

Article

Practical Considerations for Continuous Monitoring of Hexavalent Chromium in Wastewater Using a Microbial Fuel Cell Biosensor: Biosensor Fabrication, Sample Pretreatment, and Bacterial Community Analysis

Guey-Horng Wang ¹, Chiu-Yu Cheng ² and Ying-Chien Chung ^{2,*} 

¹ Research Center of Natural Cosmeceuticals Engineering, Xiamen Medical College, Xiamen 361008, China

² Department of Biological Science and Technology, China University of Science and Technology, Taipei 115, Taiwan

* Correspondence: ycchung@cc.cust.edu.tw; Tel.: +886-22782-1862

Abstract

Hexavalent chromium (Cr(VI)) is a high-priority environmental pollutant due to its strong oxidizing properties, which cause DNA damage and other severe health effects. Conventional detection methods are often costly and lack real-time monitoring capabilities, creating a strong demand for cost-effective, real-time biosensors that meet industrial requirements. In this study, we developed a novel biosensor for continuous Cr(VI) monitoring using a single-chamber microbial fuel cell (MFC). The biological element is an engineered *Escherichia coli* strain (*ChrA-ChrB-E. coli*), constructed by introducing Cr(VI)-resistant (*ChrA*) and Cr(VI)-reducing (*ChrB*) genes. The presence of Cr(VI) affects bacterial metabolism and electron transfer within the MFC, generating a measurable signal proportional to the contaminant's concentration. The biosensor demonstrated robust performance and characteristics. The recombinant strain retained functional activity after 450 days of storage at -20°C . The system exhibited high sensitivity and excellent linearity ($R^2 \geq 0.999$) across a broad Cr(VI) concentration range of 0.015–200 mg/L. During continuous monitoring of chrome tanning and electroplating wastewater, measurements deviated by less than 2.33% from the standard diphenylcarbazide (DPC) method; electroplating deviation was further reduced to -0.69% with EDTA pretreatment. In fishery water, the deviation was higher (-7.12%) due to dissolved oxygen (DO) interference but was reduced to -0.75% after mechanical stirring to remove DO. The biofilm bacterial community remained highly stable over six months in both wastewater types, with the inoculated *ChrA-ChrB-E. coli* strain maintaining dominance ($>99.6\%$). These results substantiate the feasibility of using this biosensor for continuous, online, real-time detection of Cr(VI) in actual wastewater environments.

Keywords: actual wastewater; Cr(VI)-reducing gene; cryogenic temperature; *Exiguobacterium aestuarii*; *Ochrobactrum anthropi*



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1. Introduction

Hexavalent chromium (Cr(VI)) is extensively used in various industrial applications and plays a crucial role in numerous manufacturing processes. Due to its strong oxidizing properties, Cr(VI) can penetrate cellular membranes and, once inside the cell, is reduced to trivalent chromium (Cr(III)). This reduction process generates free radicals and induces oxidative stress, leading to DNA damage and mutations, protein denaturation, enzyme

inactivation, and potentially triggering apoptosis [1]. Consequently, Cr(VI) exhibits carcinogenic, mutagenic, and teratogenic effects. Chronic exposure to this compound has been associated with serious health issues, including cancer, immune system dysfunction, and allergic reactions. Given the substantial environmental and public health risks posed by Cr(VI) contamination, it has been internationally recognized as a priority metal for remediation efforts [1,2]. This designation underscores the urgent need to develop and implement effective strategies for the management, monitoring, and prevention of Cr(VI) pollution.

International standards governing permissible concentrations of Cr(VI) are quite stringent. Typically, the maximum allowable levels of Cr(VI) are set at 0.05 mg/L for natural water bodies, 0.5 mg/L for municipal wastewater, and 0.02–0.25 mg/L for industrial effluents. To enable continuous monitoring of these trace amounts, the development of rapid detection and analytical methodologies is essential. Conventional techniques for Cr(VI) determination predominantly employ physical, chemical, or electrochemical approaches, which offer high accuracy and sensitivity. However, these methods require substantial initial investment, involve operational complexity, have limitations in real-time monitoring capabilities, and provide insufficient data on bioavailability [3]. Therefore, cost-effective, user-friendly biosensors capable of online monitoring are more suitable to meet the demands of industrial applications.

