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The performance of a novel sleeve-type CW-MFC-CW system for acid orange 7 (AO7) removal and electricity generation with leaves as a carbon source on winter days

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ABSTRACT

A novel design of microbial fuel cell coupled with constructed wetland and cascading constructed wetland (CW-MFC-CW) planted with reeds was established for simultaneously treating wastewater containing acid orange 7 (AO7) and electricity generation with a soak solution of *Platanus acerifolia* leaf litter as the carbon source. The main purposes of this study were to explore the effectiveness of this novel system with soak solution as a carbon source for AO7 decolorization, further degradation and bioelectricity generation in winter. For the whole system, the AO7 decolorization rate reached approximately 100% and COD removal efficiency exceeded 65% when the AO7 concentration varied between 10 and 150 mg/L. Compared with the outer cylinder (CW), the inner cylinder (CW-MFC) had the greater contribution to AO7 and COD removal; however, the removal efficiency declined when AO7 influent concentration increased. In contrast to the inner cylinder, the removal efficiency of AO7 and COD in outer cylinder increased from 0% and 11% to 14% and 26%, respectively with AO7 influent increase from 10 mg/L to 150 mg/L, which indicated that the sleeve-type CW-MFC-CW system created a favorable environment for the further degradation of the decolorization by-products. For electricity generation, the highest voltage and current output reached 0.19 V and 1.13 mA, respectively.

Keywords: Constructed wetland; Microbial fuel cell; Carbon source; Decolorization; COD removal; Acid orange 7

1. Introduction

Azo dyes, which are aromatic compounds with one or more -N=N-groups, are widely present in the wastewater of different industries, including the textile industry, food industry, and paper industry [1]. The use of these dyes is discouraged because of their inherent color as well as their toxic and carcinogenic decolorization products. The discharge of azo dyes into surface water leads to aesthetic problems and prevents light and oxygen from entering the water, adversely affecting aquatic organisms [2]. A range of physicochemical methods exist to remove the color from wastewater containing dyes, such as coagulation, flocculation, absorption [3,4] and advanced oxidation processes [5], which not only require substantial quantities of chemicals and produce notable amounts of sludge, necessitating further handling and disposal [6], but also consume considerable energy and are costly.

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As a result, biological processing of these wastewater has drawn increasing attention because of its lower cost, reduced sludge production and environmental friendliness. Biological treatments include aerobic, anaerobic and aerobic-anaerobic coupled processes. Although these methods can achieve a high decolorization rate, the coupled approach is the best choice for improving water quality. As examples of biological treatment technology, microbial fuel cell (MFC) technology and constructed wetland (CW) technology have considerable potential for treating dye-containing wastewater. In recent years, researchers [7–12] have successfully employed MFCs for the decolorization of azo dyes and bioelectricity generation. However, though these systems achieved high decolorization, they provided inadequate COD removal and were costly. CWs, which are complex systems, include plants, microorganisms and substrates. These systems have been successfully used for treating sewage containing azo dyes but require long hydraulic retention times (HRT)for dye degradation [1,13–16].

Based on the same principle and complementarity, Yadav et al. [17], who constructed the first CW-MFC coupled system, successfully degraded high-concentration methyl blue while generating power output. The coupled system made up for the shortcomings of each component alone. For CW, the addition of MFC improved its performance in degrading the dyes and shortened the HRT and relieved the problem of clogging [18]. CW, in turn, created an excellent microbial environment for MFC and decreased its cost. The CW-MFC system consisted of the following: anode region, transition region, cathode area, macrophytes and external circuit. The operating principle of the coupled system was the same as that of MFC. At the anode, an organic co-substrate is oxidized by electrochemically active microorganisms. Subsequently, the microorganisms transfer the electrons resulting from this oxidation to the anode via extracellular electron transfer, and the electrons then pass through an external circuit to the cathode, combining with the oxygen and protons and thus producing a current [17]. Unlike MFC, the anode region was where azo dyes accepted electrons and protons, leading to the degradation of the azo bond. A reduction in the azo bond results in the formation of colorless and biodegradable aromatic amines [19]. To some extent, the azo dyes compete with the anode electrode in accepting the electrons [2]. Reviewing recent research, we found that CW-MFC had better adaptability for degrading different types of dyes from simple to complex, and CW-MFC could promote the further degradation of azo dye decolorization products. In addition, CW-MFC was easier to establish and operate than MFCs [20,21].

