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Performance and microbial characteristics of anaerobic/anoxic/aerobic (A^2O)-biofilm system for electroplating wastewater treatment: a pilot scale study

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ABSTRACT

A pilot scale study was conducted to explore the performance and microbial characteristics of anaerobic/anoxic/aerobic (A^2O)-biofilm system in electroplating wastewater treatment. The results showed that the A^2O-biofilm system had a good removal performance for organics, nitrogen and phosphorus in electroplating wastewater, and the average concentrations of chemical oxygen demand, total phosphorus, and total nitrogen reached 28, 0.27, and 10 mg/L. High throughput sequencing showed that electroplating wastewater resulted in the microbial richness and diversity significantly decreased. Proteobacteria and Bacteroides were the dominant phyla in the biofilm process, while the abundance of Chloroflexi and Planctomycetes demonstrated a significant increase in the middle and later stages of biofilm formative process. At the genus level, Nitrosospira, Zoogloea and Haliangium were the dominant genera, among which Nitrosospira significantly increased. Aerobic chemoheterotrophy was always in the lead position which was a main metabolic function. What's more, aerobic nitrite oxidation, nitrification, aerobic chemoheterotrophy and mark iron oxidation were significantly higher in the late stage than those in the early stage.

Keywords: Anaerobic/anoxic/aerobic; Biofilm system; Electroplating wastewater; Microbial mechanisms

1. Introduction

With the rapid development of electronic manufacturing industry, electroplating industry even became the pillar industry in some large and medium-sized cities [1]. However, the effluents from electroplating production such as plating cleaning wastewater and waste electroplating liquid are featured with containing heavy metal, nitrogen (N) and phosphorus (P) [2,3]. The newly released Emission Standard of Pollutants for Electroplating (GB 21900-2008) [4] has issued higher requirements for the discharge of heavy metals, organic matter and N and P in electroplating wastewater. Therefore, the research of new electroplating wastewater treatment counts for improving the wastewater treatment efficiency.

Electroplating wastewater treatment technology mainly includes advanced oxidation, adsorption, membrane separation technology and so on [5]. For the control of heavy metals and refractory organic matter, coagulation and advanced oxidation technologies have been confirmed effective in engineering application [6]. Biological method is one of the commonly used methods to remove N and P, such

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as anaerobic-anoxic-aerobic (A^2O) process and anoxic-aerobic (AO) process. In order to resist the impact of refractory organic matter and heavy metals, and improve the removal efficiency of N and P, the biofilm-coupled activated sludge process has become one of the effective ways [7,8].

A^2O-biofilm system is a process of biofilm coexistence by adding fillers as the carrier of microbial attachment in the A^2O activated sludge system, to form a coexistence system of suspended activated sludge and attached biofilm. It is the combination of both advantages that plays an inevitable role in greatly improving the denitrification efficiency, and reducing the carbon source input and oxygen demand [9]. Zhang et al. [10] confirmed that it was feasible to achieve nitration and high-efficient nutrient removal by controlling temperature, dissolved oxygen (DO) in A^2O-moving bed biofilm reactor (A^2O-MBBR). Wang et al. [11] constructed A^2O-biofilm system to enhance the efficiency of municipal wastewater treatment at low temperature.

What's more, the introduction of plating wastewater may lead to changes in the abundance of dominant functional bacteria and microbial succession. The presence of many denitrifying phyla, such as Firmicutes and Nitrospirae ensured the stability at low temperature [10]. In addition, it demonstrated that the effect was closely associated with functional bacteria in a MBBR system [12]. However, few studies paid attention to analyzing the role of A^2O-biofilm system in respect of plating wastewater treatment and the microbial characteristics.

Therefore, in order to provide technical support for the wide application of wastewater treatment in electroplating enterprises, A^2O-biofilm system was constructed to study the removal performance of organic matter, N and P, and discuss the effects of A^2O-biofilm system on microbial community composition and diversity. What's more, the removal performances of pollutants in anaerobic, anoxic and aerobic units were compared.

