and 6.0 V), these genera were inhibited markedly. In addition, the introduction of applied voltage dramatically increased the abundance of *Pseudomonas* and *Hydrogenophaga*. These two genera were well-known electroactive bacteria that possessed high adaptability to various environmental conditions (Li et al., 2022; Zheng et al., 2019). The existence of high abundance of electroactive bacteria in the system facilitated to accelerate the electron transfer, thus promoting the degradation of pollutants. Meanwhile, considering that aniline degradation, nitrification and denitrification all took place in this system, it was necessary to pay attention to changes in the abundance of functional genera under different applied voltages. Several genera with the potential ability of aniline degradation were detected in the five reactors, including *norank_f_Microscillaceae* (Bowman et al., 2017), *Luteococcus* (Feng et al., 2022), *Rhodococcus* (Prieto et al., 2002), *unclassified_f_Chitinophagaceae* (Hou et al., 2021). Except for *norank_f_Microscillaceae*, the abundance of the other three genera increased with the applied voltage. It implied that these genera possessed strong tolerance to voltage and play an important role in aniline degradation. Although there were several aniline-degrading bacteria with high abundance existing in Y4 and Y5, the high voltage condition prevented them from degrading aniline efficiently. The macroscopic manifestation of this inhibition was that the degradation rate of aniline in the system was reduced and the secretion of EPS and sludge activity (SOUR and DHA) in Y4 and Y5 were inhibited. Meanwhile, as autotrophic bacteria, the abundance of *Nitrosomonas* and *Nitrospira* was far blow in all five reactors than heterotrophic bacteria on account of its sensitivity to aniline toxicity. The abundance of *Nitrosomonas*, belonging to Ammonia Oxidizing Bacteria, was 0.16% (Y1), 0.24% (Y2), 0.07% (Y3), 0.03% (Y4), 0.01% (Y5). And the abundance of *Nitrospira*, belonging to Nitrite Oxidizing Bacteria, was 0.005% (Y1), 0.48% (Y2), 0.002% (Y3), 0.001% (Y4), 0.003% (Y5). The abundance of nitrifying bacteria in Y2 was much higher than other reactors, especially Y4 and Y5. Obviously, the excellent nitrification performance of Y2 was attributed to the high abundance of autotrophic nitrifying bacteria, while Y4 and Y5 was just the opposite. High applied voltage severely inhibited nitrifying bacteria and hindered subsequent nitrification and denitrification process. Accordingly, the enrichment for denitrifying bacteria in Y4 and Y5 was affected by reason of the low nitrate substrate concentration. *Ferruginibacter* (Wang et al., 2020), the most abundant denitrifying bacteria in Y3 (12.92%), was inhibited under the high applied voltages (Y4-1.03%, Y5-1.21%). So did the other denitrifying bacteria, such as *Terrimonas* (Pishgar et al., 2019b) and *Dokdonella* (Wang et al., 2019). In addition, it could also be noted that *Thauera*, a typical genus with denitrifying function (Pishgar et al., 2019a), was enriched in Y2 (6.15%). However, compared with denitrifiers, heterotrophic nitrifying-aerobic denitrifying bacteria (HN-ADB) could be met with adequate survival resources in the system with sufficient DO and NH_4^+-N. *Pseudomonas* and *Rhodococcus* were reported to have the ability of heterotrophic nitrification and aerobic denitrification (Li, 2013; Zhang et al., 2011). The abundance of *Rhodococcus* achieved to 7.01% in Y5 and maintain to less than 3% in the other four reactors, and the abundance of *Pseudomonas was* 0.33% (Y1), 1.13% (Y2), 0.90% (Y3), 2.69% (Y4), 3.32% (Y5). The result suggested that conventional nitrifiers and denitrifiers were greatly inhibited led to the competitive advantages gradually occupied by HN-ADB. The enrichment of HN-ADB under high applied voltage implied that aerobic heterotrophic bacteria had great potential to obtain survival advantages under the voltage of 4.5 and 6.0 V. Nevertheless, as mentioned in section 3.2, relatively low removal rates of NH_4^+-N and aniline have been achieve by Y4 and Y5 in typical cycle. Therefore, such significant survival advantages of aerobic heterotrophic microorganisms seemed not to bring the apparent improvement in aniline and nitrogen removal performance for Y4 and Y5. In a word, the abundance of functional genera changed in response to the different applied voltage, resulting in changes of the decontamination performance.

