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Fate of sulfadiazine and its corresponding resistance genes in up-flow microbial fuel cell coupled constructed wetlands: Effects of circuit operation mode and hydraulic retention time

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HIGHLIGHTS

- Closed circuit operation UCW-MFC accumulated higher SDZ and ARGs in system.
- Low HRT operation UCW-MFC contributed to accumulate SDZ and ARGs in system.
- The shift of potential ARGs hosts may be the key factor influencing the fate of ARGs.
- Community richness and diversity was higher in closed circuit UCW-MFC.
- A high HRT contributed to high microbial diversity and electricity production.

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ABSTRACT

Few studies have evaluated sulfadiazine (SDZ) removal and the potential risks of antibiotic resistance gene (ARG) accumulation in up-flow microbial fuel cell coupled constructed wetlands (UCW-MFCs). This study aims to investigate the removal of SDZ, accumulation of ARGs (*sulI*, *sulII*, *intI*), and bacterial community changes in four UCW-MFC reactors with two hydraulic retention times (HRTs) and two circuit operation modes. The results indicated that effluent from a closed circuit UCW-MFC had a lower SDZ concentration due to a higher electrode absorption capacity for SDZ and the dehydrogenase activity of the bacteria. Additionally, the ARG abundance in closed circuit system was higher than that in open circuit system. A lower HRT was correlated with a higher SDZ concentration accumulation on the electrode, which led to a higher ARG abundance. Also, an obvious increase in the ARG abundance was observed during treatment periods. The relative abundance of target ARGs in the anode was higher than in the cathode and the bottom. A cluster analysis of bacterial communities at the phyla level showed they were more significantly affected by HRT. Furthermore, a closed circuit system and a high HRT contributed to high microbial diversity. In addition, five phyla of bacteria might be potential hosts for ARGs. Enrichment of ARG potential hosts induced by SDZ may be responsible for ARG abundance increase. Electricity production characteristics of the UCW-MFCs were also affected by HRT.

1. Introduction

Increased amounts of antibiotic disposal into the environment not only results in chemical pollution, but also accelerates the process of transference and spread of antibiotic resistance genes (ARGs) between pathogenic and nonpathogenic bacteria [1]. Sulfadiazine (SDZ) is a "high priority" sulfonamide antibiotic and is essential for the treatment of bacterial infections and to stimulate animal growth [2]. SDZ has

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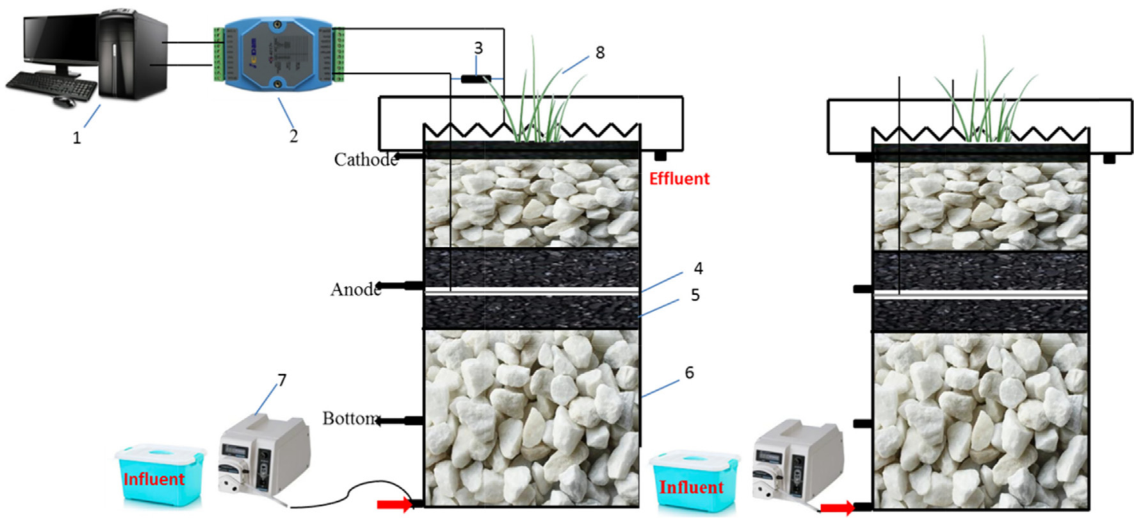

Fig. 1. Schematic diagram of the closed circuit mode UCW-MFC and open circuit mode UCW-MFC reactors (1 computer; 2 data acquisition module; 3 resistor; 4 stainless steel mesh; 5 GAC; 6 gravel; 7 peristaltic pump; 8 ipomoea aquatica).

frequently been detected in the natural environment, indicating it is difficult to eliminate [3]. The most common SDZ resistant genes found in gram-negative bacteria are *sulI* and *sulII* [4]. The *intI* is a particularly important genetic element that contributes to the spread of ARGs, and its presence and fate in the environment have been thoroughly researched [5]. Reducing SDZ disposal is essential for the reduction of toxic effects to the environment and to reduce the risks of accelerated ARGs multiplication. Therefore, cost-effective wastewater treatment technologies are required to reduce or eliminate SDZ pollution.