Biosensors consist of two fundamental components. The first comprises biosensing elements that are essential for detecting and analyzing metals. Metal regulator or sensor proteins, particularly those from the SmtB/ArsR and MerR families, play a crucial role in this process [4]. These proteins are highly versatile and can evolve to recognize a wide range of metals, including Hg, As, Cu, Zn, Pb, and Cd [5–7]. The second component involves signal output elements, which may include reporter genes or devices that generate optical or electrochemical signals [8]. Commonly used reporter genes include *lux*, *lacZ*, *crtI*, *luc*, and *gfp* [9,10]. For monitoring Cr(VI) levels in wastewater, electrochemical output devices such as microbial fuel cells (MFCs) are particularly advantageous due to their suitability for online, continuous, and on-site monitoring applications [11].

Previous biosensors for Cr(VI) often utilized bacteria capable of reducing Cr(VI) as biosensing elements. These bacterial strains include *Arthrobacter aureus*, *Bacillus cereus*, *Cellulomonas flavigena*, *Enterobacter* sp., *Exiguobacterium aurantiacum*, *Klebsiella* sp., *Micrococcus* sp., *Ochrobactrum anthropi*, *Pseudomonas stutzeri*, *Rhodobacter sphaeroides*, *Streptomyces griseus*, and *Serratia* sp. [12,13]. The reduction of Cr(VI) by these strains is mediated by genes such as *ChrB*, *YieF*, *YhdA*, or *NfsA*, facilitating detection through mechanisms involving electron transport or changes in electrical potential [14].

MFC-based biosensors have shown considerable promise for the real-time and continuous monitoring of Cr(VI) concentrations in wastewater. Xu et al. (2015) developed a Cr(VI) detection system employing a double-layer filter membrane, with the cathode coated in carbon ink and the anode coated in platinum carbon ink [15]. This configuration demonstrated a distinct negative linear correlation between Cr(VI) concentration and voltage output within the concentration range of 5–20 mg/L. Chung et al. (2016) employed a dual-chamber MFC-based biosensor wherein the anode chamber was inoculated with sludge to facilitate the oxidation of sodium acetate, while Cr(VI) served as the electron acceptor in the cathode chamber, receiving electrons from the external circuit and undergoing chemical reduction to Cr(III) [16]. Cr(VI) concentrations were determined in batch mode by monitoring voltage fluctuations. The optimal cathode pH was identified as 2.0, enabling batch detection of Cr(VI) concentrations between 0.1 and 15 mg/L. However, the use of mixed microbial consortia introduced greater variability in the measured Cr(VI) values. Wang et al. (2016) also implemented a dual-chamber MFC-based biosensor, inoculating the anode with *O. anthropi* as the biosensing element and utilizing a phosphate-saline solution as the catholyte [11].

In this system, *O. anthropi* concurrently oxidized organic substrates, releasing electrons, and reduced Cr(VI) by accepting electrons. Elevated Cr(VI) concentrations in the aqueous phase corresponded to diminished electron transfer to the cathode via the external circuit, resulting in reduced electrical charge generation. Batch analyses were conducted by tracking voltage changes, revealing a strong negative linear relationship between voltage output and Cr(VI) concentration across two ranges: 0.0125–0.3 mg/L and 0.3–5 mg/L. Generally, the analytical error remained below 10% during batch analyses; however, it increased substantially when samples contained high levels of organic matter. Wu et al. (2017) employed a dual-chamber MFC-based biosensor with *E. aestuarii* inoculated as the anodic biocatalyst [17]. This system exhibited robust adaptability to pH values ranging from 5 to 9, salinity levels between 0 and 15 g/L, and temperatures from 20 to 35 °C. Voltage output demonstrated a strong negative correlation with Cr(VI) concentrations spanning 2.5 to 60 mg/L, with a low analytical error (<6.1%), although the limit of detection (LOD) was relatively high. Zhao et al. (2018) utilized a dual-chamber sediment MFC biosensor, wherein native microbial communities in the anode functioned as biosensing elements and the upper cathode chamber facilitated Cr(VI) reduction [18]. Under optimized conditions, this system enabled batch analysis of trace Cr(VI) concentrations (0.2–0.7 mg/L) in water, achieving an analytical error below 8%.

