A new constructed wetland combined with microbial desalination cell and its application

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Abstract

Wastewater recycling can alleviate the shortage of water resources. Saline water is seldom treated with biological processes and the recycling utilization is low. Constructed wetland (CW) is a safe and economical ecological water treatment. However, the saline water treatment performance of CW is not good. Microbial desalination cell (MDC) utilizing the bioelectro-chemical principle achieves the functions of desalination and power generation. In this study, MDC was used to strengthen CW to form a composite system, MDC-CW, through optimizing design parameters. MDC-CW was applied in the treatment of salt-containing water. The average total nitrogen removal rate in MDC-CW-P1 reached 87.33% and the average COD removal rate was 92.79%. The average desalination rate of MDC-CW-P1 was 55.78% and the average voltage of MDC-CW-P1 reached 0.40 mV. It was found that planting *canna indica* in the MDC-CW was conducive to the functions of desalination and power generation. The above results were also verified by the microbial testing results of gravels in substrate, plant rhizosphere and electrodes. In addition, the decontamination of the device mainly depended on the functions of the bacteria commonly used in water treatment, such as *Proteobacteria* and *Bacteroidetes*, whereas the generation of power depended on the function of *Geobacter*. Salt ions moved spontaneously to the cathode and anode under the premise of current generation, so that the desalination function was realized under the selective isolation function of exchange membranes. The device design and laboratory application of MDC-CW experimentally accomplished the electrochemical technology and enlarged the treatment scale of CW.

Highlights

- A device with purification, desalination and power generation functions was given.
- The remarkable performance of MDC-CW was obtained.
- *Geobacter* with power generation capability was enriched in the anode of MDC-CW.

Introduction

The shortage of water resources is widely concerned in the world. Freshwater resources on the land only account for 2.53% of the total water resources on the earth and it is difficult to reuse and recycle water resources because of serious water pollution. Therefore, more and more water treatment technologies are applied in the treatment and purification of wastewater in order to deal with the water resource storage [1]. The water resource shortage is still serious in some areas. Nowadays, the treatment, purification and recycling of salt-containing water resources are also concerned. The large quantity of salt-containing water on the earth is an important option to deal with the water resource storage [2]. However, due to the influences of salt ions, the common treatment processes are not applicable and conventional physical and chemical technologies may cause secondary water pollution [3]. The applications of green and pollution-free biological methods in the treatment of salt-containing water are largely limited for a long time because of the high content of salt ions and the strong salt stress on organisms.

Constructed wetland (CW), as an ecological, economical, recyclable, and self-restoring restoration technology, has been widely used in the treatment of various kinds of wastewater, such as domestic sewage, industrial sewage, municipal sewage, and surface wastewater [4]. CW mainly remove various pollutants by physical and chemical actions, such as the filtration and adsorption effects of substrate and precipitation effects among ions, as well as biological mechanisms of plant absorption and microorganisms [5]. The main mechanisms of CW are biological mechanisms, and its applications in the treatment of salt-containing wastewater are limited due to the salt stress. However, some bacteria with the salt tolerance and decontamination capability exist in coastal areas, near islands and in sewage sediments of factories of brine products [6]. Inoculating and domesticating these bacteria might contribute to the function enhancement of CW.

Microbial electrochemical technology is widely used in the treatment of sewage and wastewater. Microbial fuel cell technology is a relatively mature electrochemical technology with the recycling performance of water resource and can effectively remove the pollutants and generate power [7]. In order to improve the decontamination efficiency of CW, constructed wetlands were integrated with coupling microbial fuel cell. Microbial desalination cell (MDC) is a variant of microbial fuel cell. In addition to the functions of decontamination and power generation, the desalination function was further realized [8]. Firstly, combing MDC with CW effectively relieved the stress of salt ions on microorganisms and plants in CWs and largely improved the operation efficiency of CW. Secondly, the presence of salt ions increased the conductivity of water and the power generation efficiency and further improved the effect of desalination. The MDC-CW improved the treatment and purification effects of saline water and expanded the application scope of CWs, thus providing a new method for the recycling and reuse of water resources.

In this study, the parameters of CW and MDC were respectively optimized and CW and MDC were established in the same structure as a combination system. After the inoculation and domestication of salt-tolerant decontamination bacteria, the MDC-CW system was launched and then entered the normal operation stage. By adjusting the operation parameters, the MDC-CW system realized the functions of salt water purification, desalination, and power generation.

Materials And Methods

Set-up of MDC-CW in laboratory

A column (with a length of 1.2 m and a diameter of 0.3 m) was established in the laboratory. Quartz sand was added in the column as the substrate in three layers (the 50-cm thick upper layer of quartz sand with the particle diameter of 4 mm ~ 6 mm; the 50-cm thick middle layer of quartz sand with the particle diameter of 2 mm ~ 3 mm; the 10-cm thick bottom layer of quartz sand with the particle diameter of 4 mm ~ 6 mm). Electrodes (graphite felt, 0.15 m × 0.15 m) were put in the column at the middle between the middle and upper layers. The vertical distance from the anode to the bottom of the column is 35 cm and the vertical distance from the cathode to the bottom of the column is 85 cm. The vertical distance between the anode and cathode is 50 cm.

A circuit was set to connect the external resistance with the voltage collector in order to monitor the power generation efficiency of the column device in real time. The ion exchange membrane (HACH USA, 0.15 m × 0.15 m) was put between the two electrodes. The anion exchange membrane (AEM) was close to the cathode, which was 61 cm away from the bottom of the column. The cation exchange membrane (CEM) was close to the anode, which was 48 cm away from the bottom of the column. The vertical distance between the two ion exchange membranes was 24 cm. Plants (*canna indica* and *Acorus calamus*) were planted in the column in such a way that the roots were arranged at a position close to the cathode as possible.

Water inlet was set on one side at the height 115 cm above the bottom of the column. Water outlet was set on the other side at the height 5 cm above the bottom of the column. Five sampling ports were set at the horizontal positions of anode, cathode, AEM and CEM, as well as the junction between the upper layer and the middle matrix. The device is shown in Fig. 1.

Six devices were established. Three devices with *canna* were set and named MDC-CW-P1 and the other three devices with *calamus* was named MDC-CW-P2. In addition, six vertical CWs with the same size were established in triplicate and named CK-CW-P1 and CK-CW-P2.

System operation

Environmental conditions were described below. The experimental period was from April to August, 2019. The average temperature was 20 °C ~ 40 °C and the relative humidity was 60% ~ 95%. The experiments were launched at Shanghai Ocean University.

Microbial inoculation acclimation was performed as follows. The inoculum of the device was the mixture taken from the natural water body along the Dongtan Coast in Shanghai, the sludge of treatment for sea foods breeding and wastewater of brine products. The inoculum was added into the devices according to the ratio of 4:1 (v/v, inoculum/tap water) for 3 times within 60 days.

According to Pollutant Discharge Standard of Municipal Wastewater Treatment (GB18918-2016), the simulated sewage was prepared with 500 mg/L COD (glucose), TN 60 mg/L (30 mg/L NH_4^+-N and 30 mg/L NO_3^--N) in tap water and stored in a storage tank (200 L). The salinity of sewage was 5 g/L (sodium chloride) [9]. Simulated sewage was simultaneously pumped into each experimental device.