An up-flow microbial fuel cell-coupled constructed wetland (UCW-MFC) is an innovative ecosystem technology used to treat wastewater and for the simultaneous production of bioelectricity [6,7]. In a UCW-MFC, a significant dissolved oxygen concentration gradient develops vertically. Therefore, the anaerobic environment at the bottom and the aerobic environment at the top can be used as an anode and cathode, respectively [8]. Previous research has concluded that a UCW-MFC can be effectively used to degrade bio-refractory and toxic compounds due to a series of biological and physicochemical processes, including microbial degradation and sorption of the substrate [9,10]. However, few studies have assessed the removal potential of SDZ and the fate of *sul* genes and the *intI* gene that have accumulated in a closed circuit UCW-MFC compared with an open circuit UCW-MFC using different hydraulic retention times (HRTs). It is widely recognized that the anode of the closed circuit mode UCW-MFC acts as an electron acceptor, and thus the rate of anaerobic treatment can be accelerated [11]. However, it is not clear if UCW-MFCs operated in a closed circuit mode have a higher treatment efficiency of SDZ than in an open circuit mode (similar to an anaerobic reactor). Additionally, a previous study had demonstrated that the abundance of pure-culture *E. coli* and its corresponding ARGs were enhanced when the MFC was switched from an open circuit operation mode to a closed circuit mode [12]. This was attributed to the fact that electron acceptors may survive antibiotic resistance bacteria (ARB) and *sul* genes [12]. Thus the influence of a micro electric field on ARG accumulation in a closed circuit UCW-MFC is a major concern in the applications of UCW-MFCs. Potentially significant environmental risks may exist in the use of a UCW-MFC to treat SDZ wastewater. Therefore, differences in ARG abundances that have accumulated in different substrate layers between closed and open circuit operations UCW-MFC requires further exploration.

The performance of a UCW-MFC depends on the flow types, substrates types, HRT, and pollutant load. Different HRTs introduce different loads of SDZ into the system, and thus the removal efficiency of SDZ may be affected. Furthermore, the electricity generation performance can also be affected. Therefore, the effects of HRT on the performance of UCW-MFC during the SDZ wastewater treatment process requires further estimation.

It has been reported that microorganisms play important roles in SDZ removal in constructed wetlands (CWs) [13]. The presence of persistent SDZ in a CW may impose selection pressures on bacteria that capture ARGs on mobile gene elements [14]. It is important to figure out how the bacterial community in a UCW-MFC responds to the circuit operation mode and the HRT. Furthermore, the potential hosts of ARGs in a system requires investigation. To our knowledge, studies that investigate the influence of circuit operation mode and HRT on the abundance of SDZ ARGs and the overall bacterial community structure in UCW-MFCs have not been conducted.

In this study, four UCW-MFCs were constructed to treat SDZ wastewater. The aim of this study was to investigate (1) the effects of circuit operation mode and HRT on the removal efficiency of SDZ, SDZ accumulation on electrode, target *sul* genes and *intI* gene abundance, and to investigate microbial community changes in the system, (2) investigate potential hosts of ARGs based on correlations between the microbial community and ARGs, and (3) investigate the effects of HRT on the characteristics of electricity production. The results will assist researchers to understand whether the circuit mode and HRT can affect SDZ removal and ARGs abundance accumulations in UCW-MFC systems, and how this might change the bacterial community.

2. Materials and methods

2.1. Reactor design

UCW-MFC was constructed with diameters of 20 cm, and with four layers from the top to the bottom composed of a cathode layer (3 cm in thickness), a middle layer (6 cm in thickness), an anode layer (9 cm in thickness), and a bottom layer (20 cm in thickness). The main matrix of the anode and cathode consisted of granular activated carbon (GAC) (2–5 mm in diameter), and a stainless steel mesh was buried into the GAC to strengthen the electron transfer [15]. The bottom and middle layers were filled with gravel. In start-up period of UCW-MFCs, eight strains of ipomoea aquatic were planted in the cathode layer after they had been cultivated for 1 month at an ambient temperature of 28 °C. Three sampling points were selected vertically along the reactors to collect the corresponding samples: bottom layer, anode layer, and cathode layer (Fig. 1). The external resistor used to connect the anode and cathode was 1000 Ω. All experiments were operated continuously

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