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A novel microbial electrolysis cell (MEC) reactor for biological sulfate-rich wastewater treatment using intermittent supply of electric field

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ABSTRACT

Microbial electrolysis cells (MEC), coupled with sulfate-reducing bacteria (SRB), was used to degrade sulfate-rich wastewater that is deficient in electron donors. However, because of the impact of electric double layers (EDLs), salt crystals formed on the electrode, thereby potentially retarding the continuity of sulfate removal during the whole operation. Here, an improved MEC reactor using intermittent electric field was established. It works better in sulfate removal for a longer period, which was higher than the conventional MEC reactor by 2.18-fold after 10 days. Observation on the activity of lactic dehydrogenase (LDH) and ATP revealed that the formation of salt crystals on the electrode led to plasmatorrhexis. Conversely, improved reactor contributed to extracellular substances production and prevented the salt crystal formation, which was conducive to biofilm formation as further verified by detection through SEM. Electrochemical impedance spectroscopy tests showed that the cathodic microorganisms accelerated electron transfer whereas the salt crystals increased the charge transfer resistance. High-throughput sequencing analysis illustrated that improved reactor could maintain the competitiveness of SRB in the microbial community for a longer period. Moreover, the improved reactor resulted in high species diversity, thereby showing the significant resistance of the microorganisms to arduous environments.

1. Introduction

The discharge of large quantities of untreated sulfate-rich wastewater has been considered as a major source of environmental pollution [1]. Sulfate contamination can indirectly lead to several environmental effects, which include ecological instability, acid-mine drainage, corrosion problems and H_2S toxicity [2–5]. Therefore, it is essential to treat sulfate-rich wastewater prior to being released into the environment. Sulfate-contaminated wastewater is normally treated using physicochemical and biological methods. Biological sulfate reduction using sulfate-reducing bacteria (SRB) is advantageous due to its high efficiency as well as low capital and operation costs. However, sulfate-rich wastewater is usually deficient in electron donors [6], thus the process requires an external carbon and energy source, which may considerably increase its total expenses. Though UASB reactor performs very well via SRB in sulfate removal, it often requires high COD/SO_4^{2-}. It is reported that UASB reactor achieved sulfate removal efficiency of 89.2% ± 4.93% under the optimal COD/SO_4^{2-} of ratio 4.0. However, when the COD/SO_4^{2-} ratio was changed to 2.0, the sulfate removal efficiency rapidly decreased to 48.3% ± 6.95% [7]. Moreover, regarding groundwater treatment, direct contact of water with organic matter should be avoided to maintain drinking water biostability [8]. Hence, other means of resolving the issue concerning electron donor deficiency became a critical factor for maintaining stable operations of SRB systems.

Recently, bioelectrochemical systems (BESs), which catalyze electrochemical reactions by microorganism adherence on the working electrode [9,10], have rapidly caught the attention of scientific experts. Microbial electrolysis cell (MEC), an example of BESs, presents a wide range of applications in wastewater treatment and other energy recycling fields [11]. Hydrogen is a usual product of MEC system and is identified as an electron donor during sulfate reduction [6]; and compared to other electron donors,

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hydrogen is more attractive because its free energy of sulfate reduction is highly favorable over that of methanogenesis [12]. Furthermore, previous study has shown that many bacteria can use the electrode as electron donors. A pure culture of *Geobacter metallireducens* is reported to exhibit an ability to reduce nitrate to nitrite through the electrodes that serve as the sole electron donor using the expected stoichiometry of electron consumption [13]. Application of MEC in the removal of nitrogen [14], hydrogen [15], and methane production[16] has already been reported. MEC can provide extra electron donors for the bacteria in the biochemical system. Thus, MEC offers a new solution for sulfate-rich wastewater treatment that is deficient in electron donors. Previous studies on the role of MEC in contamination degradation mainly focused on aspects such as, the reactor type, electrode type, reaction conditions, and so on [14–16]. In practice application, continuous operation performance of MEC reactor is essential to industry treatment, in this study we aim to prolong the performance of sulfate removal in MEC reactor. Recently, the reduction of sulfate as an electron acceptor at bacteria-catalyzed cathodes without electron donor was reported [17]. However, this research only exhibits the impact of applied voltage, but profound study on the mechanism has not been revealed yet. Moreover, this study has shown that applied voltage could also exert an inhibitory effect on the microorganism, which may impede the continuous operation of MEC, but the reason for this phenomenon has not been demonstrated. In this study, we tried to couple MEC with SRB to achieve an efficient sulfate removal method and find out the optimal operation condition for continuous operation. Several bio-electrochemical experiments were conducted to evaluate the electrochemical activities of biocathodes, optimize the sulfate reduction process, and reveal the principal mechanisms in electron transfer. Lactic dehydrogenase (LDH), ATP activity, and the variation in extracellular secretion were explored to elucidate the effect mechanism of MEC. Scanning electron microscope (SEM) was utilized to visualize biofilm morphology. The electrochemical performance of the biocathodes was assessed using electrochemical impedance spectroscopy (EIS). Subsequently, 454 GS-FLX pyrosequencing technology was used in the MEC system to investigate the changes in the bacterial community and its composition. In this study, efforts focused on exploring an optimum operation condition that can improve MEC reactor's continuous operation and mechanism that is responsible for sulfate removal in organic deficient wastewater.

