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Conductive microbial cellulose as a novel biocathode for Cr (VI) bioreduction

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ABSTRACT

In the present study, microbial cellulose (MC) as a carbohydrate polymer was made conductive by oxidative polymerization with aniline. Sulfate-reducing bacteria (SRB) were immobilized on the surface of the conductive biopolymer, and this was used as a biocathode in a bioreduction process to reduce Cr (VI) as a model of heavy metals. The results of Fourier transform infrared analysis confirmed that the polyaniline was distributed on the cellulose surface. The maximum tensile stress of the conductive biopolymer was obtained 23 MPa using calculating Young's modulus. A current density of 60 mA/m^2 was determined as optimal, and an increase in pH from 5 to 7 significantly reduced the required time for reduction of Cr (VI). The system reached >99% removal of Cr (VI) within 1.5 h at pH 7. Kinetic experiment studies showed a high constant rate (mean K_{obs} 0.78, R^2 0.95). The results showed that the conductive MC can be used as an appropriate bioelectrode to reduce Cr (VI) in bioelectrochemical processes. It is expected that experimental results could be used as a reference for the utilization of MC in bioelectrochemical systems.

1. Introduction

Nowadays, MC as a carbohydrate biopolymer has gained particular attention, not only for its exclusive nature, but also for its wide applications. The unique characteristics make MC as an applicable carbohydrate biopolymer to use in medical and environmental applications (Rezaee, Pourtagi, Hossini, & loloi, 2015; Vasconcelos et al., 2017; Corsello et al., 2017). The characteristics of MC include high tensile strength, light weight, stability and durability, water holding capacity, and high stability in humidity (Rezaee, Godini, & Bakhtou, 2008; Rezaee, Godini, Dehestani et al., 2008). MC does not have inherent electrical conductivity. There is an increasing need to synthesize biocompatible fibers with excellent mechanical and electrical performance for electrochemical and biomedical applications (Chen, Yu et al., 2015; Chen, Zhang et al., 2015). There are some reports that cellulose can be conducted by some polymers which show a wide range of applications in many fields (Chen, Yu et al., 2015; Chen, Zhang et al., 2015). Polyaniline (PANI) is a conjugated polymer with some attractive properties such as high stability, high electrical conductivity, simple preparation and low cost of production. Also cellular conducting polymer polyaniline has been used in removal of environmental pollutants by many researches (Janaki, Oh, Vijayaraghavan, Kim, & Kim, 2012). Recently, much attention has been paid to bioelectrochemical processes for the reduction and removal of heavy metals and getting energy from bioelectrochemical systems (Wang and Ren, 2014). Bioelectrochemical systems use microorganisms to catalyze the oxidation-reduction reactions on an electrode. The use of bioelectrochemical systems in removing heavy metals such as copper, lead, and zinc has been reported with high efficiency (Tao et al., 2014). Sulfate-reducing bacteria (SRB) are anaerobic bacteria that are used in environmental biotechnology. However, use of these bacteria can be problematic as they are corrosive to some metals used in industrial functions (Muyzer and Stams, 2008). Another important application of these bacteria is reduction, precipitation, and removal of heavy metals in the biological systems (Wang and Ren, 2014). This type of precipitation has some advantages including the small amount of remaining sludge, less solubility of the produced sulfides at acidic pH, efficiency, and cost-effectiveness. This method can not only remove heavy and toxic metals, but also recover precious metals from the sulfide precipitations (Cohen, 2006; Viggi et al., 2010). Sulfate reducing bacteria are promising option for the reduction and recovery of metals and decontamination of the environment using bioelectrochemical processes. But due to the corrosion of metal electrodes,

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Table 1
Characteristics of virgin MC.

1.5	(mm) Thickness
170	(nm) Average diameter fibers
0.34 ± 0.02	(g) Weight

replacing new electrodes is needed (Zhang, Wang, & Zhang, 2012). Sulfate reducing bacteria do not use cellulose as a source of carbon and energy, thus, electrodes prepared with conductive MC are appropriate biocathode for bioelectrochemical processes. In this study, we presented a conductive film based on MC/PAni which was used as a biocathode in a bioelectrochemical system for the bioreduction of Cr (VI). Hence, MC/PAni with low electrical and good mechanical resistance was proposed as an electrode to utilize in bioelectrochemical systems.

2. Experimental

2.1. Production of microbial cellulose

The MC was synthesized by cultivating *Acetobacter xylinum* in Hestrin-Schramm (HS) medium according to the procedure described elsewhere (Rezaee, Godini, Dehestani et al., 2008). Briefly, the HS medium was made of 2% glucose, 0.5% peptone, 0.5% yeast extract, 0.27% disodium phosphate, 0.115% citric acid, and 2.5% agar. *Acetobacter xylinum* produced biofilm on the surface of the culture medium in static conditions. The properties of the raw cellulose are presented in Table 1. To treat the cellulose membranes, 2% sodium dodecyl sulfate and 5% NaOH were used in 80 °C water. Then, the cellulose membranes were rinsed with 1 M acetic acid and distilled water.