2. Materials and methods

2.1. Characteristics of seed sludge and electroplating wastewater

The organic wastewater mainly came from the workshop of pretreatment degrease and oil removal of electroplating parts, and the seed sludge was collected from a wastewater treatment station of an electroplating enterprise in Shenzhen. The wastewater produced by pretreatment was taken as the experimental raw water (water quality indexes are shown in Table 1). What's more, trace elements and alkalinity were added to the raw water (7.14 g alkalinity per gram of NH_4^+–N into NO_3^-–N, measured by $CaCO_3$). In the experiment, 455.8 mg·$NaHCO_3$, 10 mg·$CaCl_2$, 10 mg·$MgSO_4$ and 5 mg·$FeSO_4$ per liter of wastewater were added to simulate the normal physiological and biochemical reaction of microorganisms in the actual electroplating wastewater. The effluent quality of this study shall meet the discharge standards stipulated in the Emission Standard of Pollutants for Electroplating (GB21900-2008) [13] and the pipe requirements of downstream urban sewage treatment plants. The water quality indicators are shown in Table 1.

2.2. Experimental devices

A^2O-biofilm coupling system with a total effective volume of 54 L is shown in Fig. 1, where the effective volume of the anaerobic tank was 9.45 L, the hypoxic tank was 18.23 L, and the aerobic tank was 26.32 L. The aeration system was composed of external electromagnetic air pump, gas flowmeter and microhole aerated aeration strip laid at the bottom of the aerobic tank. The aeration volume was adjusted according to the concentration of DO in the aerobic tank. Each reaction tank was installed with temperature control device, and the temperature of the reactor was maintained at 24°C–26°C.

The reactor adopted continuous water intake, hydraulic retention time was 24 h and nitrification liquid reflux ratio reached 200%. What's more, sludge reflux ratio was 100%, and DO of aeration tank was controlled at 2–3 mg/L. The aerobic tank was filled with sponge biological filler (the filler filling rate was 30%), and the water inlet, internal recycle flow and sludge recycle flow were controlled by the peristaltic pump. The pH of aerobic tank was controlled between 6 and 7, and the pH of anoxic tank was controlled between 7.5 and 8.5.

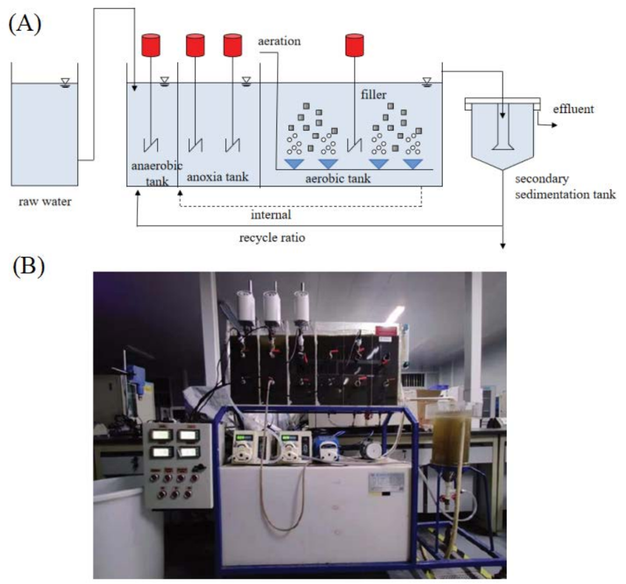

Fig. 1. A^2O-biofilm process (A) and device (B).

Table 1
Experimental influent water quality and discharge standards

Water quality index	Chemical oxygen demand	Biochemical oxygen demand	Total nitrogen	NH_4^+–N	Total phosphorus
Influent water (mg/L)	380 ± 23	80 ± 15	54 ± 5	38 ± 5	3 ± 1
Discharge standards (mg/L)	80	/	20	15	1

In the start-up process of biofilm, a certain amount of seed sludge was added into A^{2}O-biofilm process. The inoculated sludge and raw water were mixed at a ratio of 1:4 (volume ratio). Anaerobic and anoxic tanks started stirring, meanwhile aerobic tank added packing and adjusted the DO in 2–4 mg/L. Continuous water intake and initiation of internal recycle flow and sludge recycle flow was conducted for 90 days. At the initial stage of operation (the first 24 days), domestic sewage was introduced until the reaction system achieved stable, and then electroplating wastewater was introduced.