3.4.3. Network correlation analysis

The co-occurrence network analysis of the top 50 genera under low applied voltage (0–3.0 V) and high applied voltage (3.0–6.0 V) was presented to explore the impact of the applied voltage on the relationship between microbial community (Fig. 6). The distribution range of degree-clustering of genera under low and high applied voltage was similar (the clustering indexes were all in the range of 0.5–0.95) (Wang et al., 2022). believed that the indexes of clustering and degree were positively correlated with the importance of nodes. Although the microbial community was significantly changed at high applied voltage, the new dominant bacteria selected by the higher applied voltage could reconstruct the abundant interrelationships comparable with low applied voltage. Besides, under the impact of low and high applied voltage, there was no great difference in degree centrality, total centrality and betweenness of system (see supplementary data). This demonstrated that there were always new key genera selected to act as important "node" and "intermediary" under the microbial community succession, which was caused by the increase of voltage. Even if a complete microbial network comparable with the biosystem of low voltage was established, the ideal performance could not be achieved under the high applied voltage.

As a dominant aniline-degrading bacteria, *norank_f_Microscillaceae* formed cooperative relations with 11 genera and competitive relations with other 11 genera under low voltage conditions. However, its competitors increased and collaborators decreased under high voltage conditions. It could be seen that the cooperative advantage of this genus was greatly weakened under high voltage. Similarly, the relationship of another important aniline-degrading bacteria, *unclassified_f_chitinophagaceae*, was revealed to be simple at high voltage (cooperators: competitors was 6: 2) and complex at low voltage (cooperators: competitors was 11: 11) (Mougi and Kondoh, 2012). suggested that the increase of network complexity might enhance the stability of microbial community. Moreover, the electroactive bacteria, *Pseudomonas* and *Hydrogenophaga*, tended to form the competitive relationship with other genera under the higher applied voltage. The increased competition might be responsible to the deterioration of its function. In addition, the relationships between some genera with high abundance and other genera were no obvious difference at different voltages, such as *Pseudoxanthomonas* and *Luteococcus*. It implied that there were various key bacteria acting as key nodes under the ranges of different voltages, but their overall microbial communities were relatively similar. The distribution of denitrifying bacteria also proved this reason. It seemed that the number of links between dominant denitrifiers and other genera did not change significantly under various applied voltage, such as *Dokdonella*, *Ferruginibacter*, *Arenimonas*, *norank_f_Saprospiraceae*. Thus, a node in a network would be occupied by a new genus when the original genus in this node was eliminated by the impact of applied voltage. Consequently, although the microorganisms were significantly selected by the impact of different applied voltage, the overall microbial network would not be affected obviously.

3.5. Metagenomic analysis of performance enhancement mechanism under weak electrical stimulation

3.5.1. Changes of metabolic function

Metagenomics is a new microbial research method that takes the microbial genome in environmental samples as the research object, functional gene screening and sequencing analysis as the research means, and microbial diversity, population structure, evolutionary relationship, functional activity, cooperative relationship, and relationship with the environment as the research purpose. To further elucidate the enhancement mechanism of system performance under applied voltage of 1.5V, sludge samples from Y1 and Y2 on the last day of operation were collected for metagenomic sequencing. The metabolic pathways of Y1 and Y2 were predicted by Kyoto Encyclopedia of Genes and Genomes (KEGG) database to find out the changes of metabolic function (see supplementary data). The top five functions were metabolic pathways, biosynthesis of secondary metabolites, microbial