Although the above CW-MFC system exhibited an improved treatment efficiency of dyes in wastewater, certain shortcomings remained, for example, the effluent water had a higher COD concentration because of less dissolved oxygen in the cathode area and more toxic byproducts , such as aromatic amines, which could be further degraded into organics with smaller molecular structure and less toxic under sufficient dissolved oxygen [9,14], lower power output due to competition for electrons between dyes and anode electrodes, and a higher cost due to the addition of glucose. To solve these problems, in this study, a completely new sleeve-type coupled system was constructed to increase the dissolved oxygen in the effluent water from the cathode area by dropping the water into the outer cylinder, and further COD removal could occur because of the macrophyte and microorganism in the outer areas. In addition, alternative carbon resources were studied. It is known that azo dyes have a special structure that makes them resistant to biodegradation; thus, co-substrates are required for the breakdown of the nitrogen–nitrogen double bond (-N=N-) [14,22–24]. Commonly used co-substrates are glucose, sucrose, acetate et al., and glucose is considered as the best carbon resource [25]. The addition of ma large amount of glucose increases the cost of the whole system; thus, finding an effective and inexpensive carbon source is necessary. It was well known that plant carbon sources, such as wheat straw, reed, cattail and others, have been successfully utilized as alternatives to glucose for denitrification in CWs [26–29]. Recently, researchers [29] have found that the efficiency of a soak solution of *Platanus acerifolia* leaf litter (the soak solution) as a carbon source for denitrification was similar to that of glucose, mainly because the soak solution contained a large amount of fumaric acid, an organic acid with a lower molecular weight than that of glucose, which was easier to be utilized by microorganism. In addition, *P. acerifolia* is one of the most common street tree species of the urban area in China, and there are abundant *P. acerifolia* fallen leaves every year. The fallen leaves and pruning branches in winter lead to a litter disposal problem. Hence, it was hoped to reuse the waste and implement a green strategy for addressing the plant material problem while obtaining a natural organic carbon source.

Therefore, the purposes of this paper were to verify the efficiency of this new sleeve-type system and simultaneously explore the feasibility of plant carbon resources as a co-substrate for azo dye decolorization and bioelectricity generation.

2. Material and methods

2.1. Plant carbon source

The litter of *P. acerifolia* leaves was gathered from Hohai University in the Gulou District of Nanjing City. The litter was cleaned, cut into small pieces approximately 2 cm × 2 cm, and air dried for use. The soak solution of *P. acerifolia* leaf litter was the final form of the alternate carbon source, which was made by mixing leaves and deionized water at a weight ratio of 1:100. According to the measurement, the COD concentration of the soak solution reached approximately 1000 mg/L on the 20th day, and the soak solution was used as the carbon source that was added into influent water after diluted. In this experiment, the total COD concentration was maintained at 300 ± 10 mg/L.

2.2. CW-MFC-CW system construction

In this experiment, a new CW-MFC-CW coupled system was established and the configuration is shown in Fig. 1. The system consisted of two parts of inner cylinder and outer cylinder. The inner cylinder was a CW-MFC coupled