2.3. Chemical analytical methods

The conventional water quality indicators referred to SEPAC [13]. Chemical oxygen demand (COD) was determined by potassium dichromate fast digestion spectrophotometric measurement, NH_4^+–N by Nessler's reagent spectrophotometry, total nitrogen (TN) by alkaline potassium persulfate digestion ultraviolet spectrophotometry, and total phosphorus (TP) by ammonium molybdate spectrophotometry. NO_3^-–N and NO_2^-–N were detected by phenolic disonic acid spectrophotometry and molecular absorption spectrophotometry, respectively.

2.4. Microbiological analytical methods

Six DNA templates were extracted via the Power Soil DNA Kit (Mobio, CA, USA). Before analyzed by PCR with 338 F (50-ACTCCTACGGGAGGCAGCA-30) and 806 R (50-GGACTACCAGGGTATCTAAT-30) as PCR primers, the purified DNA was preserved at − 20°C. The amplicons were sequenced at Majorbio Company (Shanghai, China). The amplification method was performed based on the study by Huang et al. [14].

Sequences within the threshold range (97% similarity) were grouped and different samples were divided into operational taxonomic units (OTUs) by Usearch software (7.1http://drive5.com/uparse/version). Graphical representation of the relative abundance of microbial diversity at phylum and genus levels was counted depended on the taxonomic data.

The functional prediction of 16S rRNA was based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) (http://www.genome.jp/kegg/) and the unsupervised homologous group (eggNOG) (http://eggnog.embl.de/) database. Using PICRUSt software (http://huttenhower.sph.harvard.edu/galaxy/root?tool_id=PICRUSt_ standardized/) to standardize the detection of 16S marker genes. According to the greengene id of each OTU, the clustering information of homologous group (COG) and KEGG homology (KO) was calculated. COG functional information and KO, pathway annotations were from eggnog and KEGG databases. In addition, metabolic functions of microbial communities were predicted by FAPROTAX and BUGBASE.

2.5. Statistical analysis

The above indicators were to be sampled in three groups, the average values were calculated and displayed in diagram. Origin 2018 software was introduced into drawing figures.

3. Results and discussion

3.1. COD removal performance

The removal performance of COD in A^{2}O-biofilm process is shown in Fig. 2. The COD concentration in the effluent fluctuated slightly at the early stage of operation (the ratio of domestic sewage and electroplating wastewater was 1). The highest COD concentration in effluent was 34.56 mg/L on the 14th day, and the lowest value was 12.30 mg/L on the 17th day. The average COD concentration during the first 23 days was 21.64 mg/L with the average removal efficiency was 94.30%. The domestic sewage and pretreated electroplating wastewater were fully degraded in the A^{2}O-biofilm process, because the pretreatment of electroplating wastewater removed a certain amount of organic matter and reduced the loading of the biochemical process.

On the 24th day, the electroplating wastewater was completely flowed into A^{2}O-biofilm process without domestic sewage. However, it was shown that the effluent COD concentration increased significantly, reaching 63.52 mg/L on the 25th day, and the removal efficiency was only 83.28% that approached the discharge limit. After that, the A^{2}O-biofilm system gradually adapted to the refractory electroplating wastewater, and the COD concentration fluctuated at about 60 mg/L. During the 24th day to 44th day, the average COD removal efficiency was only 85.50%.

Starting from 45th day, the effluent concentration recovered to a lower level and the COD concentration in the effluent was basically about 30 mg/L with an average removal efficiency of 92.23%. It could meet the third table of electroplating pollutant discharge standard (GB21900-2008). From the 79th day, the COD concentration in effluent kept at about 30 mg/L, and the average value was 28.03 mg/L with the average removal efficiency was 92.62%.

The initial stage of the reaction was the domestication stage with low content of refractory organics in the simulated electroplating wastewater, and the activated sludge and biofilm system had strong adaptability to it, and little impact appeared on the biological system. When the concentration of refractory organics with long-term toxicity increased, the COD removal performance became worse [15]. With the recovery of microbial system and the enrichment of resistant microorganisms, the treatment performance was restored. Then the biofilm system began to exert its anti-shock and recovery ability. What's more, microorganism adapted to the refractory organic matter and survived in the harsh environment, and began to reproduce.