2.2. (MC/PAni)

Aniline and ammonium persulfate (APS) as initiators and hydrochloric acid as the doping factor, as well as other consuming materials, were purchased from Merck. Aniline was double distilled under a vacuum before use and kept under argon gas. The prepared MC was placed in a flask containing a mixture of 1 M HCl and isopropyl alcohol in which 2 ml aniline monomer was dissolved, and then it was gently stirred. Samples were polymerized by an aniline oxidative polymerization process (1:1 mol ratio) at 5 °C; four hours was allowed for polymerization (Hu, Chen, Yang, Liu, & Wang, 2011). After polymerization, a dark green layer of PAni was formed on the surface of the cellulose. To remove any remaining impurities, the samples were washed with distilled water. To neutralize the pH, the samples were washed in methanol, followed by ammonium solution (Namazi, Kabiri, & Entezami, 2002). The samples were dried in a 60 °C oven to reach a constant weight.

2.3. Cultivation of sulfate-reducing bacteria

The *Desulfovibrio desulfuricans* were isolated from mineral springs in Ramsar, Iran. In order to grow and enrich the bacteria, Postgate medium B containing microelements was used. If necessary, the pH of the culture medium was adjusted by 1 M NaOH.

2.4. Startup and operation of the bioelectrochemical reactor

The experiments were performed in a glass reactor with a height of 28 cm and volume of 2 l. The contents of the reactor were stirred by a magnetic stirrer at a speed of 100 rpm. The experiments were conducted at laboratory temperature (25 ± 2 °C). The biofilm was prepared as follow: The conductive MC/PAni was placed in a stainless steel mesh frame with the same dimension. The steel mesh was cleaned with acetone and distilled water before covering. The covered conductive MC/PAni was used as the cathode in the bioelectrochemical reactor with a diameter of 10 cm. The anode was stainless steel mesh with dimensions of 20 cm by 5 cm, and was placed in the central part of the biocathode. The bioreactor were inoculated with 200 ml of enriched of SRB and operated in batch mode. It was fed by Postgate medium B include lactate as a carbon source and sulfate as an electron acceptor for ten days. During this time, biofilm formed on the cathode. To adapt the SRB with the electric current, 1 mA of current was connected to the bioelectrochemical system. A DC power supply was used to apply direct current. The effect of Cr (VI) concentrations (10–200 mg/L), current density (10, 30, and 60 mA/m^2), and pH (5, 6, 7) on the performance of bioreactor were evaluated.

2.5. Analysis

The samples were analyzed according to standard methods for the Examination of Water and Wastewater (APHA, 2005). To investigate the effects of the interaction between the MC and polyaniline, the samples were analyzed by Fourier transform infrared spectrometry (FTIR; Thermonicolet, Nexus670). The electrical resistance of MC/PAni samples was measured by a 505 APPA digital multimeter using the two-point method (the distance between the two points was 10 cm). Two samples were prepared under the experimental conditions and the electrical resistance was measured at three different points on each surface. An average of 6 measurements was reported (Saravanan, He, & Yonghao, 2014). The mechanical resistance of MC/PAni samples was measured by compression testing and using SANTAM-STM-20 on samples of 10 mm thickness and width. Before carrying out the experiments, the samples were filtered through a 0.45 μm filter. To determine the concentration of Cr (VI), diphenylcarbazide was used at 540 nm. Stock chromium (VI) solution was prepared by dissolving $K_2Cr_2O_7$ in distilled water. The intensity of absorption of remaining Cr (VI) was measured by a UV–vis 9200 spectrophotometer, and the removal of chromium was calculated based on the Eq. (1):

$$\frac{C_i - C_e}{C_i} \times 100 \quad (1)$$

Where C_i and C_e are the initial and remaining concentration of Cr (VI). The pH and redox potential (ORP) were measured by a pH and ORP meter (Eutech, Singapore). To determine the concentration of total chromium in the wastewater and sludge of the bioreactor, inductively coupled plasma (ICP) analysis was done by the ICPS-7000 ver.2 instrument. Before the ICP test, 0.5 g of sediment from the reactor was accurately weighed. It was then digested in a mixture of concentrated hydrochloric and nitric acid for an hour. After reaching ambient temperature, the solution was centrifuged and then diluted to a final volume.

The morphology and chemical composition of the biofilm were investigated using emission scanning electron microscopy (SEM) coupled with energy dispersive spectroscopy (EDS). To fix the grown biofilm to the conductive MC/PAni surface, the specimens were immersed for 1 h in a 3% glutaraldehyde solution, dehydrated with successively five ethanol solutions (10 min each): 30%, 50%, 75%, 90%, and 100%, after which the specimens were air dried after immersion in HMDS (Sigma, USA) for 20 min and then gold coated.

3. Results and discussion

3.1. Characteristics of MC/PAni

The polymerization of aniline was done on a layer of wet MC. Aniline hydrochloride can penetrate throughout the internal MC network. Hydrogen bonding can occur between the cellulose