After the inoculation period, the influent and effluent were continuously sampled for the determination of water quality indicators and the devices were discharged once every 7 days within 70 days.

Sampling and testing

Water samples

Influent and effluent were collected from the sampling port of each device in triplicate and stored into 50-mL bottles. After sampling, the physical and chemical indicators (temperature, oxidation reduction potential (ORP), dissolved oxygen (DO) and pH) were tested immediately with multiple parameter instrument (HACH, USA). Water samples were filtered (0.22-μm filter membrane) and then COD, TN, NH_4^+-N, NO_3^--N and NO_2^--N concentrations were determined according to the Standard Determination Method of Water and Wastewater by America health and Public Association (AHPA) in 2012. The performance of power generation was monitored by a data collector. The salinity was also determined with tap water as correcting samples. The desalination performance was calculated with salt removal rate.

Microbial samples

A total of 20 g of quartz sand samples were collected at the bottom of each device. Plant roots in triplicate devices were harvested and mixed. The electrodes in triplicate devices were collected as well. The surface biofilm of quartz sand, roots and electrodes were exfoliated into the solution by ultrasonic vibration. Then 0.22-μm filtration membrane was used to filter the microbial solution and the microbial DNA information on the membrane was extracted, amplified by PCR, and then measured. Operational taxa unit (OTU) was used to express the microbial abundances.

Electrode and membrane samples

The electrode and ion exchange membrane were treated for the observation under a scanning electron microscope (SEM). The electrode sample was placed in a glutaraldehyde solution (2.5%, pH 6.8) and allowed to stand for 12 h at 4 °C. Then, the samples were washed for 3 times (15 min each time) with PBS buffer solution (0.1 M, pH 6). Dehydration was performed for 15 min with 25%, 50%, 70%, 85%, 95% and 100% ethanol, respectively. The samples were treated with the mixture of 100% ethanol and isoamyl acetate (v/v, 1:1) for 30 min and then displaced with pure isoamyl acetate for 2 h. Finally, the samples were dried in a freeze-dryer.

Data analysis

The data of water quality samples measured in the experiments were analyzed in SPSS and Prism (ANOVA test with $P \leq 0.05$). The experimental data were the mean values and the error bars were plotted with the water quality data from repeated experiments.

Polarization curve and power density curves were plotted according to the following steps. Firstly, the MDC-CW devices were set as the open circuit voltage mode, so that open circuit potential (OCV) was obtained after stabilization. The resistor was then plugged in and the resistance was incrementally increased from 5 to 10000 Ω (each resistance value was maintained for 30 min). Then, current (*I*) was calculated as.

$$I(A) = \frac{U}{R} \quad (1)$$

where *U* indicates voltage (*v*) and *R* indicates resistance (Ω).

Finally, the polarization curve was obtained with the values of voltage and current and the linear part of the curve was fitted. The linear slope represents the total internal resistance of the MDC-CW devices.

$$P \ \ (\mathrm{w/m^3}) = \frac{U \times I}{V} = \frac{U^2}{R \times V} \quad (2)$$

where *P* indicates the power density; *V* indicates the effective working area of electrode (m^3).

Results And Discussion

General performance of MDC-CWs

Pollutant removal performance

During the experimental period, the DO, pH and ORP in influent were respectively 7.21 mg/L ~ 7.87 mg/L, 7.07 ~ 7.19 and 147 mV ~ 163 mV. The DO, pH and ORP in effluent were respectively 1.07 mg/L ~ 1.98 mg/L, 6.83 ~ 7.11 and − 114 mV ~ -93 mV.

The MDC-CWs operated well with the stable removal performance in 10 cycles. Within 7-day hydraulic retention time (HRT), the concentration of organic matters (COD) decreased continuously. Especially from Day 2 to Day 4 in the HRT, the decrease of COD concentration was significant, indicating that the pollutant removal performance of MDC-CWs was significantly improved in the period. Until Day 7 in the HRT, the optimal pollutant removal performance was realized and maintained stably. The trend of TN removal performance was consistent with that of COD removal performance. The COD and TN in effluent met the water quality criteria (Class 1A from DB31/199–2018). Among various MDC-CWs with different plants, the treatment with *canna* (P1) showed the higher removal performance, but the difference in the pollutant removal performance between MDC-CW-P1 and MDC-CW-P2 was not significant. The device in this study showed a better pollutant removal performance of saline wastewater under the salt stress.

Desalination

The SEM images of AEM and CEM in MDC-CWs are shown in Fig. 3. Particles and crystals were attached onto the surface of membranes, indicating that MDC-CWs had the desalination function.

During the experimental period, the desalination effect of MDC-CW devices was stable within 10 cycles (Fig. 3(a)). At the beginning of the experiment, the enhancement effect of MDC on the desalination performance of CW was not significant. In the late experimental period, the desalting performance of MDC-CW gradually increased due to the action of current and peaked on Day 5 in the HRT and then tended to be stable. The average desalination performances of MDC-CW-P1 and MDC-CW-P2 in 10 repeated cycles were respectively 55.78% and 52.58%. The average desalination performances of CK-CW-P1 and CK-CW-P2 were respectively 17.56% and 19.36%. The salinity values in effluent from MDC-CW-P1 and MDC-CW-P2 were respectively 2.211 g/L and 2.371 g/L. The desalination efficiency of MDC-CW with *canna* was slightly higher than that of MDC-CW with *calamus*, but the difference was not significant.

CW has a certain interception and adsorption effect on salt ions in saline water, but the effect was not obvious. CW treatment was enhanced by MDC. With ion exchange membrane and electrode added in the CW system, cations and anions in saline wastewater spontaneously moved to both electrodes due to the existence of current in the device [10]. AEM and CEM acted as an interception medium to effectively separate cations and anions and finally realized the desalination function [7].

Power generation

The power generation capacity of MDC-CW is one of the indicators to measure the start-up and operation performances of the device. The generation of a current could not only realize the synchronous removal of various pollutants in the system, but also promote the desalination performance of the device [11]. Microorganisms were successfully attached onto the electrodes of the MDC-CW device and the attachment of the biofilm played an important role in the operation of the system (Fig. 4).

The polarization curve and power density curve are shown in Figs. 5(a) and 5(b). According to the linear fitting result of the polarization curve, the internal resistances of MDC-CW-P1 and MDC-CW-P2 were 574.38 Ω (R^2 = 0.9551) and 536.37 Ω (R^2 = 0.9503), respectively.

During the experimental period with 10 repeated cycles, the power generation performance of the MDC-CW device was stable. At the beginning, the power generation was low. Then, the power generation performance was gradually increased on Day 3 in HRT and peaked on Day 5 in HRT. The average voltage of MDC-CW-P1 was 0.40 mV (ranged from 0.33 mV ~ 0.45 mV) on Day 5 in HRT, whereas the average voltage of MDC-CW-P2 was 0.39 mV (ranged from 0.34 mV ~ 0.45 mV) on Day 5 in HRT. The voltage peak was basically consistent with the peaks of COD removal and desalination performance. MDC-CW-P1 had the slightly higher power generation performance than MDC-CW-P2, but the difference was not significant.