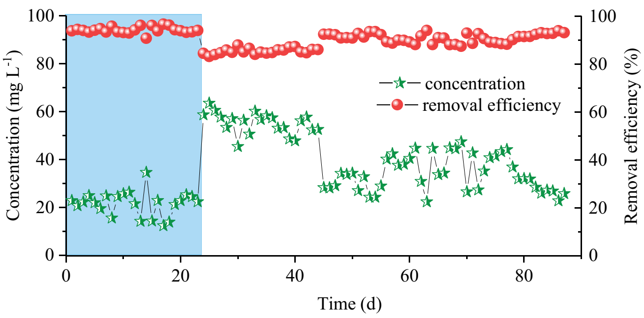

Fig. 2. Change of chemical oxygen demand concentration.

Furthermore, water quality gradually improved and the system entered the recovery period. During this period, the quality of effluent also fluctuated to some extent due to the change of community structure under the new environmental pressure selection, and the new competition evolution in the community [16]. After 30 days, the system tended to be stable with good biodegradation effect, and the effluent quality reached the standard.

3.2. Nitrogen removal performance

3.2.1. Total nitrogen

The TN removal performance of A^2O-biofilm process is shown in Fig. 3A. The TN concentration in effluent from the biochemical tank fluctuated between 10~20 mg/L in the early phase of operation and reached 22.59 mg/L on the 20th day, while its removal efficiency was only maintained at 61.05% (failure to meet the standard). On the 7th day, its effluent was 4.12 mg/L with the removal efficiency of 92.88%. The average TN effluent in the first 23 days was 21.64 mg/L with an average removal efficiency of 94.30%. The electroplating wastewater entering the biochemical tank on the 24th day led to the TN effluent concentration increased, and the average TN removal efficiency was only 76.37% from the 24th day to the 43rd day, which was significantly lower compared with the effluent of the first 23 days. At 44th day, the TN concentration started to be maintained at a low level (the TN effluent concentration was basically stable at about 10 mg/L, which met water quality standards).

3.2.2. NH_4^+–N

The NH_4^+–N removal performance of A^2O-biofilm process is shown in Fig. 3B. The concentration of NH_4^+–N in the effluent of biochemical tank maintained lower than 3 mg/L in the early stage of operation. The highest NH_4^+–N concentration was 2.18 mg/L with 94.26% removal efficiency on the 10th day, while the lowest was 0.09 mg/L with 99% removal efficiency on the 2nd day. The average effluent NH_4^+–N was 0.67 mg/L (98.22% removal efficiency on average) in the first 23 days. It meant that the biofilm system was beneficial for NH_4^+–N removal. In fact, the removal of NH_4^+–N mainly depended on ammonia-oxidizing bacteria in the aerobic tank, while the role of biofilm was to provide ammonia-oxidizing bacteria (AOB) enrichment sites. It enhanced the nitrification capacity of the system and facilitated the removal of NH_4^+–N [17].

NH_4^+–N concentration did not fluctuate much after entering electroplating wastewater on the 24th day. There was an increasing trend of NH_4^+–N effluent concentration from 32nd day to 48th day, which was higher than the average level of the previous 23 days. The average NH_4^+–N concentration at this stage was 2.34 mg/L with 93.85% removal efficiency. Compared with the previous period, a decrease trend appeared, but the NH_4^+–N was not influenced by the change of influent water quality. It might be that the poisonous function of intermediates from the conversion of refractory organic matter contained in electroplating wastewater affected AOB and resulted in a large fluctuation for NH_4^+–N removal [18].