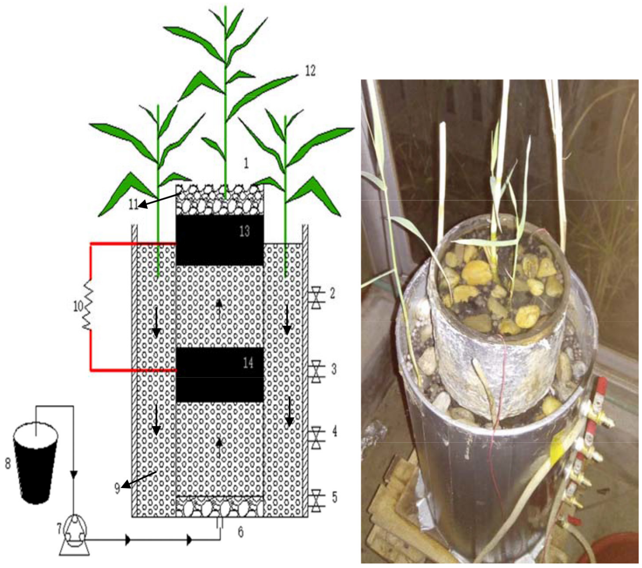

Fig. 1. Configuration of the sleeve-type CW-MFC-CW system [1,2,3,4,5–sampling ports; 6-inlet; 7-peristaltic pump; 8-wastewater tank 9-ceramsite; 10-extemal resistance; 11-cobblestone; 12-reeds; 13-cathode; 14-anode].

system and the outer cylinder was a simple CW system. In this study, the effective treatment volume ratio of the inner and outer cylinders was approximately 2:3. This kind of design could effectively reduce the land use.

The synthetic wastewater was pumped into the reactor from the bottom of the inner cylinder. On account of the design that the inner cylinder positioned 8 cm higher than the outer cylinder, the influent wastewater could fall from the top of the inner cylinder into the outer cylinder, which should increase the dissolved oxygen of the effluent water from the cathode area in inner cylinder.

The inner cylinder was made of a polyacrylic plastic cylinder with an internal diameter of 15 cm and a height of 48 cm. There were six layers from the bottom to top. The bottom layer contained two types of material: cobblestone (diameter of 16–32 mm) and ceramsite (diameter of 5–8 mm), with depths of 4 cm and 10 cm, respectively. The anode and cathode layers had the same design: 8 cm depth of granular activated carbon (GAC, diameter of 2–4 mm) and a stainless steel wire mesh (buried in the electrodes), which could enhance the electron transfer. The middle ceramsite layer between the anode and cathode layers had a height of approximately 14 cm, and the cobblestone layer above the cathode layer had a depth of approximately 4 cm. The volume of the inner cylinder was approximately 8.3 L and the liquid volume approximately was 2 L. The outer cylinder had the same material as the inner cylinder (internal diameter of 20 cm and height of 40 cm) and was filled with ceramsite (depth of 32 cm).

In this study, reeds were chosen and planted into the surface layer as the system plants. An external circuit with an external resistance of 1000 Ω was used as a link between the anode and cathode and was connected by copper conductors. The reactor contained five sampling ports (as shown in Fig. 1).

2.3. Inoculation and operating conditions

This system was inoculated using anaerobic sludge collected from the North City Wastewater Treatment Plant (Nanjing, China). During domestication, glucose (600 mg/L) was used as the only carbon resource for the microorganisms and was added directly to the nutrient and buffer solution. The composition was as follows: NH_4Cl (0.31 g/L), NaH_2PO_4 (4.97 g/L), Na_2HPO_4 (2.75 g/L), KCl (0.13 g/L), $NaHCO_3$ (3.13 g/L) and 0.1 mL concentrated trace element solution [30], which was pumped continuously into the reactor from the water inlet at the bottom of the reactor. The system was acclimated for approximately 30 d until the output of voltage was stable.

After the domestication, acid orange 7 (AO7) was added at 10 mg/L and the glucose was replaced simultaneously by the soak solution as the carbon source. To eliminate the influence of adsorption actions by GAC and acclimate the microorganisms to AO7, the system was continuously supplied with 10mg/L AO7 influent for over 20 d. Subsequently, the AO7 concentration was increased to 50, 70, 100, 120 and 150 mg/L in turn, and each concentration was maintained for about 12 d. The hydraulic retention time (HRT) was 2 d. The experiment was carried out during the winter (the room temperature was 4°C ± 2°C).