It was believed that the consumption of COD not only contributed to the denitrification, but also served as the substrate of MDC to provide the electron donor for electricity-producing bacteria [12]. When the device related to external circuit, electrons moved from anode to cathode, thus achieving power generation. When the potential difference between anode and cathode in the MDC-CW device was the highest, the desalination performance of the system was the highest because the existence of potential difference between anode and cathode led to the uneven distribution of redox potential gradient in the system. In this way, cations and anions moved spontaneously through the AEM and CEM to anode and cathode and finally the desalination function of the MDC-CW system was achieved.

Microbial conditions

Gravels

In total, 779,041 high-quality sequences were extracted with the fragments of V4 & V5 standard regions as the primers. Microbial diversity analysis results are provided in Table 1.

Table 1
Microbial diversity indices in all CW treatments.

Treatments	Ace	Chao	Coverage	Shannon	Simpson	Sobs
Gravels						
CK-CW-P1	2868.128	2942.666	0.979	5.711	0.014	2170
CK-CW-P2	2961.125	2938.408	0.969	5.626	0.027	2070
MDC-CW-P1	3117.430	3054.262	0.974	6.148	0.028	2330
MDC-CW-P2	3349.760	3313.881	0.981	6.235	0.027	2610
Electrodes						
MDC-CW-P1-Cathode	2937.818	2934.617	0.979	5.582	0.016	2092
MDC-CW-P1-Anode	3225.344	3256.118	0.978	6.012	0.011	2475
MDC-CW-P2-Cathnod	3009.287	2946.455	0.977	5.947	0.017	2291
MDC-CW-P2-Anode	3373.066	3358	0.980	6.252	0.016	2602
Plant roots						
CK-CW-P1	2320.695	2377.631	0.984	5.580	0.009	1758
CK-CW-P2	2373.638	2410.213	0.985	5.895	0.009	1873
MDC-CW-P1	2477.869	2453.155	0.980	5.590	0.015	1478
MDC-CW-P2	2422.064	2688.096	0.984	5.895	0.015	1509

The microbial diversity indices of MDC-CW devices were higher than those of CK-CW devices, indicating that the microbial diversity in CW was significantly increased by MDC. The evenness (Simpson index) of microbial distribution in MDC-CW was better than that in CK-CW as well.

The microbial community distributions at the phylum level and genus level are provided in Fig. 6. The microorganisms in MDC-CW-P1 device were in good conditions. The abundances of *Proteobacteria* and

Bacteroidetes reached 60%, displaying the good enrichment effect. The enrichment of abundant functional strains was the main reason for the better wastewater treatment performance of the MDC-CW-P1. In the MDC-CW-P2 device, the total abundance of *Proteobacteria* and *Bacteroidetes* was less than 50%. The distributions of microbial community in CK-CW-P1 and CK-CW-P2 were similar, suggesting that P1 plants could promote the enrichment of functional microbes in MDC-CW. In the treatments with P2 (MDC-CW-P2 and CK-CW-P2), the abundance of *Firmicutes* was relatively high, suggesting the promotion effect between plants (P2) and bacteria (*Trichococcus* at the genus level). In addition, *Trichococcus* (genus level) belonging to *Firmicutes* (phylum level) is chemotrophic bacteria, which could over-absorb and store the energy to resist the extreme environment. The treatment with P2 was conducive to the enrichment of *Trichococcus* and further helped the system to resist the external stress. However, the symbiosis between *Trichococcus* and other functional bacteria (such as *Proteobacteria* and *Bacteroidetes*) was not good. Therefore, it was believed that the enrichment of *Trichococcus* could improve the adaptability of the treatment to the environment other than the wastewater treatment performance of the device.

According to the function prediction results of microorganisms (Fig. 7), the function distributions of microorganisms in all the devices were similar and the abundance of microorganisms with the functions including the transport and metabolism of amino acids was high. Amino acid transport and metabolism is the basic function responsible for the biological mechanism in CW treatments. Carbon and nitrogen cycles can be realized by the conversion and metabolism of amino acids. Therefore, this result could be considered as an evidence of the improvement effect of MDC on the CW performance. It is a meaningful attempt to utilize the basic function of biological treatment in MDC-CW device.

Electrodes

The microbes in the electrodes of MDC-CW treatments were extracted and analyzed. The microbial diversity results are shown in Table 1. The values of microbial diversity indices in MDC-CW-P1 were higher than those in MDC-CW-P2. The difference may be ascribed to plants [13], indicating that the better synergistic relationship between plants and bacteria in MDC-CW-P2.

The distribution of microflora in the electrodes of MDC-CW devices is shown in Fig. 8. The functional bacteria with the highest abundance (about 55%) enriched in the anode were *Proteobacteria* and *Bacteroidetes*, which were functional bacteria for wastewater treatment. The enrichment of these bacteria promoted the removal performance of nitrogen and organic matters in MDC-CW treatment. On the cathode of MDC-CW-P1, the abundance of microorganisms was not as high as that on the cathode of MDC-CW-P2. It was believed that in the nitrification zone of the cathode on the upper part of MDC-CW, more nitrogenous pollutants were degraded and transformed by microorganisms at other parts of the device, rather than microorganisms on the electrodes. However, the power generation performance of MDC-CW-P1 did not decrease, suggesting that electron acceptors such as oxygen existed in the nitrification zone of the cathode and realized the electron circulation. As indicated in the distribution of microbial community at the genus level, the abundance of *Geobater* in MDC-CW-P1 was high. *Geobater* is a kind of bacteria with power generation function [14]. The enrichment of *Geobater* was obvious, especially on the anode of MDC-CW devices.

The abundance of *Geobacter* on the cathode of MDC-CW-P2 device was significantly higher than that on the cathode of MDC-CW-P1 device, whereas the abundance of *Geobacter* in the anode of the MDC-CW-P1 was significantly higher than that in the anode of MDC-CW-P2 (Fig. 9). In this study, the power generation status of MDC-CW-P1 was slightly higher than that of MDC-CW-P2 due to the enrichment of *Geobacter* in the anode of MDC-CW-P1 (Fig. 5). However, the difference in electricity generation efficiency was not obvious and the main reason might be the shortage of electronic accepter on the cathode. The different distributions of microbial community might be ascribed to the plant species in treatments [15]. *Canna* and *calamus* are both wetland plants, but they affected the accumulation of bacteria to different degrees. The study on the rhizosphere bacteria will be conducive to understanding the treatment performance, power production capacity and desalination capability of MDC-CW.

Plants

After collecting and testing plant rhizosphere microorganisms, the microbial diversity was obtained (Table 1). It was found that the microbial diversity indexes of MDC-CW devices were higher than those of the CK-CW devices. MDC could increase the diversity of microbial community in plant rhizosphere.

The microbial community distribution of plant rhizosphere is shown in Fig. 10. The abundances of *Proteobacteria* and *Bacteroidetes* in MDC-CW were higher than those in CK-CW. The total abundance of *Proteobacteria* and *Bacteroidetes* in MDC-CW-P1 was about 70% and the abundance of *Firmicutes* with the too high energy consumption ability was relatively low. Therefore, the MDC-CW-P1 was the optimal treatment in terms of the microbial community condition in plant rhizosphere. The results of microbial abundance and distribution in the plant rhizosphere at the genus level indicated that *Aquabacterium* with the nitrate nitrogen metabolism function was enriched in the MDC-CW-P1. In the aerobic and anaerobic zones of the plant rhizosphere, the enrichment of *Aquabacterium* was conducive to alleviating the accumulation of nitrate nitrogen in the nitrification zone of CW [2].