The effluent NH_4^+–N concentration showed large fluctuations of 5.44 mg/L (61st day) and 4.53 mg/L (67th day) with 85.69% and 86.55% removal efficiency from 60th day to 69th day, respectively. The average removal efficiency of NH_4^+–N was 91.79% with the average effluent concentration of 3.12 mg/L. NH_4^+–N concentration did not produce the same real-time response to water quality change as COD concentrations, but the long-term poisonous nature of the refractory organic material remained and influenced the NH_4^+–N effluent. The system soon returned to stability and the NH_4^+–N effluent was stable at about 1 mg/L from 69th day to 87th day. The average effluent NH_4^+–N concentration was 0.45 mg/L (the average removal efficiency was 98.82%) during this period. The effluent quality basically returned to the previous level and the system operated stably.

3.3. TP removal performance

The removal performance of TP by A^2O-biofilm process is shown in Fig. 4. The TP concentration in effluent fluctuated around 0.2 mg/L at the early stage of operation, and reached the highest effluent concentration of 0.39 mg/L at the 2nd day with the removal efficiency of 87.00%, and the lowest was 0.07 mg/L and the removal efficiency was 97.67% at the 7th day. In the first 23 days, the average concentration of TP reached 0.28 mg/L, and the average removal efficiency was 90.75%. When adding electroplating wastewater

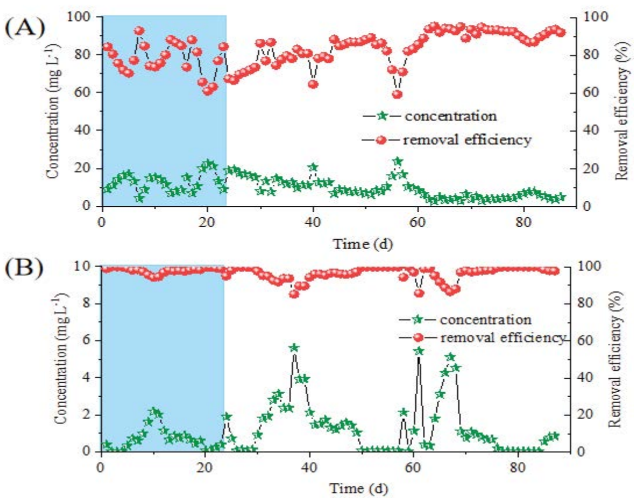

Fig. 3. Change of total nitrogen (A) and NH_4^+–N (B) concentration.

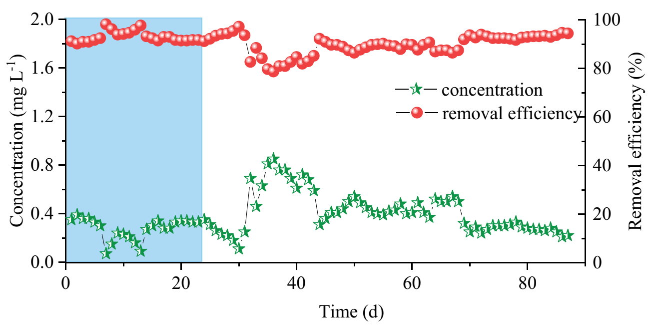

Fig. 4. Change of total phosphorus concentration.

on the 24th day, the concentration of TP remained decrease. However, the average concentration of TP was 0.65 mg/L and the average removal efficiency was only 78.21% in the 31st day to 43rd day, which was significantly lower than that in the earlier period. At the beginning of the 44th day, the total phosphorus removal function of the system recovered and the concentration of total phosphorus began to decrease to a lower level. From 44th day to 69th day, the average concentration of TP was 0.44 mg/L. From 70th day to 87th day, the average value was 0.27 mg/L, and the effluent quality was basically restored to the previous level.

3.4. Characteristics of microbial community

3.4.1. Richness and diversity

Table 2 shows the richness and diversity of microbial communities. Microbial community richness were evaluated by ACE index and Chao index, meanwhile the Shannon index and Simpson index were used to characterize the microbial community diversity. The first 5 days were the beginning of biofilm formation, and the microorganisms were basically from the activated sludge adsorbed on the filler from the 5th day to the 10th day, and the richness and diversity decreased. With the enrichment of activated sludge on the carrier and the formation of biofilm, ACE, Chao1, Shannon and Simpson indexes increased on the 15th day, indicating that the biodiversity tended to be complex during biofilm hanging. Since 20th day to 30th day, ACE and Chao1 indexes decreased, which indicated that the species abundance was decreasing. Besides, Shannon and Simpson indexes also demonstrated decrease trend since 15th day, because some microorganisms were gradually eliminated due to competition in the biofilm community.