2.4. Analysis and calculation

Decolorization efficiency of AO7 was determined by monitoring the decrease of absorbance at a maximum wavelength of 488 nm with a spectrophotometer (BT100-2J).

Decolorization activity was calculated by the following Eq. (1):

$$\text{Decolorization rate} = (A - B)/A \times 100\% \tag{1}$$

where A is the AO7 concentration of influent and B is the AO7 concentration of effluent (mg/L).

The removal efficiency of aniline compounds was determined by monitoring the decrease of absorbance at a maximum wavelength of 545 nm with a spectrophotometer (BT100-2J).

Removal efficiency was calculated by the following Eq. (2):

$$\text{Removal rate} = (C - D)/C \times 100\% \tag{2}$$

where C is the aniline compounds concentration of effluent in sampling port 1 and D is the aniline compounds concentration of effluent in other sampling ports (mg/L).

The soluble chemical oxygen demand (COD) was measured using the UV spectrophotometer method (UV 1801).

The COD removal efficiency could be calculated by using the following Eq. (3):

$$\text{COD} = (\text{COD}_i - \text{COD}_e)/\times 100\% \tag{3}$$

where COD_i is the COD concentration of effluent in, and COD_e is the COD concentration of effluent (mg/L).

The voltage (V) and current (I) were measured every 30 min with a digital multimeter (Mastech, MS8232).

3. Results and discussion

3.1. Wastewater treatment performance

3.1.1. Decolorization

The influence of AO7 influent concentration on decolorization performance of the novel CW-MFC-CW system was showed in Fig. 2. As shown in Fig. 2a, it was obvious that the AO7 effluent concentration of inner cylinder increased with the influent concentration increasing from 10 mg/L to 150 mg/L. However, the AO7 effluent concentration of outer cylinder was kept at a very low level during the whole experimental period indicating that the novel CW-MFC-CW system presented a satisfactory decolorization performance. Fig. 2b intuitively describes that AO7 decolorization rate of the system reached 100% within the range of experimental AO7 concentration. Especially, the inner cylinder made the greatest contribution to AO7 decolorization. As seen in Fig. 2b, the highest decolorization rate in the inner area was 100% when the AO7 influent concentration was 10 mg/L, and the lowest decolorization rate was more than 85% when the AO7 influent concentration was up to 150 mg/L, which was approximately 70% higher than that of the outer cylinder, even though the volume of inner area was only 40% of the whole volume. The decolorization rate was greater in the inner cylinder of the reactors than in the outer cylinder of the reactors, most likely because of the consumption of carbon sources. It was known that azo dyes had a special structure that made them resistant to be biodegraded; thus, carbon resources are required for the breakdown of the – N=N–bond [24], therefore, a sufficient amount of carbon sources could increase the AO7 decolorization rate. And the soaking solution added in influent as organic carbon source was firstly utilized by the microorganisms in the inner cylinder to help break down the – N=N–bond, which indicated that the soak solution was an effective alternative carbon source for AO7 decolorization. On the other hand, according to the previous study, the anode region at the bottom of the device was in anaerobic or anoxic environment, which also provided favorite environmental conditions for the break of the –N=N–bond [9,31,32]. In addition, the existence of the GAC in anode and cathode area also contributed to the decolorization of AO7 in the inner cylinder. GAC, which has a high specific surface area, significantly benefited biofilm growth. AO7 was first adsorbed onto the GAC biofilm, and then, it was oxidized by the microorganisms on the biofilm with the breakdown of the –N=N– bond; thus, the adsorption capacity of GAC remained balanced during the biodegradation process.

Although the inner cylinder had the highest contribution to the decolorization rate, the decolorization rate of the inner area declined when the AO7 influent concentration increased because of the toxicity of the azo dye itself and the operating conditions of the inner cylinder area. However, complete decolorization of the whole system was achieved with the existence of the outer cylinder area. A greater burden was placed upon the outer cylinder when the AO7 concentration was higher; the highest decolorization of the outer cylinder area was 14% when the AO7 influent concentration was 150 mg/L, which indicated that the design of the outer cylinder (the CW system) was effective, especially for the higher concentration of AO7 influent.