2. Material and methods

2.1. Experimental set-up

The reactor design and operation was detailed in the previous study [18]. Graphite rod was used as electrode in the reactor. The specific surface area of all the electrodes were the same, so we just used current briefly in this study instead of current density. Cathodes were inoculated 25 mL SRB suspension and filled with 245 mL basal medium. The concrete components of basal medium were shown before [18]. 1 mL/L of trace element (the concrete elements are shown in Table S1) was added to supply trace element. Ethanol was added afterwards to ensure that the ratio of [COD]/[SO_4^{2-}] in the reactor was 2. The anolyte in MEC contain 1 g/L of sodium acetate in a 100 mM bicarbonate buffer solution. Moreover, the headspace of reactors was flushed with N_2/CO_2 (80/20) to maintain an anaerobic condition. The reactors were incubated at 32 °C and operated in a batch mode. Each batch cycle lasted for 5 days. Five batches were carried out under each current. The electric operation conditions of different MEC reactors is shown in Fig. S1.

2.2. Analytical methods

The chemical oxygen demand (COD) was periodically qualified and analyzed twice according to standard methods [19]. Sulfate concentration in the aqueous phase was measured using an IC-5000$^+$ ion with an IonPac AS18 anion column after filtration with 0.22 μm pore size membranes. The working current of the suppressor was 100 mA. The crystal phase was characterized by X-ray diffraction (XRD). The morphologies and structures of the carbon electrode were examined by scanning electron microscopy (SEM).

LDH and ATP analytical methods are shown below [20]. Extracellular protein content was determined according to a BCA protein assay kit (Beyotime Institute of Biotechnology, China). The phenol/sulfuric acid method [21] was used to qualify the polysaccharide.

2.3. Electrochemical tests

Electrochemical impedance spectroscopy (EIS) was conducted in the same two-chamber MECs using an electrochemical analyzer (Autolab PGSTAT302N, Metrohm, Switzerland) to assess and compare the electrochemical activities of the biocathodes. All tests were conducted in the same two-chamber reactors used for starting up the biocathodes, with the cathode as the working electrode, a Pt mesh (10 mm × 10 mm) as the counter electrode, and an Ag/AgCl reference electrode placed in the cathode chamber. Each reactor chamber was filled with PBS (pH = 7). The frequency range was 100 kHz to 10 Hz with a sinusoidal perturbation of 5 mV amplitude. Charge transfer resistances were obtained by fitting spectra to an equivalent circuit (Fig. S2). Charge transfer elements were used here to calculate the total charge transfer resistance ($R_{ct} = R_{ct1} + R_{ct2}$).

2.4. Bacterial 16S rDNA amplification and high-throughput sequencing

The samples were collected from reactors. After extraction, the corresponding bacteria of all bands were sequenced by Majorbio Biotech Co., Ltd (Shanghai, China). A 194bp product spanning the V3 region of 16S rDNA was amplified from nucleic acid samples by polymerase chain reaction (PCR) and using the primer pair 338F (5′CCTACGGGAGGCAGCAG-3′) and 518R (5′-ATTACCGCGGCTGCTGG-3′) [22]. Nucleotide sequences were compared and classified by using BLAST program on the NCBI website. Phylogenetic analysis was conducted by the neighbor-joining method using MEGA [23].

3. Results and discussion

3.1. MEC operation and performance in short period

In our previous study [18], we have studied the relationship between current intensity with MEC reactor performance in 5 days' operation time. The outcome has proved high current resulted in low COD removal. The COD represented the added organic matter, so this change proved to be more economical in terms of cost. However, the rapid decline of COD removal efficiency at 3.5 mA possibly indicated an inhibition in the anaerobic treatment process, as seen with the large-scale death of bacteria in the reactor. Sulfate removal efficiency reached its peak when the current applied was 1.5 mA. Sulfate removal was improved with the application of electric current, with the optimal value of 1.5 mA. Although less ethanol was consumed, sulfate removal efficiency was significantly higher [18]. The formation of biofilm can decrease the overpotential of the electrode, leading to smoother production of hydrogen [24]. Hydrogen is a common byproduct of the MEC system and is identified as an