3.4.2. Community composition

3.4.2.1. At the phylum level

The relative abundance distribution plot of the microorganisms at the phylum level is shown in Fig. 5A. Proteobacteria, Nitrospirae, Bacteroidetes and Acidobacteria and Chloroflexi took the lead in the electroplating wastewater group. On the contrary, in the domestic sewage group, Proteobacteria, Nitrospirae, Bacteroidetes and Acidobacteria were dominant.

From 5th day to 30th day, Proteobacteria and Bacteroidetes accounted for 33.28%–83.26% and 9.03%–16.81%, 48.23%–80.00% and 10.68%~25.94%, respectively. These phyla maintained a high proportion during the whole hanging period, which could reach more than 90%, the dominant in biofilm process. Previous research on Proteobacteria was shown that it was the largest of bacteria, including many bacteria that could fix nitrogen [19]. It participated in the degradation process of biological nitrogen and phosphorus removal and organic pollutants, accounting for the dominant bacterial group of biofilm process. Bacteroidetes secreted abundant adhesion proteins, contributing to the microorganisms on the carrier surfaces, which was an important role for biofilm formation [20]. Proteobacteria and Bacteroidetes were the highest abundance, with excellent biological nitrogen and carbon removal functions.

Acidobacteria kept 2.19%~14.50% and about 10% at 15th day in all samples. It was a gram-negative bacterium, which could decompose macromolecular organic matter and produce amorphous extracellular polysaccharide-like substances [21]. What's more, it was an important bacterium in biofilm process. Deinococcus-Thermus occupied a considerable proportion (8.84%~10.41%), which was verified that it could autotrophic and fix carbon during the reducing tricarboxylic acid cycle, and oxidize sulfur compounds and hydrogen with oxygen or nitrate as electron acceptor to obtain energy [22].

Chloroflexi and Planctomycetes increased significantly in the middle and late biological membranes while Nitrospirae decreased. Chloroflexi not only promoted the formation of sludge flocs particles, but also degraded macromolecular organic pollutants into small molecular organic pollutants [23]. Planctomycetes oxidized NH_4^+ to N_2 by NO_2^-–N, carrying on anaerobic ammonia oxidation reaction and playing a certain short-range nitrification denitrification [24].

3.4.2.2. At the genus level

The distribution plot of the relative abundance of the microorganisms at the genus level is shown in Fig. 5B. The dominant bacteria of biofilm were Nitrosospira, Zoogloea, Haliangium and Sphaerotilus, while the dominant bacteria in group B were Nitrosospira, Zoogloea and Haliangium.

The richness of Nitrosospira increased significantly, reaching 19.96% at the 20th day. The community diversity increased significantly in the later period and decreased during the long-term operation. It could be deduced that Nitrosospira become the dominant species in the enrichment process, and had a high richness, which made the system a good ability of nitrification and denitrification.

Zoogloea and Haliangium are the dominant genera of biofilm process, occupying dominant position (11.47%) in the early stage of group A (17.45% in group B). Haliangium was the core genus of biological nitrogen removal [25]. Zoogloea could secrete bacterial pellet to stabilize the structure of biological flocs and ensure the decomposition of organic matter in wastewater on biofilm by sufficient biomass on biofilm [26]. The proportion of Sphaerotilus in the late stage

Table 2
Microbial community diversity index

Sample	ACE[a]	Chao1[b]	Shannon[c]	Simpson[d]
A1	592.058	534.375	6.727	0.974
A2	521.994	522.519	5.125	0.923
A3	609.952	611.810	6.645	0.971
A4	562.382	574.500	6.005	0.949
A5	553.314	550.622	5.583	0.942
A6	530.908	562.889	5.610	0.931

[a,b]Community richness. A higher number represented more richness.
[c,d]Community diversity. A higher Shannon index represented more diversity, Simpson was on the contrary.
A1: the sample on the 5th day; A2: the sample on the 10th day; A3: the sample on the 15th day; A4: the sample on the 20th day; A5: the sample on the 25th day; A6: the sample on the 30th day.