To enable continuous monitoring of Cr(VI) in water, Wu et al. (2019) developed a three-stage, single-chamber MFC-based biosensor system, using *E. aestuarii* introduced into the anode chamber as the biosensing element [19]. Their findings demonstrated that this system could continuously detect Cr(VI) concentrations between 5 and 90 mg/L, with a liquid retention time (LRT) of 2 min, an analytical error below 7%, and an LOD of 0.5 mg/L. This research highlights the potential of this MFC for ongoing, broad-range Cr(VI) monitoring in water or as an early warning device. Lazzarini Behrmann et al. (2020) employed a single-chamber MFC-based system as the signal output platform, immobilizing *Pseudomonas veronii* 2E in the anode as the biosensing element [20]. Their results showed that the system could continuously measure Cr(VI) levels from 4 to 18.5 mg/L. However, they did not evaluate how various environmental factors might affect its analytical performance, nor did they test it with real Cr(VI)-containing wastewater. Chang et al. (2023) utilized a computer numerical control-fabricated laminar-flow microfluidic MFC to detect Cr(VI) in water, incorporating domesticated anaerobic sludge (mainly *Geobacter* spp.) as the biosensing element [21]. The current output correlated strongly with Cr(VI) concentrations in the ranges of 0.1–1 mg/L and 1–10 mg/L ($R^2 \geq 0.97$). Unfortunately, this system has yet to be tested with actual industrial wastewater. To shorten the biosensor's response time, Liu et al. (2025) introduced the c-di-GMP gene module into *Shewanella oneidensis* cells [22]. By exposing the cells to near-infrared light, they triggered the production of c-di-GMP—a key regulator of biofilm formation—thereby accelerating biofilm growth and enhancing sensitivity to Cr(VI). Their results showed that the batch sensing time of this system could be significantly reduced from 30 min to 3 min. To broaden the detection range and achieve an LOD of 0.02 mg/L for Cr(VI) in wastewater, Wang et al. (2025) used recombinant *E. coli* (named *ChrA-ChrB-E. coli*), containing *ChrA* and *ChrB* gene plasmids, as the biosensing element in a single-chamber MFC for continuous Cr(VI) monitoring [23]. This system demonstrated the ability to continuously detect Cr(VI) concentrations from 0.0075 to 200 mg/L under specific conditions.

In this study, a single-chamber MFC-based biosensor inoculated with *ChrA-ChrB E. coli* is proposed for continuous online monitoring of Cr(VI) levels. From a practical perspective regarding the biosensing element, this research introduces several advancements. It evaluates how different cryogenic storage temperatures and durations affect the detection of Cr(VI), aiming to enable the future use of thawed biosensor elements directly, thereby

minimizing the need for frequent preparation of fresh elements. Regarding the biosensor system, it will be applied directly to detect Cr(VI) in various actual wastewater samples, facilitating broader practical validation. The study also investigates how different water quality conditions influence Cr(VI) detection, affect the long-term stability of bacterial communities, and explores the potential of pretreatment methods to improve detection accuracy. Finally, the analytical performance of the biosensor will be compared with existing methods to assess its effectiveness.

2. Materials and Methods

2.1. Materials

The strains *Ochrobactrum anthropi* YC152 and *Exiguobacterium aestuarii* YC211 were provided by Professor Chung's laboratory at the China University of Science and Technology [11,17]. *E. coli* BL21(DE3) was obtained from Novagen (Darmstadt, Germany). Unless otherwise specified, all media, biochemical reagents, and chemical reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Biosensing Element

The protocols for culturing *O. anthropi* and *E. aestuarii*, as well as for chromosome extraction and purification, were adapted from the procedures previously reported by Wang et al. (2025) [23]. The construction of the recombinant *E. coli* biosensing element, incorporating both the *ChrA* and *ChrB* genes, is briefly outlined below. Gene fragments corresponding to *ChrA* and *ChrB* gene fragments were amplified via polymerase chain reaction (PCR) using the primers *NcoI-ChrA-f*, *XhoI-ChrA-r*, *NcoI-ChrB-f*, and *NotI-ChrB-r*. Following amplification, the PCR products were digested with the restriction enzymes *NcoI*, *XhoI*, and *NotI*, as described by He et al. (2018) [24]. The complete PCR protocol, recombinant plasmid assembly, plasmid transformation into *E. coli*, and subsequent cultivation procedures adhered to the methodologies outlined by Wang et al. (2025) [23]. The recombinant plasmids harboring the *ChrA* and *ChrB* genes were designated pET21a-*ChrA* and pET28a-*ChrB*, respectively (see Figure 1). Freshly prepared recombinant *E. coli* strains containing both pET21a-*ChrA* and pET28a-*ChrB* were designated as the *ChrA-ChrB-E. coli* biosensor.