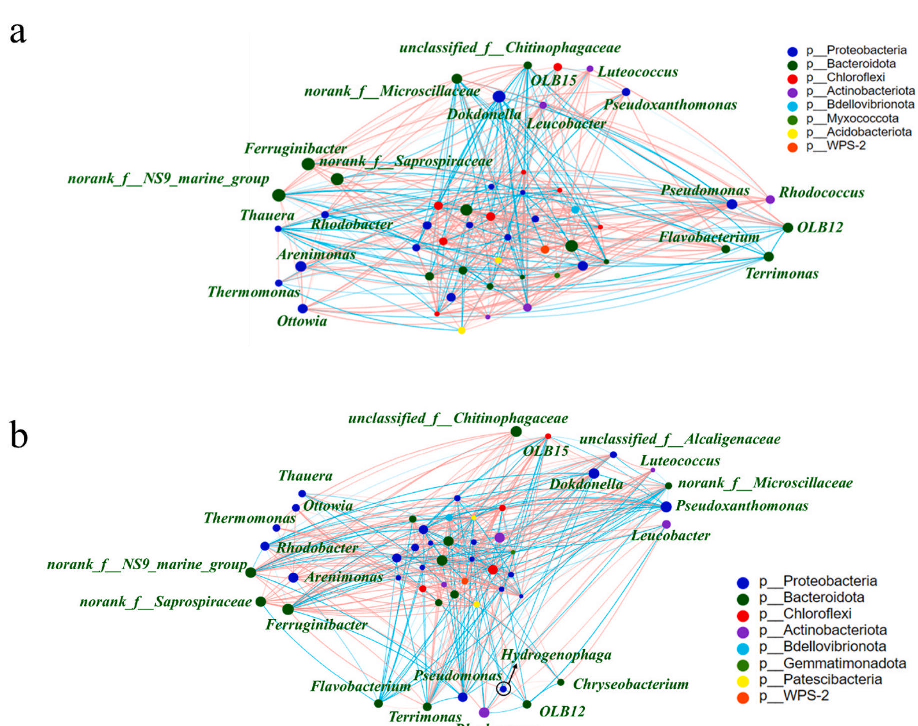

Fig. 6. The co-occurrence network analysis of the top 50 genera under low applied voltage of 0–3.0V (a) and high applied voltage of 3.0–6.0V (b). The red line represents the positive correlation and the blue line represents the negative correlation. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

metabolism in diverse environments, biosynthesis of amino acids and carbon metabolism. There was no significant difference in the abundance of the five metabolic functions between the two systems, not exceeding 0.1%. Furthermore, the abundance of benzoate metabolism (the aniline metabolic pathway was included in benzoate metabolism) and nitrogen metabolism in the two samples was 0.51% (Y1), 0.58% (Y2) and 0.43% (Y1), 0.51% (Y2). The functions of aniline degradation and nitrogen metabolism under weak electrical stimulation (1.5 V) were enhanced.

The contributions of the top ten genera to benzoate metabolism and nitrogen metabolism were conducted to establish the relationship between key pathways and functional flora (Fig. 7a). In the control group (Y1), *Candidatus_Microthrix* was the major contributor to benzoate and nitrogen metabolism, while other genus contributed comparatively less. However, the contributions of dominant genera to these two functions were evenly distributed in the Y2. *Thauera*, the most abundant genera of Y2 (6.15%), has the highest contribution to benzoate and nitrogen metabolism. A large number of intermediate products during aniline degradation could be consumed by *Thauera* to denitrify efficiently. In addition, *Dokdonella*, *unclassified_f_Chitinophagaceae*, *Ferruginibacter* and *Pseudomonas* had greater contributions to benzoate and nitrogen metabolism simultaneously in Y2, but exactly the opposite was Y1. It indicated that the utilization rate of aniline degradation intermediates by denitrifying bacteria could be improved under the voltage of 1.5 V. Thus, the denitrifying and aniline-degrading capacity of Y2 was promoted simultaneously without supplementary carbon source. In addition, the total contribution of the top ten genera to benzoate metabolism (Y1-79702, Y2-129420) and nitrogen metabolism (Y1-82274, Y2-108828) suggested that Y2 had better performance of aniline degradation and nitrogen removal than Y1 due to the higher species contribution.