Microbial function prediction results of plant rhizosphere were similar in all the CW devices (Fig. 11). The functions of carbohydrate transport and metabolism of plant rhizosphere microorganisms in the treatments with P2 (MDC-CW-P2 and CK-CW-P2) were slightly stronger than those of plant rhizosphere microorganisms in the treatments with P1 (MDC-CW-P1 and CK-CW-P1). The difference may be ascribed to microbial species [16], such as *Firmicutes* enrichment.

Conclusion

In this study, a new MDC-CW treatment device with multiple functions of decontamination, desalination and power generation was proposed. The functions of the device were successfully realized by optimizing the construction parameters of MDC-CW. The performances of decontamination, desalination and electricity production were remarkable. The pollutant removal performance of the device mainly depended on biological mechanisms. The reinforcement effect of MDC on CW could optimize the distribution of microbial community in the system and increase functional microorganisms. The enrichment of electrogenic bacteria was the cause for a current generated in the device. The generation of a current was the premise of promoting salt ion migration and desalination. MDC-CW is a new exploration of CW for saline water treatment.

Declarations

Competing of interests: The authors declare no competing of interests

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Figures

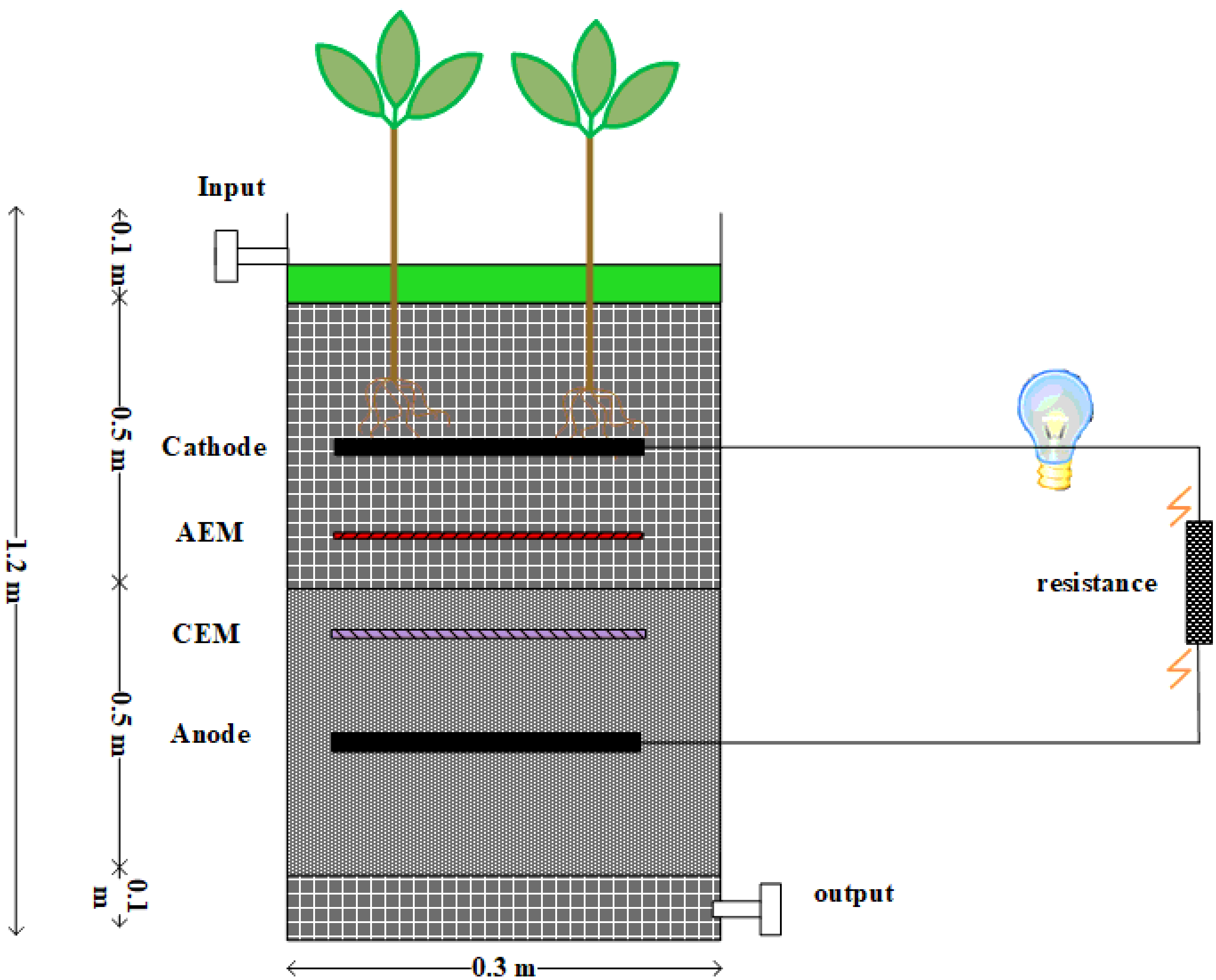

Figure 1

Sketch of MDC-CW.

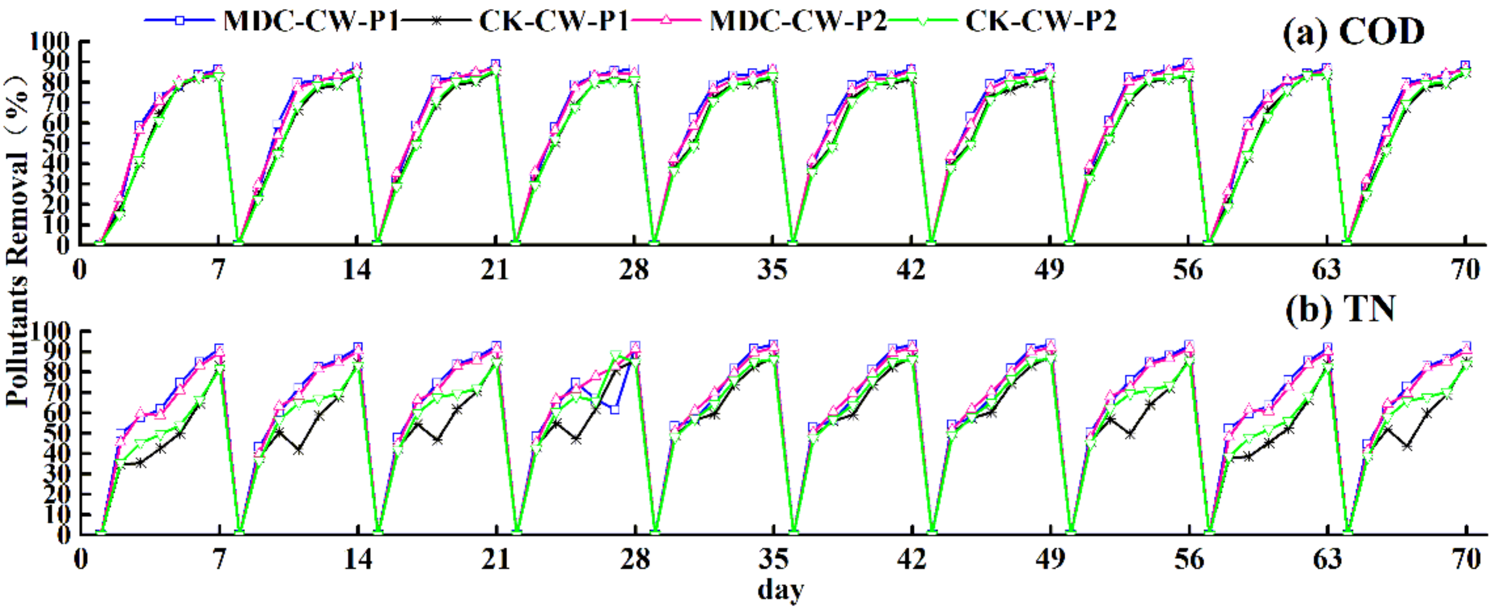

Figure 2

Pollutant removal performance of MDC-CWs.