To reduce the logistical challenges associated with the frequent preparation of fresh biosensors, overnight cultures of the recombinant cells were mixed in equal volumes with 30% glycerol and stored at either $-20\text{ }^{\circ}\text{C}$ or $-80\text{ }^{\circ}\text{C}$; these preparations were referred to as frozen cells. The frozen cells were subsequently thawed after storage intervals of 90, 270, 450, and 630 days (hereafter referred to as thawed cells) and used as biosensing elements. The procedures for immobilizing the biosensing elements within the MFC and the operation of the single-chamber MFC-based biosensor are comprehensively described in Section 2.3.

2.3. Construction of a Single-Chamber MFC-Based Biosensor

The biosensor apparatus is designed as a single-chamber system fabricated from acrylic, featuring an internal cubic volume of $4 \times 4 \times 4\text{ cm}^3$ (see Figure 2). Centrally located within the chamber is the anode, constructed from graphite felt with a surface area of 18 cm^2 , which serves as the material for immobilizing the biosensing element. The air cathode consists of a tri-layer configuration comprising a catalyst layer, a micro porous layer, and a gas diffusion layer, prepared according to protocols established in prior studies [25]. These layers collectively function to prevent liquid leakage, enhance electrical conductivity, provide corrosion resistance, facilitate exposure to ambient air, and receive electrons from the external circuit. Positioned above the device is an inlet pipe that permits the introduction of wastewater and inoculum, as well as facilitates water quality assessment.

Conversely, an outlet pipe located beneath the device allows for effluent discharge and subsequent analysis of water quality and bacterial populations. To mitigate environmental interferences, a buffer tank was installed upstream of the biosensor. Pretreatment of the influent water was conducted as necessary, including adjustments to pH (5–8), temperature (25–45 °C), chemical amendments, and flow rate modulation, following guidelines from previous research [23]. Wastewater was subsequently delivered to the biosensor at a controlled flow rate via a peristaltic pump. Electrochemical parameters of the biosensor were continuously monitored using a Model 2700 multimeter (Keithley Instruments, Solon, OH, USA). The immobilization of the *ChrA–ChrB–E. coli* biosensing element onto the anode was performed according to the methodology described by Wang et al. (2025) [23].