When the soak solution was used as the carbon source, AO7 could be decolorized completely within the range of experimental AO7 influent concentration when the HRT was 2 d (the inner cylinder area with less than 24 h and the outer cylinder with more than 24 h), and the decolorization rate was similar to that employed by Yadav [17] and Fang [31,33] (all used glucose as carbon resources for decolorization). Moreover, Heng-Chong Tee [1] successfully used domestic wastewater and raw rice husk as carbon source for AO7 decolorization in newly developed horizontal subsurface-flow constructed wetland, but the time needed for decolorization was more than that in this study.

3.1.2. COD removal

Fig. 3 presents the influence of AO7 influent concentration on COD removal performance of the novel CW-MFC-CW system. As shown in Fig. 3a, it was obvious that the COD effluent concentration of inner and outer cylinder increased with the AO7 influent concentration increasing from 10 mg/L to 150 mg/L. Fig. 3b shows directly that the COD average removal rate of the whole system, the inner cylinder and outer cylinder. For the whole system, the COD average removal rate decreased from 91% to 66% with the increase of AO7 influent concentration. The

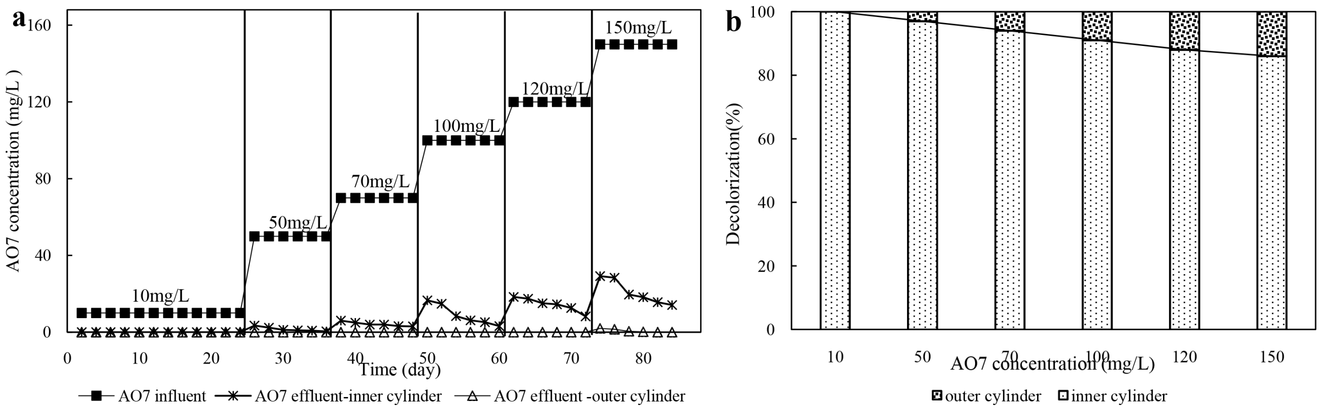

Fig. 2 (a) AO7 effluent concentration of inner and outer cylinder with different AO7 influent concentration; (b) Decolorization rate in inner and outer cylinder at various AO7 influent concentration.

