Microbial Fuel Cell Inoculated with *Ochrobactrum Tritici* KCC210 for Chromium (VI) Measurement in Electroplating Wastewater

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**Abstract.** Many methods/techniques have been developed for Cr(VI) measurement, but they are often conducted offsite or and cannot provide real-time for Cr(VI) monitoring. A microbial fuel cell (MFC) is a self-sustaining device and has great potential as a biosensor for in situ Cr(VI) measurement, especially for wastewater generated from different electroplating units. In this study, *Ochrobactrum tritici* KCC210, a facultatively anaerobic, Cr(VI)-reducing, and exoelectrogenic bacterium, was isolated and inoculated into the MFC to evaluate its feasibility as a Cr(VI) biosensor. The results indicated that *O. tritici* KCC210 exhibited high adaptability to pH, and temperature under anaerobic conditions. The maximum power density of the MFC biosensor was 17.5±0.9 mW/m² at 2,000 Ω. A good linear relationship was observed between the Cr(VI) concentration (10–80 mg/L) and voltage output. The stable performance of the MFC biosensor indicated its potential as a reliable biosensor system. Moreover, the developed MFC biosensor is a simple device that can accurately measure Cr(VI) concentrations in the actual electroplating wastewater generated from different electroplating units within 15 min with low deviations (–1.8% to 7.8%) in comparison with the values determined using standard method. Thus, the MFC biosensor can measure Cr(VI) concentrations in situ in the effluents and has potential as an early warning detection device.

1. **Introduction**

Chromium has been widely used in various industries, such as metal plating, wood preservation, tanning, and steel manufacturing, as well as in the military industry [1, 2]. Generally, chromium exists in water in two oxidation forms: hexavalent [Cr(VI)] and trivalent [Cr(III)] [3]. Cr(VI) is more toxic and mobile than Cr(III). Thus, Cr(VI) is considered toxic because it can cross cell membranes when the ambient pH exceeds 6 [4]. These toxic Cr(VI) compounds have been proved that cause health...
problems such as severe diarrhea, pulmonary congestions, and liver damage [5]. The U.S. Environmental Protection Agency (US EPA) identified Cr(VI) as one of the 17 chemicals posing the greatest threat to human health [6].

Removal of heavy metal treatment typically uses physical and chemical methods such as adsorption, precipitation, reverse osmosis, and ion exchange [7]. These methodologies exhibit high removal efficiency but may involve expensive equipment, and long treatment times [8]. The accidental release of industrial effluents without proper treatment would pose a serious threat to the environment during a long-term treatment process. Thus, the measurement of Cr(VI) concentrations in water or wastewater is crucial for environmental risk and health-related considerations.

Several chemical analysis methods, including ion chromatography (IC), atomic absorption spectrometry (AA), inductively coupled plasma mass spectroscopy (ICP-MS), and colorimetric methods, are currently being used for the measurement of chromium in water samples [9]. These techniques exhibit high accuracy and selectivity, however, they are usually complicated, time consuming, and expensive. Moreover, they often require sophisticated instrumentation and inadequate for use outside the laboratory. Hence, they have low applicability in routine measurements [10]. Recently, biosensors, such as amperometric enzyme-based sensors using cytochrome c3 or urease and cell-based sensors using bacteria or V79 cells, have been developed for Cr(VI) measurement [11]. They require less complex instrumentation and shorter measurement period times compared to classical methods. However, these methods are often limited to measuring very low Cr(VI) concentrations (e.g., μg/L). The dynamic ranges restrict their use to diluted samples only [9].

There are many scientific papers illustrate that pollutants can be removed from water bodies by aquatic plant, microalgae, and microorganisms [12-15]. A microbial fuel cell (MFC) is a device that uses plant, microalgae, or microorganisms as catalysts to generate electricity from organic fuels. Thus, it provides a potential approach for powering of electronic sensors or/and for the generation of renewable energy [16]. MFCs have been developed as powering electronic sensors to analyze pollutants (BOD, COD, toxins) and monitoring state variables for system control [17]. Thus, an MFC has great potential in measuring the Cr(VI) concentrations in water, if the appropriate bacteria are used. In this study, Ochrobactrum tritici KCC210, a facultatively anaerobic, Cr(VI)-reducing, and exoelectrogenic bacterium, was isolated from electroplating wastewater. It was inoculated into an MFC to evaluate its feasibility as a Cr(VI) biosensor. Crucial operating parameters were established to optimize the performance of the MFC. The relationship between the Cr(VI) concentration and voltage output was investigated. Cr(VI) concentrations in actual electroplating wastewater were in-situ measured using the MFC-based biosensor and ex-situ measured using standard colorimetric methods to illustrate the feasibility and accuracy of the self-sustaining device.