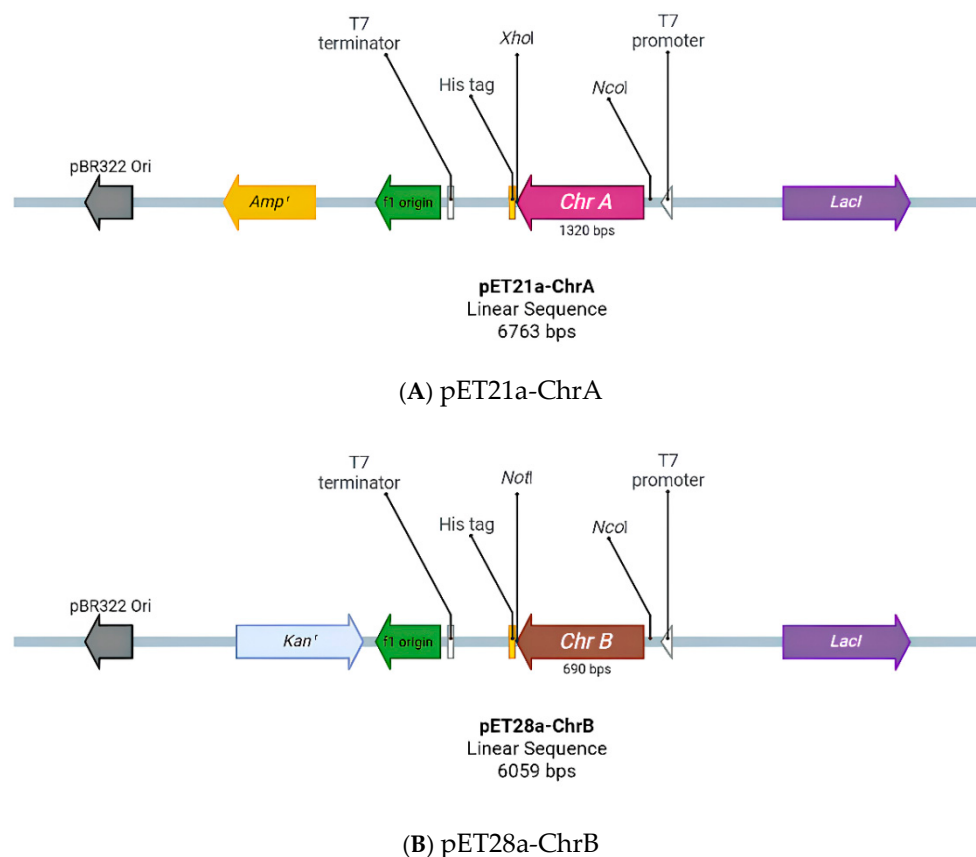


Figure 1. Schematic linear maps of the plasmids used in this study.

2.4. Effects of Cryogenic Temperature and Storage Duration on the Continuous Monitoring of Cr(VI) in Artificial Wastewater

To evaluate the practicality of using thawed cells as biosensing elements, we prepared artificial wastewater consisting of Tris-buffered mineral salts medium (TMM) supplemented with 30 mg/L acetic acid and Cr(VI) at concentrations of either 5 mg/L or 50 mg/L. This solution was continuously introduced into a single-chamber MFC-based biosensor for 1 h, maintaining an operating temperature of 35 °C, an external resistance of 800 Ω , and an LRT of 2 min. Here, LRT refers to the average duration a liquid molecule remains within the system, calculated by dividing the liquid volume by the flow rate. These settings correspond to the optimal conditions determined in our previous research [23]. Using the voltage output versus Cr(VI) concentration calibration curve derived from freshly prepared cells as the biosensing reference, we measured the Cr(VI) concentration, calculated the mean and deviation, and assessed deviations relative to the initial time point (freshly prepared cells).

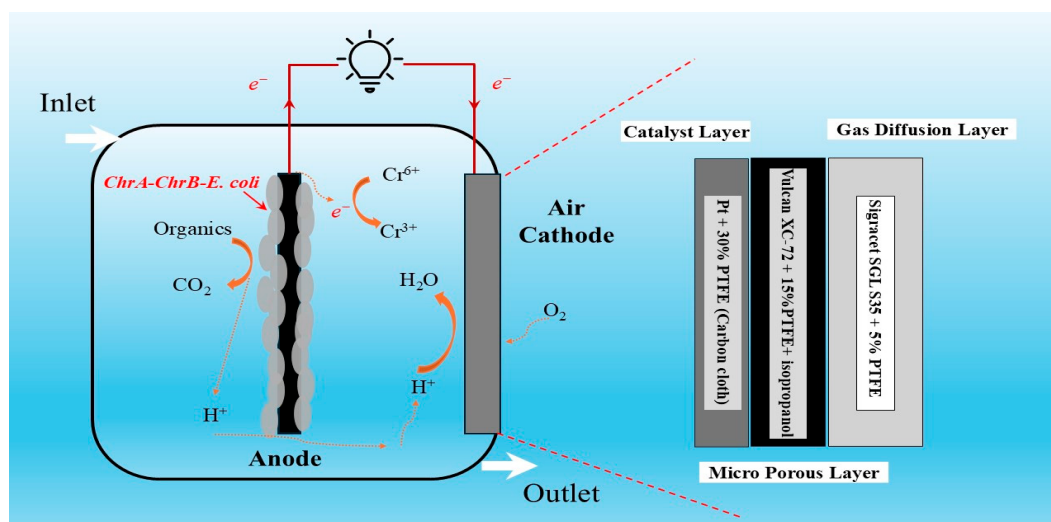


Figure 2. Schematic diagram of a single-chamber MFC-based biosensor. The mechanism for detecting Cr^{6+} using the biosensor inoculated with *ChrA-ChrB-E. coli* is as follows: Wastewater containing organic compounds and Cr^{6+} enters the anode chamber, where the *ChrA-ChrB-E. coli* strain oxidizes the organic matter, generating electrons. These electrons move through an external circuit to the air cathode, where they react with O_2 , producing a voltage output. Because Cr^{6+} is present in the wastewater, *ChrA-ChrB-E. coli* also utilizes electrons derived from the oxidation of organic matter to reduce Cr^{6+} to Cr^{3+} . This reduction decreases the number of electrons reaching the cathode, causing a drop in voltage output. Additionally, the voltage output shows a linear negative correlation with the inlet concentration of Cr^{6+} .