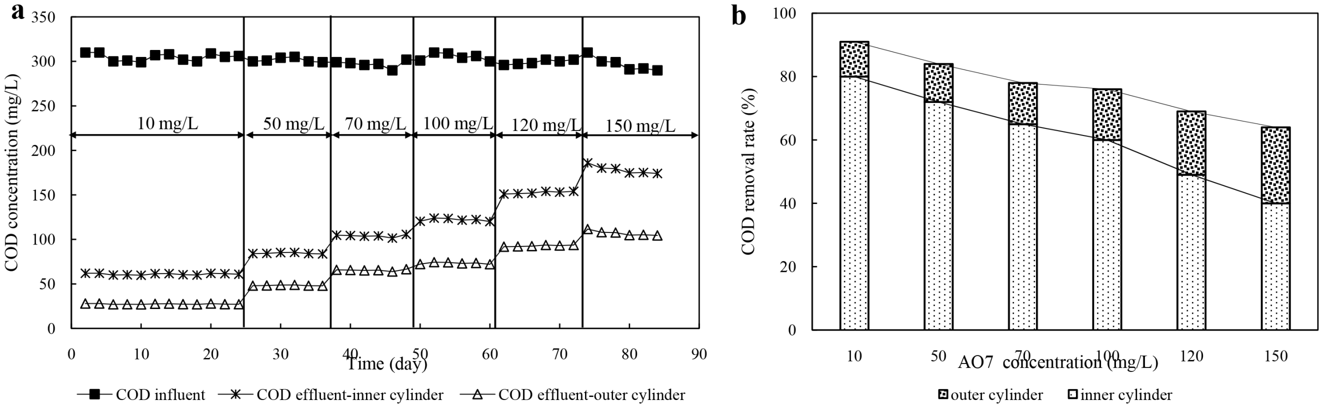

Fig. 3 (a) COD effluent concentration of inner and outer cylinder with different AO7 influent concentration; (b) COD removal rate in inner and outer cylinder at various AO7 influent concentration.

highest COD removal efficiency was over 90% (inner cylinder: 80%; outer cylinder: 11%) when AO7 concentration was 10 mg/L, and the lowest COD removal efficiency was 66% (inner cylinder: 40%; outer cylinder: 26%) when AO7 concentration was 150 mg/L. Compared with the outer cylinder, the inner cylinder made the greater contribution to COD removal, however, the removal efficiency declined when AO7 influent concentration increased. In contrast to the inner cylinder, the COD removal efficiency in outer cylinder had a marked increase from 11% to 26% when the AO7 influent concentration increase from 10 mg/L to 150 mg/L.

As previously mentioned, the AO7 decolorization rate of inner cylinder reached approximately 85% of the total decolorization rate (in Fig. 2b), then there would be a great deal of intermediate and by-products of decolorization, such as aniline and sulfonated aromatic amines in the effluent water, most of which are toxic. Therefore, it was very necessary to achieve the complete removal of AO7 as possible to avoid environment pollution. According to the previous study, the low dissolved oxygen in the cathode region was the main reason to inhibit the further degradation of these intermediate and by-products [34,35].

In this study, the outlet of the inner cylinder was designed 8 cm higher than the top of the outer cylinder and the purpose was to increase the DO concentration in water when the effluent of inner cylinder fell into the outer cylinder and to further degrade those intermediate and by-products. To confirm this idea, the concentration variation of DO, aniline compounds and COD in effluent of the inner cylinder were monitored along the distance of the outer cylinder. Since the higher the influent concentration of AO7, the more contribution made by the outer cylinder, three AO7 concentrations of 100, 120 and 150 mg/L were chosen to carry out and the results are shown in Figs. 4 and 5.

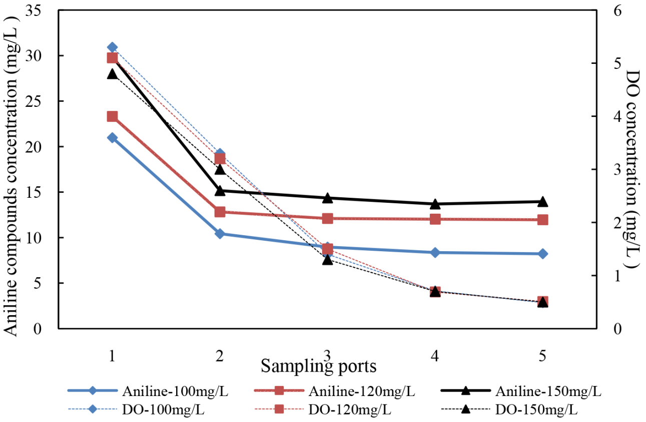

Fig. 4. Variation of the aniline compounds and DO concentration along the distance of the outer cylinder.