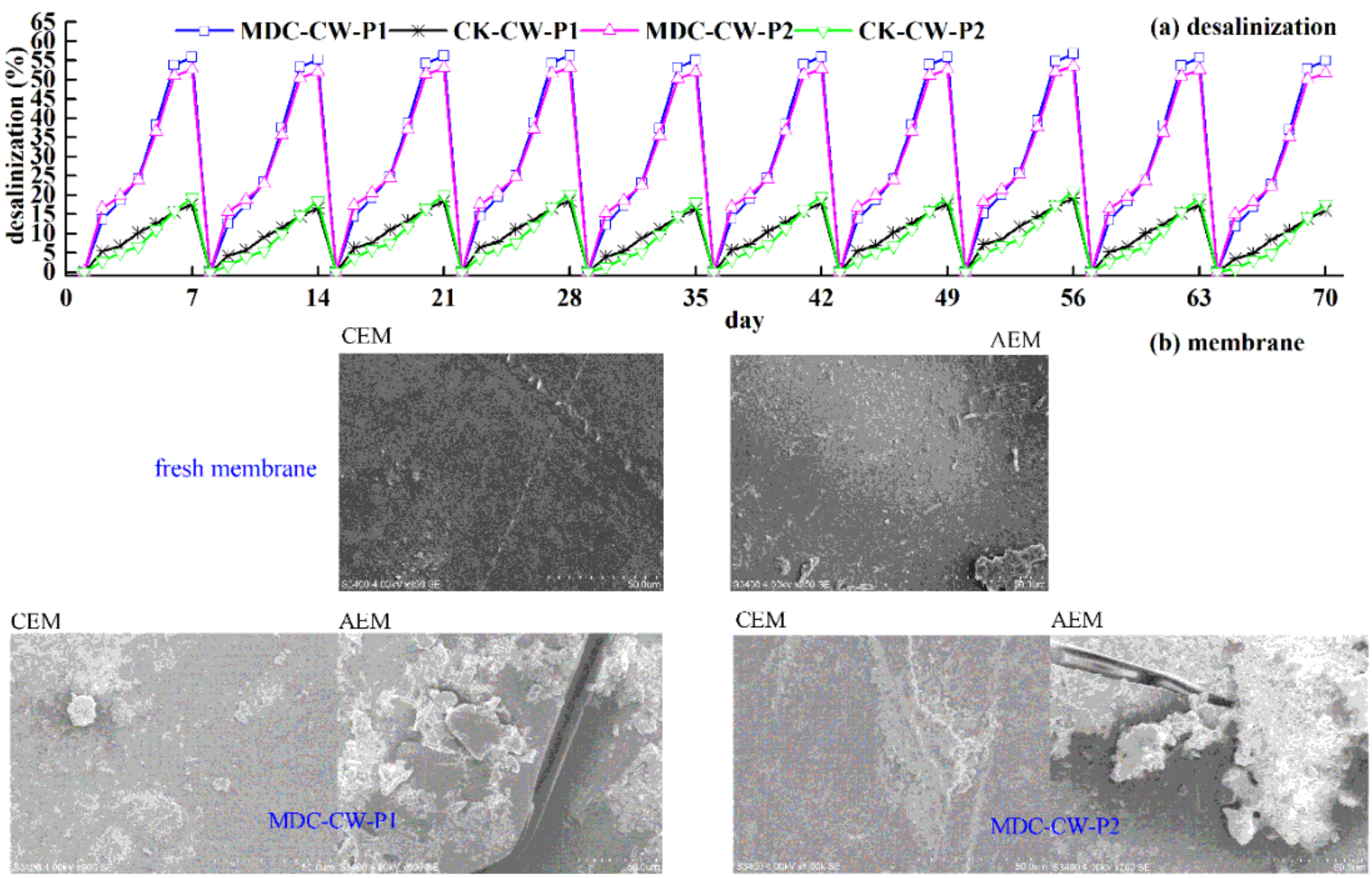

Figure 3

(a) Desalination of MDC-CW devices; (b) SEM of AEM and CEM in MDC-CW devices.

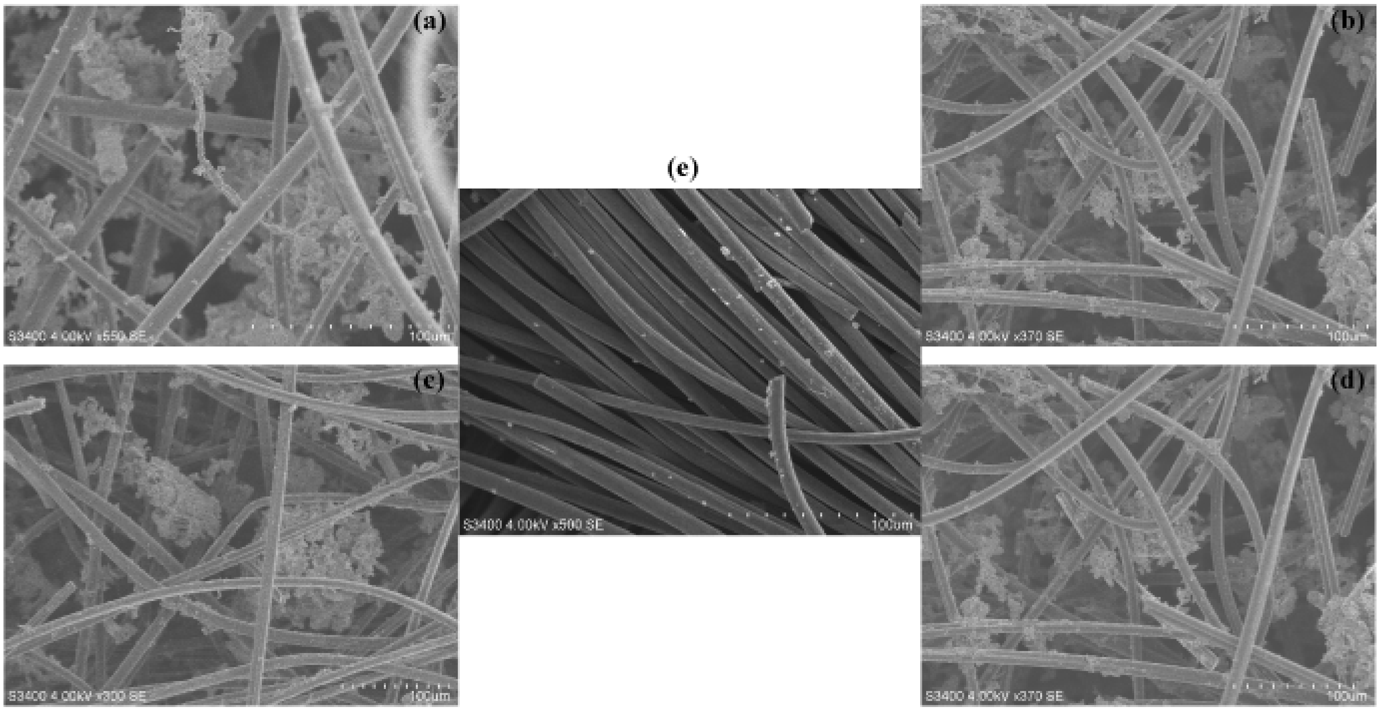

Figure 4

(a) Anode SEM image of MDC-CW-P1; (b) Cathode SEM image of MDC-CW-P1;(C) Anode SEM image of MDC-CW-P2;(d) Cathode SEM image of MDC-CW-P2; (e) SEM image of original electrode.