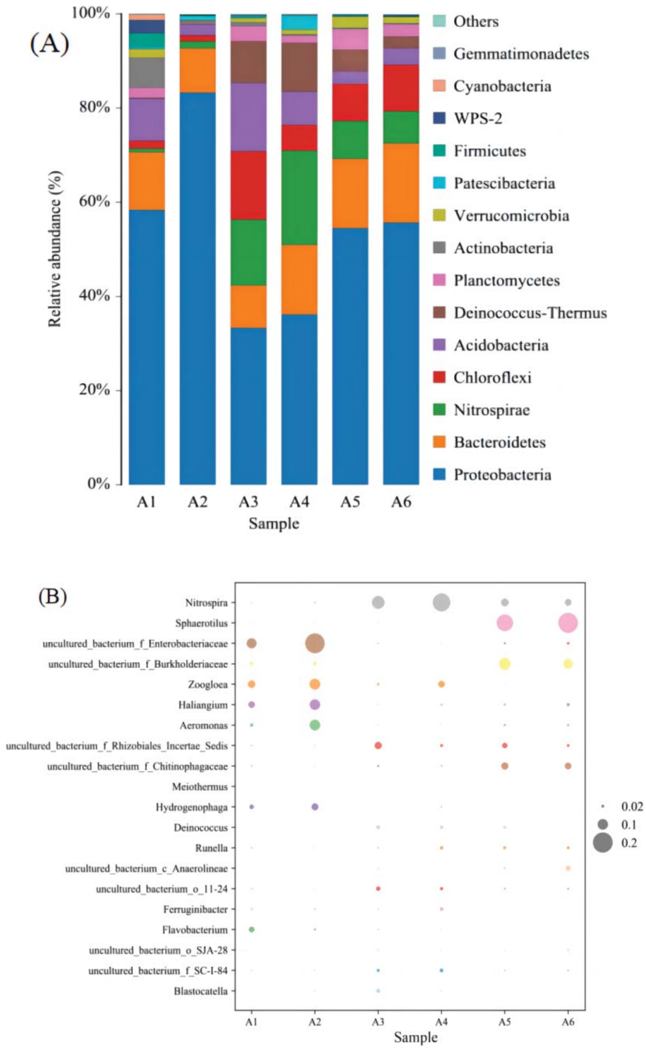

Fig. 5. Microbial community composition at phylum level (A) and genus level (B) (A1: the sample on the 5th day; A2: the sample on the 10th day; A3: the sample on the 15th day; A4: the sample on the 20th day; A5: the sample on the 25th day; A6: the sample on the 30th day).