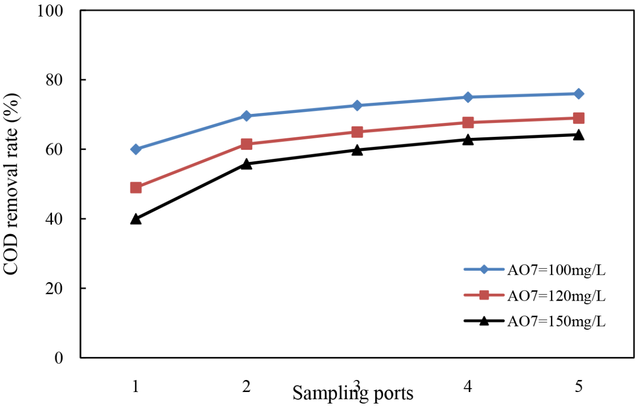

Fig. 5. Variation of the COD concentration along the distance of the outer cylinder.

As seen in Fig. 4, the concentration of aniline compounds declined sharply from sampling port 1 to sampling port 2 and then followed with a very slight decline along the distance. And the DO concentration of sampling port 2 was about 3 mg/L, which was higher than the 1.22 mg/L of the surface layer CW-MFC system reported in the previous study [31], which confirmed our hypothesis, namely the falling process enriched more oxygen into water. The higher concentration of DO in water promoted the further degradation of aniline compounds. In addition, a significant increase in the removal rate of COD from sampling port 1 to sampling port 2 is also shown in Fig. 5, which was in accordance with the variation of aniline compounds. However, the removal of COD was slightly different from that of aniline compounds removal after the sampling port 2 and the removal rate of COD further increased along the remaining distance of the outer cylinder. This suggested that some other organic intermediates, except aniline compounds, were removed from the water due to the higher concentration of DO. These findings indicated that the sleeve-type CW-MFC-CW system effectively created a favorable environment for the further degradation of these by-products of decolorization.

In this experiment, the COD arose from two sources: the soak solution and the AO7 (Table 1) and the total COD concentration was maintained at 300 ± 10 mg/L. Fig. 3 shows that the removal rate of COD decreased with the increase of AO7 concentration, which also meant that it increased with the increase of the proportion of the soak solution in COD. Fig. 6 presents the correlation analysis result between COD removal efficiency and the proportion of the soak solution and the result showed that the correlation ship of the two was a significantly positive linear correlation ($r^2 = 0.9508$, $p < 0.05$). It was found that the difference of COD removal efficiency between the soak solution proportion of 0.6 and 0.7 was no remarkable and both of the COD removal efficiency reached 80%. However, with the soak solution proportion decreased less than 0.6 (AO7 concentration: 100 mg/L), the COD removal efficiency decreased less than 70%. This indicated that when the total COD influent concentration was about 300 mg/L, the influent concentration of AO7 should not be higher than 100mg/L, which was similar to the result reported by Fang for the study of glucose as a carbon source [36].

The COD removal rate in this experiment was lower than the reported in previous studies, mainly because this experiment was conducted in winter and the microbial activity was lower in the cold season. In addition, it was reported that the plants in constructed wetland could increase the removal of azo dyes by approximately 25% [1]. However, in this experiment, the plants withered and played a less role in COD removal efficiency due to the seasonal limitation.

Table 1
COD concentration corresponding to AO7 concentration

AO7 concentration (mg/L)	COD concentration (mg/L)
10	22.1
50	63
70	87.9
100	120.9
120	145.3
150	179.7

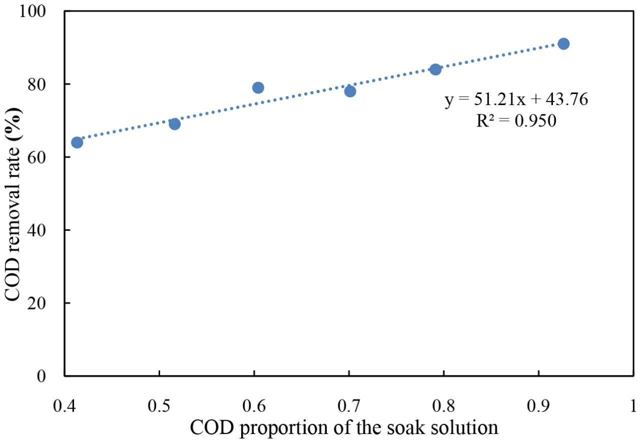

Fig. 6. COD removal efficiency at different COD proportions of the soak solution.