2. Materials and Methods

2.1. Bacterial Strains, Cultivation, and Identification

Sediments were collected from an electroplating wastewater treatment plant in Tainan County, Taiwan (22°47’N/120°15’E). The collected samples were centrifuged at 10,000g for 20 min and the obtained precipitates were inoculated in a 3 L working volume in a chemostat. Nutrient broth (Difco Co., USA) supplemented with Na₂Cr₂O₇ (99%, Sigma–Aldrich Co., USA), called NBCr medium, was continuously fed to the chemostat. The NBCr medium containing 30–120 mg/L of Cr(VI) was
progressively fed into the system to acclimate the Cr(VI)-reducing bacteria under the anaerobic conditions. After a 30-day acclimation period, KCC210 bacterium predominated in the chemostat and was selected for subsequent experiments. To identify the isolated KCC210 bacterium, cell lysis, DNA extraction, 16S rRNA gene amplification, and sequencing were performed as described previously [18]. The KCC210 bacterium was identified as *Ochrobactrum tritici* (98.2% similarity).

2.2. Factors Affecting Cr(VI) Removal by the *Ochrobactrum Tritici* KCC210

To evaluate the effects of different operating conditions on Cr(VI) removal by the KCC210 bacterium, different pH values (4–10) and culture temperatures (15–45 °C) were used in anaerobic conditions. In this study, the initial cell number of KCC210 bacterium was $3.8 \times 10^7$ cfu/mL and initial Cr(VI) concentration was 50 mg/L. The Cr(VI) removal efficiency were analyzed after 24-h cultivation periods. All the experiments were conducted at least in triplicate to evaluate the accuracy and reproducibility of the obtained results.

2.3. MFC Construction and Its Operation

The schematic of the dual-chambered MFC is presented in Figure 1. The MFC was comprised of a 6 cm × 6 cm × 6 cm acrylic cube. The anode and cathode compartments had working volumes of 62 mL each. Anode and cathode compartments were physically separated using a PEM (Nafion 117, DuPont Co., USA) with a surface area of 25 cm$^2$. Graphite felt (25 cm$^2$ surface area) was used for the electrodes and an OK line connected the electrodes through a variable resistor. NBCr medium (50 mg/L Cr(VI)) containing $3.8 \times 10^7$ cfu/mL of the KCC210 bacterium was continuously recycled in the anode compartment of the MFC with a 1,000 Ω resistor for cell enrichment at 5-day retention time under anaerobic conditions. The anode compartment was kept anoxic by purging with nitrogen gas (99.5%, Sanfu Co. Taiwan). The catholyte consisted of 100 mM phosphate-buffered saline (Sigma–Aldrich Co., USA). When the potential of the inoculated MFC reached a steady state (after approximately 41 days of operation), the biofilm in the anode of the MFC was considered stable or mature.

To understand the operating characteristics of the MFC biosensor, 1/100 NB was used and the circuit was adjusted using variable resistance (700–8,000 Ω) to evaluate the relationship between current density and power density in a batch mode. In these batch experiments, the voltage and power density of the MFC were analyzed after 15 min operating periods. After obtaining the optimal external resistance, 1 mL Cr(VI) with a final concentration in the range of 1–100 mg/L was added to 1/100 NB to analyze the relationship between voltage output and Cr(VI) concentration.
2.4. Cr(VI) Measurement in Actual Electroplating Wastewater

Cr(VI) concentrations in the actual electroplating wastewater were measured using the MFC biosensor and a standard colorimetric method [19]. Wastewater samples A–C were obtained from the effluents of different electroplating units. To measure Cr(VI) concentration in the actual wastewater, 61.5 mL of actual electroplating wastewater was mixed with 0.5 mL of 124/100 NB to maintain the NB concentration in the anolyte of the MFC. Based on the established standard curve between voltage output and Cr(VI) concentration, the Cr(VI) concentrations in the actual electroplating wastewater could be analyzed [16]. All the experiments were conducted using 4 separate MFCs and each analysis was conducted in triplicate.

2.5. Analysis

The colorimetric method for Cr(VI) measurement was performed as described previously [19]. The MFC potential was measured using a multimeter (Model 2700, Keithley Instruments, Inc., USA). The measured voltage ($V$) was converted to current ($I$) according to the following equation: voltage ($V$) = current ($I$) $\times$ resistance ($R$). The power ($P$) was calculated as $P = I \times V$ and then normalized using the surface area of the anode.

To understand the changes in the bacterial community of the MFC, the biofilm at the anode was collected for bacterial community analysis using NGS. Cell lysis, DNA extraction, PCR amplification, and 454 pyrosequencing were conducted according to the methods described by Naz et al. [20].