3.5.2. Response of key functional genes

Metagenomic sequencing can not only reveal the changes of microbial community structure, but also reveal the changes of systematic metabolic pathways and the abundance of key functional genes. Combined with the gene data of the metagenomic sequencing results and the information of benzoate metabolism and nitrogen metabolism in KEGG database, the pathway of aniline degradation and nitrogen metabolism was obtained (Fig. 7b). The first step of aniline degradation was to decompose into NH_4^+-N and catechol. Therefore, it was necessary to reveal the functional gene abundance of catechol metabolism (ortho and meta metabolism) and conventional nitrogen metabolic pathway. The results showed that the applied voltage did not seem to affect the main metabolism pathway of aniline degradation and both Y1 and Y2 preferred to degrade aniline through the ortho pathway. Moreover, it should be noted that the gene abundance of Y2 was 2–10 times higher than that of Y1 in most steps of both ortho and meta metabolism pathway. Such a disparity of functional gene abundance implied that compared with the control, most of the functional bacteria responsible for aniline-degrading pathway proliferated significantly under the applied voltage of 1.5 V. This phenomenon also agreed with the best aniline-degrading performance of Y2 in section 3.2. In contrast, the voltage of 1.5 V seemed to obviously stimulate few genes with the function of nitrification or denitrification, such as hao (Y1-104, Y2-586), norBC (Y1-3888, Y2-8156) and nosZ (Y1-2812, Y2-6444). It

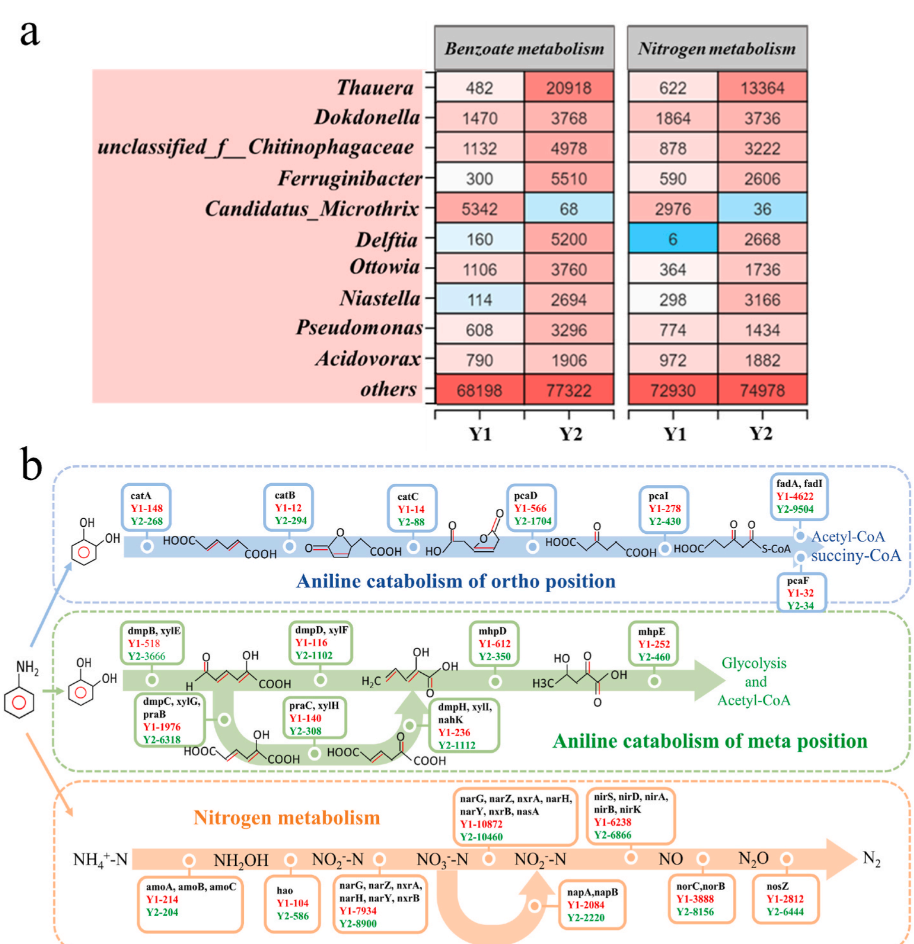

Fig. 7. (a) The contributions of the top ten genera to benzoate metabolism and nitrogen metabolism; (b) The potential pathway of aniline degradation and nitrogen metabolism and the differences in the abundance of functional genes at each step.

demonstrated that the 1.5 V applied voltage could improve the nitrification by stimulating the massive replication of genes (determined by metagenomics data) which controlled oxidation of hydroxylamine, and enhance the denitrification performance by strengthening the replication of nitrate reducing gene. This indicated that the incentive strategies of 1.5 V applied voltage for aniline degradation and nitrogen metabolism were different. In general, the introduction of voltage enhanced the related functional genes to enhance the decontamination function of the system.