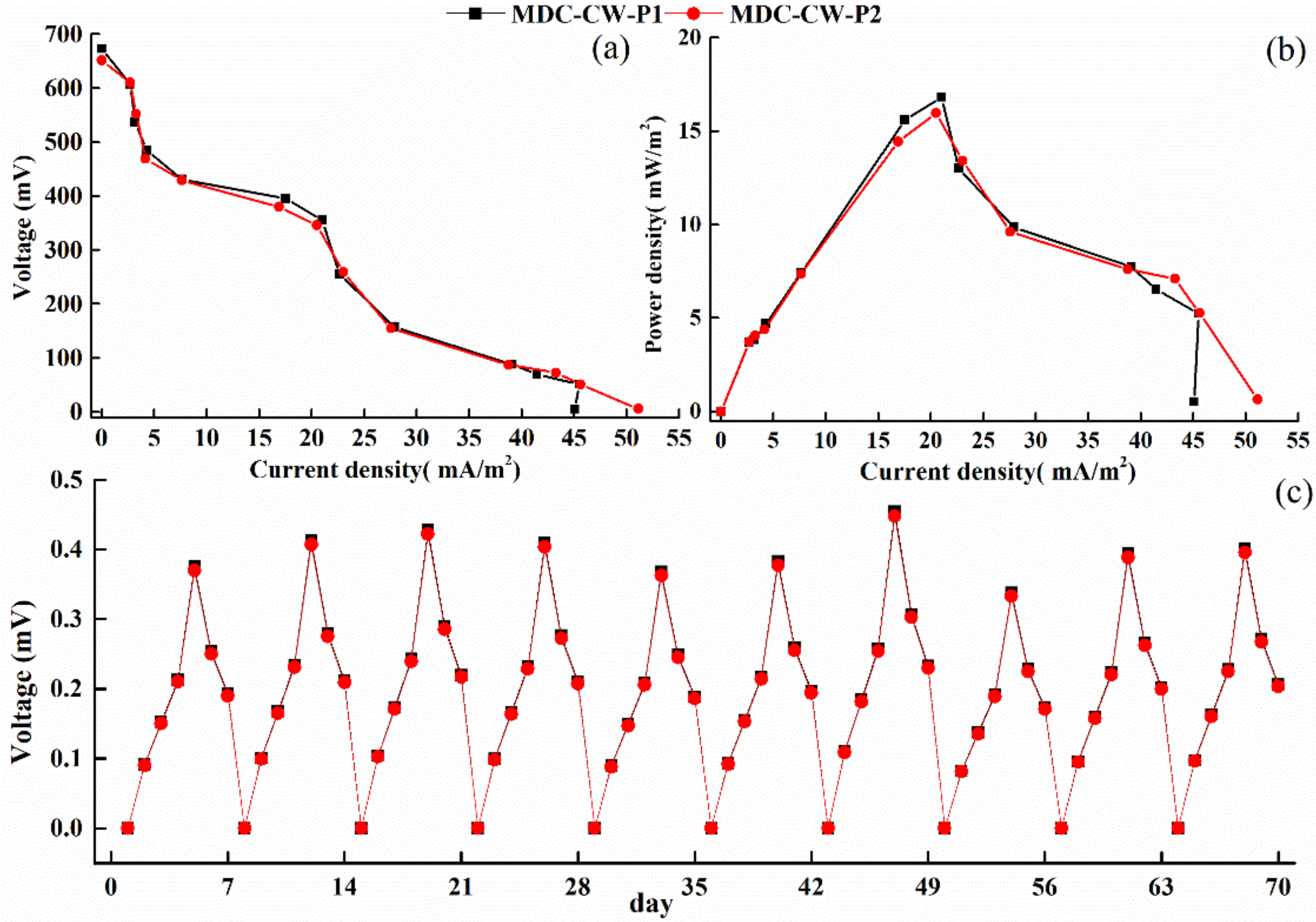

Figure 5

(a) Polarization curve, (b) Power density curves, and (c) voltages of MDC-CWs during the experimental period.

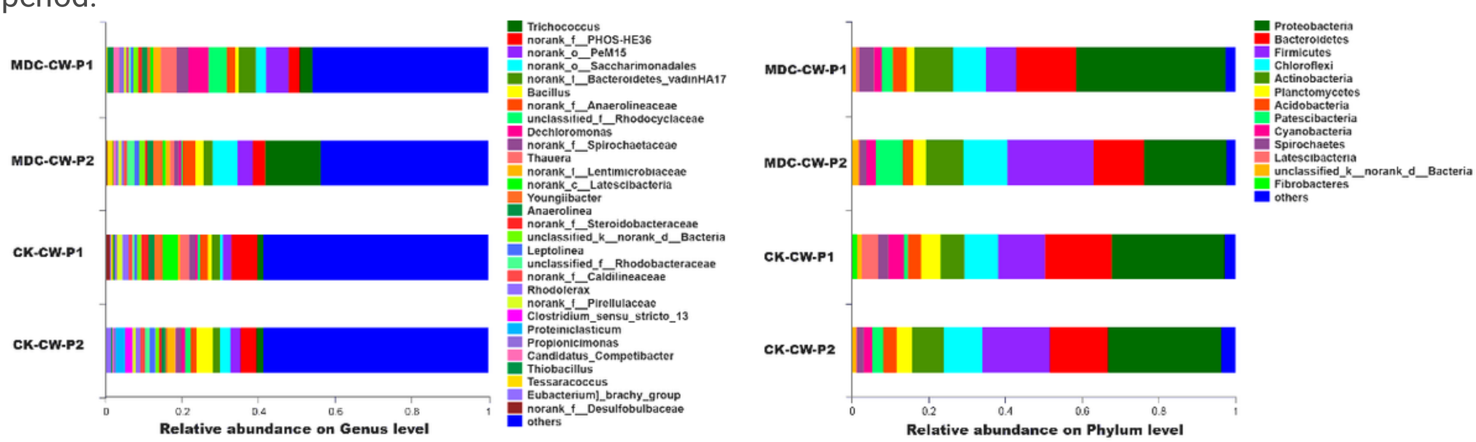

Figure 6

Relative abundances of microbial community in gravels of all CW treatments.