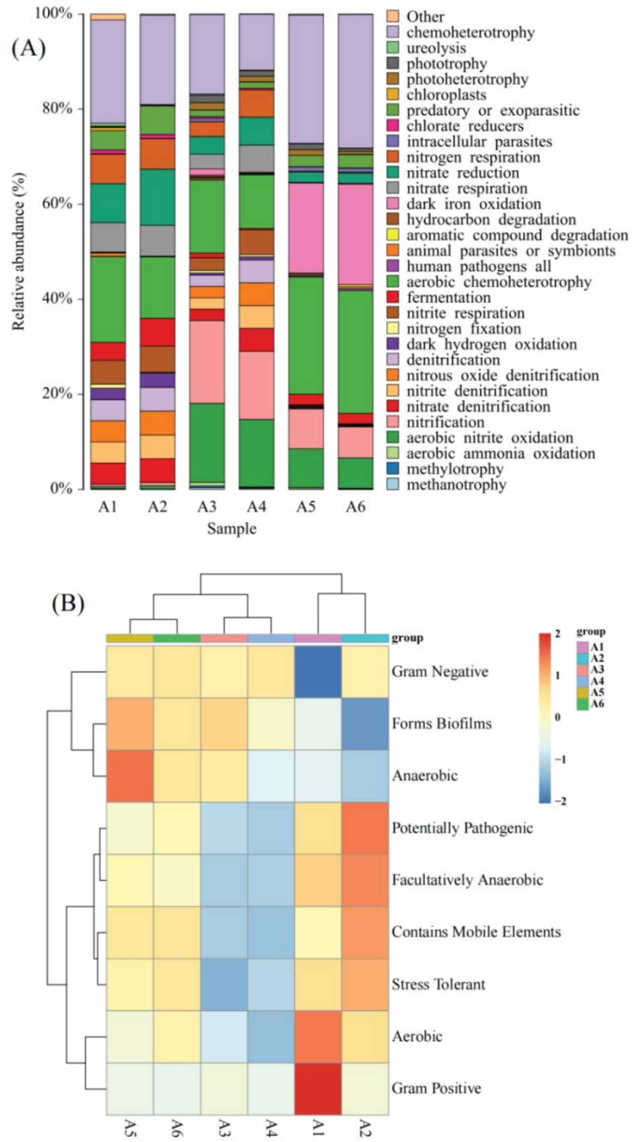

Fig. 6. Functional characteristics of microbial community predicted by FAPROTAX (A) and BUGBASE (B) (A1: the sample on the 5th day; A2: the sample on the 10th day; A3: the sample on the 15th day; A4: the sample on the 20th day; A5: the sample on the 25th day; A6: the sample on the 30th day).

of hanging film increased significantly (18.52%~22.44%). It was a gram-negative obligate aerobic bacterium, the cell morphology of which is a filament with a sheath, generally attached to the solid, so that the filaments can be adsorbed to the matrix [27]. The strong adsorption of its filaments may be the dominant bacterial genus on biopacking.

In addition, other bacteria also had a certain proportion of bacteria, such as Hydrogenophaga (2.14%), Aeromonas (3.18%), Flavobacterium (1.42%), Ferruginibacter (1.57%), Denitratisoma (0.68%), etc. Hydrogenophaga was an aerobic facultative autotrophic bacterium with oxygen as the terminal electron acceptor for biological anaerobic nitrate respiration reaction to decompose organic acids so as to perform denitrification [28]. Aeromonas, Flavobacterium are important biological carbon removal in sewage [29,30]. Ferruginibacter, as an uncommon denitrifying functional bacteria, was found in the undercurrent constructed wetland [31]. Denitratisoma is an abundant and potentially important denitrifier in the microbial community, capable of reducing NO_3^- to N_2O [32].

3.5. Microbial functional prediction

The impact of the A²O-biofilm system on the prediction of microbial functionality predicted by FAPROTAX is shown in Fig. 6A. The functional groups with a higher proportion included aerobic nitrite oxidation, nitrification, nitrite respiration, fermentation, aerobic chemoheterotrophy, dark iron oxidation, nitrate reduction, nitrogen respiration. Among, aerobic chemoheterotrophy was always in the lead position which was a main metabolic function associated with dissolved organic matter transform [33]. Aerobic nitrite oxidation, nitrification, aerobic chemoheterotrophy, mark iron oxidation were significantly higher in the late stage than in the early stage, especially mark iron oxidation. As the biofilm formed, the nitrification showed a noteworthy increase, bringing about the efficient removal of nitrogen to a great extent [34]. In addition, the functions of nitrate respiration, nitrate reduction, and nitrogen respiration related to the nitrogen cycle had also changed accordingly, probably on account of the removal of nitrogen making carbon to nitrogen ratio change [35]. The above phenomenon demonstrated that the A^2O-biofilm system had a significant impact on microbial energy heterotrophy, carbon and nitrogen cycle, and oxidation function, possibly because Proteobacteria and Bacteroidetes were the dominant species. From Fig. 6B it is evident that anaerobic had the positive proportion with forms biofilms.

4. Conclusions

A^2O-biofilm system had a good removal performance on both organic matter, N and P in electroplating wastewater. The COD, TP and TN in effluent met the standard (GB21900-2008). High-throughput sequencing showed that Proteobacteria and Bacteroidetes were the dominant microbial community in this A^2O-biofilm process, while the proportion of Chloroflexi and Planctomycetes increased significantly in the middle and late stages of biofilm formation stage.

Declaration of competing interest

The authors declare that they have no known associative or commercial interest regarding the publication of this paper.

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