4. Conclusions

The present work revealed the influence of gradient voltages on the decontamination performance, sludge property and microbial communities in E-SBR. The degradation performance of aniline was almost not affected by different applied voltage. However, the optimal applied voltage for nitrogen removal was 1.5 V, the average removal rate of TN increased by 13.36% compared with control. Meanwhile, the content of EPS and sludge activity was increased at proper applied voltages. In addition, obvious differences in microbial community structure were observed at different applied voltages. The metabolic function and the expression of key functional genes was enhanced at 1.5V applied voltage.

CRediT authorship contribution statement

Jing He: Investigation, Visualization, Methodology, Writing – review & editing. **Qian Zhang:** Conceptualization, Supervision, Funding acquisition, Writing – review & editing. **Meng Li:** Conceptualization, Writing – review & editing. **Tingzhen Ming:** Writing – review & editing. **Jiapeng Feng:** Writing – review & editing. **Haojin Peng:** Writing – review & editing. **Junhao Su:** Writing – review & editing. **Yunjie Zhang:** Writing – review & editing.

Declaration of competing interest

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

Data availability

The authors do not have permission to share data.

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Appendix B. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jclepro.2023.136077.

References

APHA, 2005. Standard Methods for the Examination of Water and Wastewater. American Public Health Association (APHA), Washington, DC, USA.
Abdel-Rahman, M.A., Shibl, M.F., El-Demerdash, S.H., El-Nahas, A.M., 2020. Simulated kinetics of the atmospheric removal of aniline during daytime. Chemosphere 255, 127031. https://doi.org/10.1016/j.chemosphere.2020.127031.
Bowman, J.P., Hugo, C., Bernardet, J.-F., 2017. International committee on systematics of Prokaryotes subcommittee on the taxonomy of aerobic Bacteroidetes (formerly Flavobacterium and cytophaga-like bacteria). Minutes of the meeting, 30 july 2014, Montréal, Canada. Int. J. Syst. Evol. Microbiol. 67 (2), 507–509.
Cai, T., Lu, X., Zhang, Z., Li, W., Wang, N., Zhang, Y., Zhang, R., Zhen, G., 2022. Spatial distribution and nitrogen metabolism behaviors of anammox biofilms in bioelectrochemical system regulated by continuous/intermittent weak electrical stimulation. J. Clean. Prod. 336, 130486 https://doi.org/10.1016/j.jclepro.2022.130486.
Cao, B., Zhang, W., Du, Y., Wang, R., Usher, S.P., Scales, P.J., Wang, D., 2018. Compartmentalization of extracellular polymeric substances (EPS) solubilization and cake microstructure in relation to wastewater sludge dewatering behavior assisted by horizontal electric field: effect of operating conditions. Water Res. 130, 363–375. https://doi.org/10.1016/j.watres.2017.11.060.
Cardenas-Robles, A., Martinez, E., Rendon-Alcantar, I., Frontana, C., Gonzalez-Gutierrez, L., 2013. Development of an activated carbon-packed microbial bioelectrochemical system for azo dye degradation. Bioresour. Technol. 127, 37–43. https://doi.org/10.1016/j.biortech.2012.09.066.
Chen, C., Smye, S., Robinson, M., Evans, J., 2006. Membrane electroporation theories: a review. Med. Biol. Eng. Comput. 44 (1), 5–14.
Ding, A., Fan, Q., Cheng, R., Sun, G., Zhang, M., Wu, D., 2018. Impacts of applied voltage on microbial electrolysis cell-anaerobic membrane bioreactor (MEC-AnMBR) and its membrane fouling mitigation mechanism. Chem. Eng. J. 333, 630–635. https://doi.org/10.1016/j.cej.2017.09.190.
Dvořák, L., Lederer, T., Jirků, V., Masák, J., Novák, L., 2014. Removal of aniline, cyanides and diphenylguanidine from industrial wastewater using a full-scale moving bed biofilm reactor. Process Biochem. 49 (1), 102–109. https://doi.org/10.1016/j.procbio.2013.10.011.
Fan, Y., Chen, X., Yao, Z., Li, H., Wang, D., Tian, M., Xu, Z., Wan, J., 2021. A novel inhibition mechanism of aniline on nitrification: aniline degradation competes dissolved oxygen with nitrification. Sci. Total Environ. 770, 145205 https://doi.org/10.1016/j.scitotenv.2021.145205.