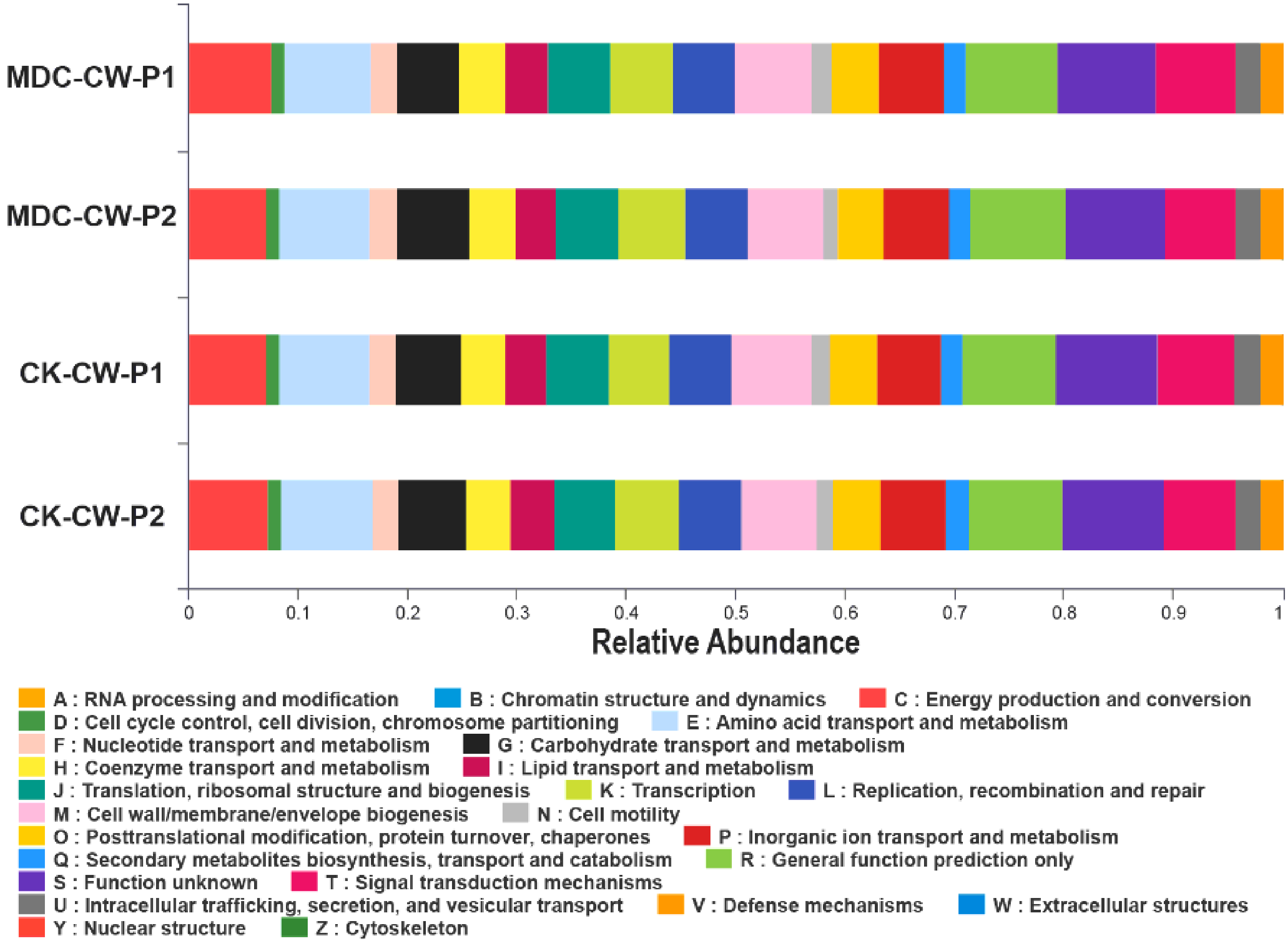

Figure 7

Prediction results of the microbial functions in gravels of all CW treatments.

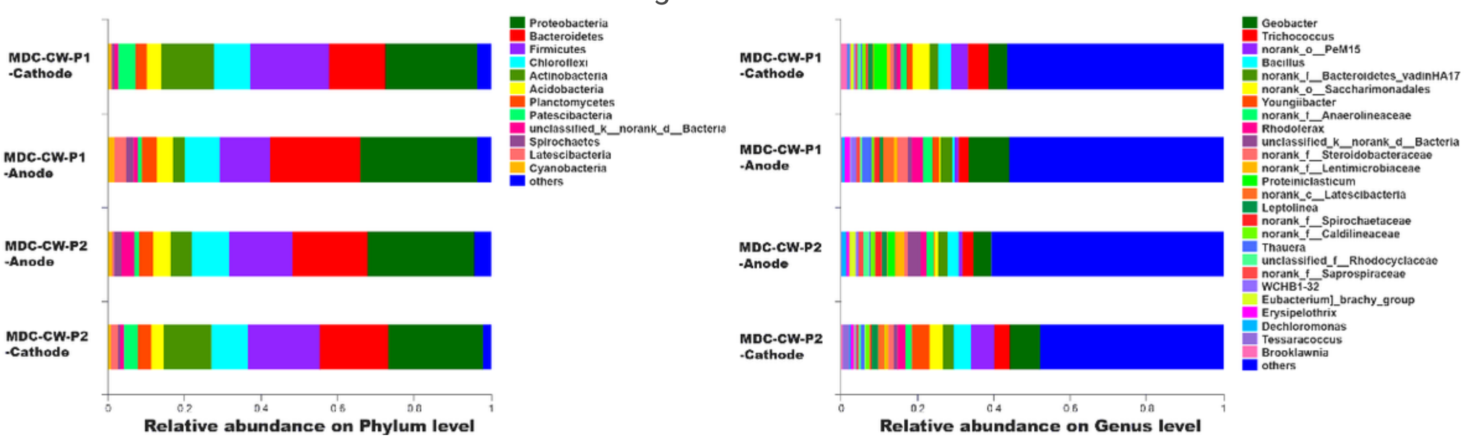

Figure 8

Relative abundances of microbial community in the electrodes of all CW treatments.