3.2. Electricity generation

In this study, the stable voltage and current output were 201 mV, 1.34 mA after the domestication period with glucose as carbon source. To investigate the effect of soak solution as carbon source on electricity generation, the voltage and current output with different AO7 concentrations were monitored during the whole experimental period.

As seen in Fig. 7, the voltage and current declined quickly to a few millivolts when the AO7 wastewater was added into the systems and the glucose was replaced by soak solution simultaneously. This was probably due to the toxicity of the AO7 and its intermediate by-products and the characteristics of the soak solution different from glucose. As described earlier (in Fig. 4), aniline compounds, reductive products of AO7, were detected in water and they were toxic to microorganisms [37,38]. Different from glucose, soak solution was a complex compound and was rich in cellulose, a kind of polysaccharide with high molecular weight, which was hard to be assimilated by electrogenic bacteria [39]. With the running of system, the electricity output began to rise slowly and continuously, which could be explained by the adaptability of the microorganisms to AO7 and the soak solution. The highest values of the voltage and current reached 190 mV and 1.13 mA, respectively, when the AO7 concentration was 150 mg/L. These values were very close to the level of glucose as the carbon source at the time of domestication period in this study and in other studies that used glucose as the carbon source [36]. This finding indicated that the soak solution had a certain electric effect when it was used as a carbon source.

It was interesting to note that the electricity output rose with the increase of AO7 concentration in this study, which was not consistent with the results of many previous studies [12,36]. We thought that mainly because AO7 and the soak solution were added to influent at the same time in this study. After the initial shock of these two substances,

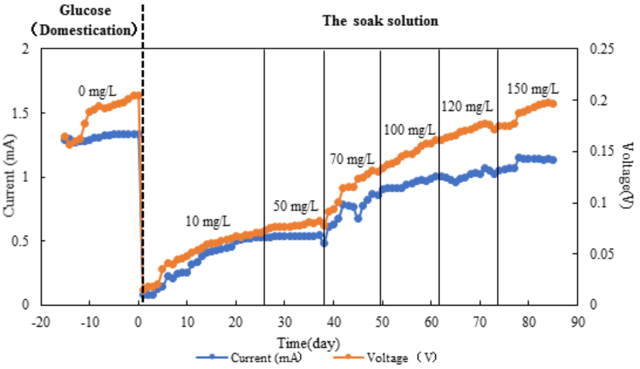

Fig. 7. Voltage and current output with different AO7 concentrations.

the electrogenic microorganisms began to adapt to this new environmental condition until they reached the stable state, which lead to the continuous rise of electricity generation. In addition, according to some studies, azo dyes or its decolorization products may become electron redox mediators, which decrease the electron transmission resistance of anode and then made the voltage output increase [40,41]. Therefore, the phenomenon of the electricity output rising with the increase of AO7 concentration in this study may be reasonable. And of course, more precise and detailed explanation need to be investigated in future study.

4. Conclusions

The novel CW-MFC-CW system with soak solution as carbon source presented a good performance of simultaneous decolorization of azo dye, COD removal and bioelectricity generation. The AO7 decolorization rate reached approximately 100% and COD removal efficiency exceeded 65% when the AO7 concentration varied between 10 and 150 mg/L, while the highest voltage and current output reached 0.19 V and 1.13 mA. The removal efficiency of AO7 and COD in outer cylinder increased from 0% and 11% to 14% and 26%, respectively with AO7 influent increase from 10 mg/L to 150 mg/L, which indicated the role of the outer cylinder was more important, especially for the higher concentration.

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