Feng, J., Zhang, Q., Tan, B., Li, M., Peng, H., He, J., Zhang, Y., Su, J., 2022. Microbial community and metabolic characteristics evaluation in start-up stage of electro-enhanced SBR for aniline wastewater treatment. J. Water Proc. Eng. 45, 102489 https://doi.org/10.1016/j.jwpe.2021.102489.
Foesel, B.U., Drake, H.L., Schramm, A., 2011. Defluviimonas denitrificans gen. nov., sp. nov., and Pararhodobacter aggregans gen. nov., sp. nov., non-phototrophic Rhodobacteraceae from the biofilter of a marine aquaculture. Syst. Appl. Microbiol. 34 (7), 498–502. https://doi.org/10.1016/j.syapm.2011.08.006.
Hou, Y., Li, B., Feng, G., Zhang, C., He, J., Li, H., Zhu, J., 2021. Responses of bacterial communities and organic matter degradation in surface sediment to Macrobrachium nipponense bioturbation. Sci. Total Environ. 759, 143534 https://doi.org/10.1016/j.scitotenv.2020.143534.
Hu, Z., Li, J., Zhang, Y., Liu, W., Wang, A., 2022. Exerting applied voltage promotes microbial activity of marine anammox bacteria for nitrogen removal in saline wastewater treatment. Water Res. 215, 118285 https://doi.org/10.1016/j.watres.2022.118285.
Jiang, Y., Wang, H., Shang, Y., Yang, K., 2016. Simultaneous removal of aniline, nitrogen and phosphorus in aniline-containing wastewater treatment by using sequencing batch reactor. Bioresour. Technol. 207, 422–429. https://doi.org/10.1016/j.biortech.2016.02.014.
Jiang, Y., Wei, L., Yang, K., Shi, X., Wang, H., 2017. Rapid formation of aniline-degrading aerobic granular sludge and investigation of its microbial community succession. J. Clean. Prod. 166, 1235–1243. https://doi.org/10.1016/j.jclepro.2017.08.134.
Li, D., Cui, F., Zhao, Z., Liu, D., Xu, Y., Li, H., Yang, X., 2014. The impact of titanium dioxide nanoparticles on biological nitrogen removal from wastewater and bacterial community shifts in activated sludge. Biodegradation 25 (2), 167–177.
Li, M., Feng, C., Zhang, Z., Lei, X., Chen, R., Yang, Y., Sugiura, N., 2009. Simultaneous reduction of nitrate and oxidation of by-products using electrochemical method. J. Hazard Mater. 171 (1), 724–730. https://doi.org/10.1016/j.jhazmat.2009.06.066.
Li, M., Yu, X.-L., Li, Y.-W., Han, W., Yu, P.-F., Lun Yeung, K., Mo, C.-H., Zhou, S.-Q., 2022. Investigating the electron shuttling characteristics of resazurin in enhancing bio-electricity generation in microbial fuel cell. Chem. Eng. J. 428, 130924 https://doi.org/10.1016/j.cej.2021.130924.
Li, M., Zhang, J., Yang, X., Zhou, Y., Zhang, L., Yang, Y., Luo, L., Yan, Q., 2021. Responses of ammonia-oxidizing microorganisms to biochar and compost amendments of heavy metals-polluted soil. J. Environ. Sci. 102, 263–272. https://doi.org/10.1016/j.jes.2020.09.029.
Li, W., 2013. Study on characteristics in the removal process of ammonia nitrogen and nitrate nitrogen by an isolated heterotrophic nitrification-aerobic denitrification strain Rhodococcus sp, 01 J. Environ. Protect. 4, 74.
Lin, X.-Q., Li, Z.-L., Liang, B., Zhai, H.-L., Cai, W.-W., Nan, J., Wang, A.-J., 2019. Accelerated microbial reductive dechlorination of 2,4,6-trichlorophenol by weak electrical stimulation. Water Res. 162, 236–245. https://doi.org/10.1016/j.watres.2019.06.068.
Liu, X., Sun, R., Hu, S., Zhong, Y., Wu, Y., 2022. Aromatic compounds releases aroused by sediment resuspension alter nitrate transformation rates and pathways during aerobic-anoxic transition. J. Hazard Mater. 424, 127365 https://doi.org/10.1016/j.jhazmat.2021.127365.
Matsunaga, T., Nakasono, S., Takamuku, T., Burgess, J.G., Nakamura, N., Sode, K., 1992. Disinfection of drinking water by using a novel electrochemical reactor employing carbon-cloth electrodes. Appl. Environ. Microbiol. 58 (2), 686–689.
Mohammed, M., Mekala, L.P., Chintalapati, S., Chintalapati, V.R., 2020. New insights into aniline toxicity: aniline exposure triggers envelope stress and extracellular polymeric substance formation in Rubrivivax benzoatilyticus JA2. J. Hazard Mater. 385, 121571 https://doi.org/10.1016/j.jhazmat.2019.121571.
Mougi, A., Kondoh, M., 2012. Diversity of interaction types and ecological community stability. Science 337 (6092), 349–351.
Peng, H., Zhang, Q., Tan, B., Li, M., Zhang, W., Feng, J., 2021. A metagenomic view of how different carbon sources enhance the aniline and simultaneous nitrogen