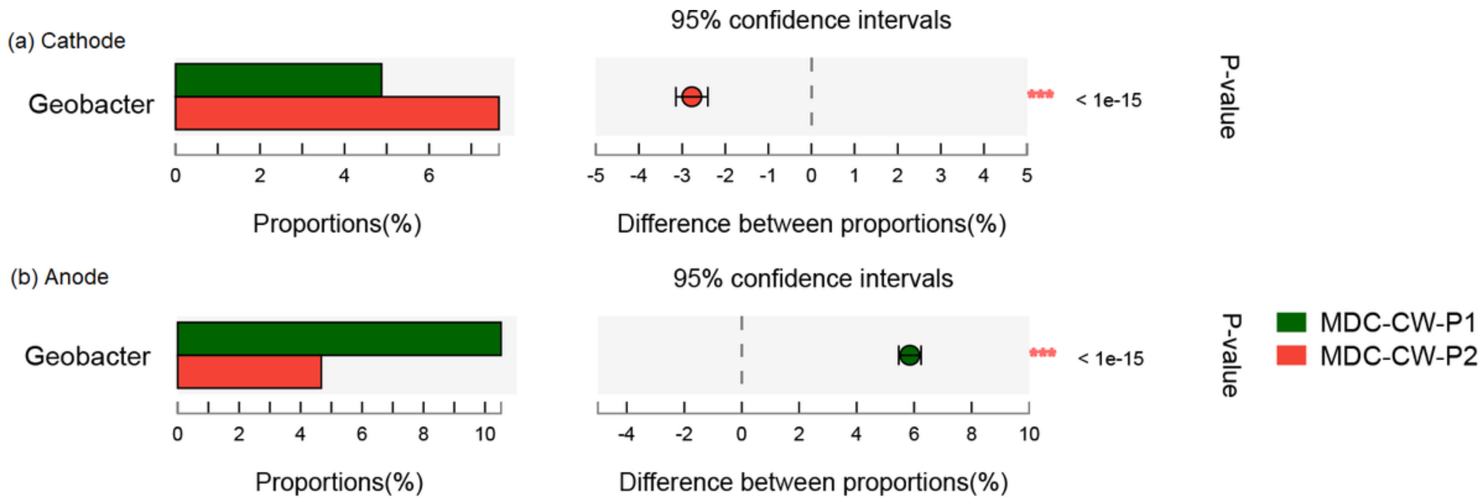

Figure 9

Significant difference analysis of specific electrogenic bacteria (Geobacter) in electrodes of MDC-CW treatments.

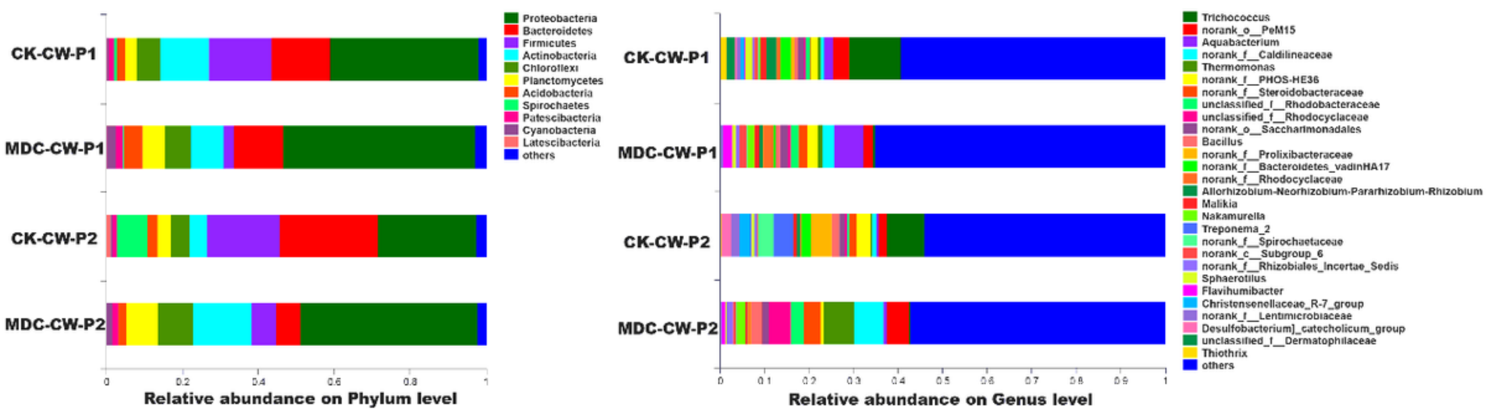

Figure 10

Relative abundances of microbial community in plant rhizosphere in all CW treatments.

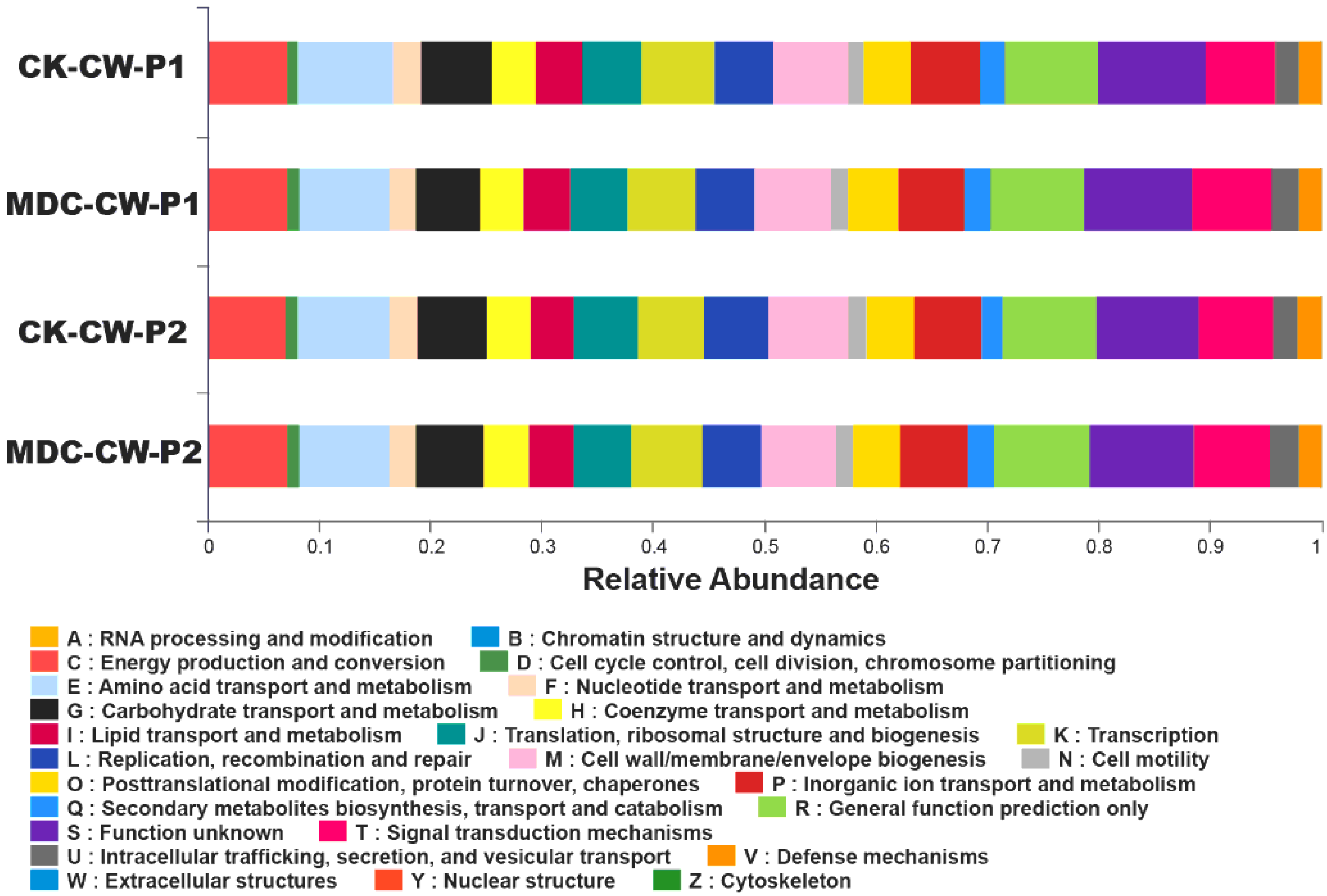

Figure 11

Prediction results of the microbial functions in plant rhizosphere in all CW treatments.