3. Results and Discussions

3.1. Effects of PH and Temperature on Cr(VI) Removal of O. tritici KCC210

As the pH in the wastewater is often variable, the effect of pH on the Cr(VI) removal efficiency of O. tritici KCC210 is evaluated. Figure 2A indicates that a pH of 5–10 led to >75% removal efficiency and an optimal pH range of pH 6–8, >92% removal efficiency could be achieved. This characteristic illustrates O. tritici KCC210 is alkalotolerant and favors the application of the MFC biosensor in
measuring Cr(VI) concentration in different water bodies [21]. Figure 2B illustrates the effects of temperature on Cr(VI) removal efficiency of *O. tritici* KCC210. The results indicated that relatively high Cr(VI) removal efficiencies (82.5%–94.6%) were found in the range of 20–35 °C. However, at 15 °C and 45 °C, relatively low removal efficiencies of 46.1% and 32.5%, respectively, were observed.

![Figure 2](image)

**Figure 2.** Effects of (A) pH (temperature: 30 °C) and (B) temperature (pH: 7) on the Cr(VI) removal efficiency of *O. tritici* KCC210 (Cr(VI) concentration was: 50 mg/L).

### 3.2. Operating Characteristics of MFC

Figure 3 indicates the MFC potential/voltage decreased as the current density increased. Under such conditions, the maximum power density was 17.5 ± 0.9 mW/m², corresponding voltage was 325 ± 6.5 mV and the optimal external resistance was 2,000 Ω. Moreover, our results indicated that the decrease in MFC voltage had two significant slopes due to the activation loss and ohmic loss. However, the mass transport loss was not observed. These results illustrate that *O. tritici* KCC210 is an exoelectrogenic bacterium and the external resistance should be set at 2,000 Ω for subsequent experiments. The maximum power density of the MFC using *O. tritici* KCC210 was smaller than that of the Cr(VI)-MFC biosensor using *O. anthropi* YC152 [9]; thus, the MFC using *O. tritici* KCC210 may narrow the Cr(VI) concentration measurement range.

Figure 4 illustrates the relationship between the Cr(VI) concentration and the MFC voltage in the MFC in a batch mode. Generally, a 15 min reaction time was required to achieve the stable voltage output. A linear relationship could be observed when the Cr(VI) concentration was ranged from 10 mg/L to 80 mg/L. The regression equation was calculated as $y$ (voltage) = $-1.3321x$ (concentration) + 317.82. When the Cr(VI) concentration was <10 mg/L or >80 mg/L, the voltage variation was too slight or dramatic to establish their relationships. These results clearly illustrate operating characteristics of the developed MFC inoculated with *O. tritici* KCC210. Using the established calibration curve, the Cr(VI) concentration in the wastewater can be rapidly measured.
Figure 3. Polarity and power curves obtained from the MFC biosensor inoculated with *O. tritici* KCC210 during the stable phase of power generation.

Figure 4. Relationship between Cr(VI) concentration and voltage output of the MFC biosensor inoculated with *O. tritici* KCC210.

3.3. Cr(VI) Measurement in Actual Electroplating Wastewater

To evaluate the feasibility of using the developed MFC biosensor to measure Cr(VI) concentrations in actual wastewater, the Cr(VI) concentrations in the samples were measured using the MFC biosensor and colorimetric method. Figure 5 indicates the biosensor-based measurements on actual electroplating wastewater were accurate in comparison with standard colorimetric method. Moreover, both exhibited low deviations (−1.8% to 7.8%). Thus, the results clearly indicate that the biosensor can be used for accurate in situ measurement of Cr(VI) concentration in the electroplating wastewater.

To understand the changes in the bacterial community of the MFC after operation, the biofilm at the anode was analyzed using NGS. The results indicated the most abundant families found in the biofilm were *Brucellaceae* (98.1%) and then *Bacillaceae* (1.2%) after treating the actual electroplating wastewater with MFC for 152 days. Together, two families accounted for 99.3% of the total bacterial sequences obtained. The originally inoculated *O. tritici* KCC210 was the most abundant species by further identification. The results clearly demonstrated that reliable MFC performance may be attributed to a stable bacterial community present during the treatment period.
4. Conclusions
In this study, a MFC-based biosensor inoculated with *O. tritici* KCC210 for the in situ determination of Cr(VI) concentration in the electroplating wastewater was developed. *O. tritici* KCC210 is facultatively anaerobic, Cr(VI)-reducing, and exoelectrogenic; thus, it is suitable for application in an MFC to determine Cr(VI). The results illustrate that the developed MFC biosensor is a simple device for measuring a wide range of Cr(VI) concentrations with high accuracy. Although the detection limit of the biosensor was larger than the maximum limit of Cr(VI) in an effluent, the biosensor was not designed to assess whether the Cr(VI) concentration was in accordance with wastewater discharge standards or not, instead of being a warming function. Thus, the application of MFC biosensors as in situ devices for Cr(VI) determination in electroplating wastewater is promising.

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6. References
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![Figure 5. Cr(VI) measurement from actual electroplating wastewater by MFC biosensor and colorimetric method.](image-url)
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