removal capacities in the aniline degradation system. Bioresour. Technol. 335, 125277 https://doi.org/10.1016/j.biortech.2021.125277.

Peng, H., Zhang, Y., Zhang, Q., Zhang, W., Li, M., Feng, J., Su, J., He, J., Zhong, M., 2022. Control of aeration time in the aniline degrading-bioreactor with the analysis of metagenomic: aniline degradation and nitrogen metabolism. Bioresour. Technol. 344, 126281 https://doi.org/10.1016/j.biortech.2021.126281.

Pishgar, R., Dominic, J.A., Sheng, Z., Tay, J.H., 2019a. Denitrification performance and microbial versatility in response to different selection pressures. Bioresour. Technol. 281, 72–83. https://doi.org/10.1016/j.biortech.2019.02.061.

Pishgar, R., Dominic, J.A., Sheng, Z., Tay, J.H., 2019b. Influence of operation mode and wastewater strength on aerobic granulation at pilot scale: startup period, granular sludge characteristics, and effluent quality. Water Res. 160, 81–96. https://doi.org/10.1016/j.watres.2019.05.026.

Prieto, M., Hidalgo, A., Rodriguez-Fernandez, C., Serra, J., Llama, M., 2002. Biodegradation of phenol in synthetic and industrial wastewater by Rhodococcus erythropolis UPV-1 immobilized in an air-stirred reactor with clarifier. Appl. Microbiol. Biotechnol. 58 (6), 853–860.

Qin, S., Liu, H., Meng, Q., Zhou, Y., Xu, S., Lichtfouse, E., Chen, Z., 2022. Enhanced nutrient removal from mixed black water by a microbial ultra-low weak electrical stimulated anaerobic-two stage anoxic/aerobic process. Chem. Eng. J. 434, 134615 https://doi.org/10.1016/j.cej.2022.134615.

Siddharth, T., Sridhar, P., Vinila, V., Tyagi, R.D., 2021. Environmental applications of microbial extracellular polymeric substance (EPS): a review. J. Environ. Manag. 287, 112307 https://doi.org/10.1016/j.jenvman.2021.112307.

Tao, N., Zhang, W., Si, L., Zhang, R., Wang, D., Guo, C., 2022. Effects of aniline on growth, oxidative and DNA damage of rice (Oryza sativa L.) seedlings. Environ. Technol. Innovat. 28, 102583 https://doi.org/10.1016/j.eti.2022.102583.

Wang, D., Li, T., Huang, K., He, X., Zhang, X.-X., 2019. Roles and correlations of functional bacteria and genes in the start-up of simultaneous anammox and denitrification system for enhanced nitrogen removal. Sci. Total Environ. 655, 1355–1363. https://doi.org/10.1016/j.scitotenv.2018.11.321.

Wang, H., Fan, Y., Zhou, M., Wang, W., Li, X., Wang, Y., 2022. Function of Fe (III)-minerals in the enhancement of anammox performance exploiting integrated network and metagenomics analyses. Water Res. 210, 117998.

Wang, S., Gao, M., She, Z., Zheng, D., Jin, C., Guo, L., Zhao, Y., Li, Z., Wang, X., 2016. Long-term effects of ZnO nanoparticles on nitrogen and phosphorus removal, microbial activity and microbial community of a sequencing batch reactor. Bioresour. Technol. 216, 428–436. https://doi.org/10.1016/j.biortech.2016.05.099.

Wang, Z., Yuan, S., Deng, Z., Wang, Y., Deng, S., Song, Y., Sun, C., Bu, N., Wang, X., 2020. Evaluating responses of nitrification and denitrification to the co-selective pressure of divalent zinc and tetracycline based on resistance genes changes. Bioresour. Technol. 314, 123769 https://doi.org/10.1016/j.biortech.2020.123769.

Yan, W., Wang, N., Wei, D., Liang, C., Chen, X., Liu, L., Shi, J., 2021. Bacterial community compositions and nitrogen metabolism function in a cattle farm wastewater treatment plant revealed by Illumina high-throughput sequencing. Environ. Sci. Pollut. Control Ser. 28 (30), 40895–40907.

Yan, Y., Lu, H., Zhang, J., Zhu, S., Wang, Y., Lei, Y., Zhang, R., Song, L., 2022. Simultaneous heterotrophic nitrification and aerobic denitrification (SND) for nitrogen removal: a review and future perspectives. Environ. Adv. 9, 100254 https://doi.org/10.1016/j.envadv.2022.100254.

Zhang, C., Chen, H., Xue, G., Liu, Y., Chen, S., Jia, C., 2021a. A critical review of the aniline transformation fate in azo dye wastewater treatment. J. Clean. Prod. 321, 128971 https://doi.org/10.1016/j.jclepro.2021.128971.

Zhang, J., Wu, P., Hao, B., Yu, Z., 2011. Heterotrophic nitrification and aerobic denitrification by the bacterium Pseudomonas stutzeri YZN-001. Bioresour. Technol. 102 (21), 9866–9869. https://doi.org/10.1016/j.biortech.2011.07.118.

Zhang, S., You, J., An, N., Zhao, J., Wang, L., Cheng, Z., Ye, J., Chen, D., Chen, J., 2018. Gaseous toluene powered microbial fuel cell: performance, microbial community, and electron transfer pathway. Chem. Eng. J. 351, 515–522. https://doi.org/10.1016/j.cej.2018.06.027.

Zhang, W., Zhang, Q., Li, M., Wang, H., Li, Y., Peng, H., Feng, J., 2021b. Microbial community and function evaluation in the start-up period of bioaugmented SBR fed with aniline wastewater. Bioresour. Technol. 319, 124148 https://doi.org/10.1016/j.biortech.2020.124148.

Zhang, Y., Angelidaki, I., 2014. Microbial electrolysis cells turning to be versatile technology: recent advances and future challenges. Water Res. 56, 11–25. https://doi.org/10.1016/j.watres.2014.02.031.

Zhang, Y., Zhang, Q., Peng, H., Wei, H., Feng, J., Su, J., He, J., 2022. An attempt to stimulate aniline degrading bioreactor by exogenous auto-inducer: decontamination performance, sludge characteristics, and microbial community structure response. Bioresour. Technol. 347, 126675 https://doi.org/10.1016/j.biortech.2022.126675.

Zheng, M., Xu, C., Zhong, D., Han, Y., Zhang, Z., Zhu, H., Han, H., 2019. Synergistic degradation on aromatic cyclic organics of coal pyrolysis wastewater by lignite activated coke-active sludge process. Chem. Eng. J. 364, 410–419. https://doi.org/10.1016/j.cej.2019.01.121.