After determining the optimal cryogenic temperature and storage duration, we re-established calibration curves for the biosensors using thawed cells to ensure precise analytical measurements under these optimal conditions. The procedure for generating these curves is summarized as follows. The operating parameters remained consistent with those previously described; however, the Cr(VI) concentration in the artificial wastewater introduced into the biosensor varied sequentially from 0.0075 to 200 mg/L, increasing stepwise from low to high concentrations. Each concentration batch was applied for at least 30 min before switching. For low Cr(VI) levels (≤ 0.5 mg/L), voltage readings were taken after 20 s; for concentrations between 0.5 and 50 mg/L, after 75 s; for 50 to 100 mg/L, after 2 min; and for 100 to 200 mg/L, after 4 min [23]. To determine the LOD concentration, we first calculated the standard deviation (SD) of the voltage readings from three blank samples. We then multiplied this SD by three and used the calibration curve to calculate the corresponding LOD concentration.

2.5. Measurement of Cr(VI) in Actual Water Samples

Wastewater containing high concentrations of Cr(VI) primarily originates from electroplating and leather processing industries. In this investigation, wastewater samples were collected from an electroplating facility in Changhua, Taiwan. The experimental setup consisted of two groups: one subjected to pretreatment with 2×10^{-5} M EDTA and the other without any pretreatment. In both groups, Cr(VI) concentrations were continuously monitored over a 60 min period with a 2 min LRT, and samples were collected every 20 min for batch colorimetric analysis of Cr(VI) .

Additionally, process wastewater from a leather manufacturing plant in Pingtung, Taiwan, was examined. This included tanning wastewater, chrome tanning rinse water, coloring and fatliquoring effluents, and finishing wastewater. Continuous short-term monitoring of Cr(VI) concentrations was conducted for 5 min with a 2 min LRT, and the

mean values were recorded. Batch colorimetric assays were also performed on collected samples to evaluate the influence of biochemical oxygen demand (BOD) on Cr(VI) detection. Furthermore, the tanning wastewater, which exhibited the highest Cr(VI) concentration among the samples, was continuously monitored for 60 min with a 2 min LRT, and samples were taken every 20 min for batch colorimetric determination.

To assess the broader applicability of the single-chamber MFC-based biosensor for continuous Cr(VI) analysis across diverse water matrices, five additional water sources were investigated: fishery water from Tainan County, domestic sewage from New Taipei City, polluted river water from Hsinchu County, chemical plant wastewater from Changhua County, and contaminated groundwater from Hsinchu County, all located in Taiwan. The procedure involved continuous Cr(VI) monitoring for 5 min with a 2 min LRT, followed by averaging the results. Batch colorimetric measurements were also performed on these samples. To mitigate interference from elevated dissolved oxygen (DO) levels, fishery water was pretreated using a submersible mixer MR21NF250 (Tsurumi Manufacturing Co., Ltd., Osaka, Japan) operating at 50 rpm. Furthermore, to validate the efficacy of mechanical stirring in facilitating oxygen removal, Cr(VI) concentrations in the fishery water were continuously monitored over a 60 min period with a 2 min LRT. Samples were also collected at 20 min intervals for batch colorimetric analysis.

2.6. Investigation of the Bacterial Community

Wastewater from electroplating and tanning facilities was continuously and separately introduced into five identically configured biosensors (five replicates), each operating at a resistance of 800 Ω with a 2 min LRT. Samples of the anode biofilm or effluent were collected after one hour and six months of operation for subsequent next-generation sequencing (NGS) analysis to characterize the bacterial community structure and evaluate the prevalence and dynamics of the inoculated bacterial strains. DNA extraction from the collected biofilm or effluent bacteria was performed using the Fast DNA Spin Kit (MP Biomedicals, Santa Ana, CA, USA). The V3-V4 hypervariable region of the eubacterial 16S ribosomal RNA gene was amplified via PCR following a previously established protocol, followed by product purification and preprocessing [17]. The 16S rRNA gene sequence data were then analyzed using QIIME 2 software. Operational taxonomic unit (OTU) clustering was performed using the UCLUST method with a 97% similarity threshold. The RDP classifier was employed to classify and identify representative sequences [19].

2.7. Analysis

DO and pH levels were measured using a portable multiparameter device (WTW, Weinheim, Germany). The BOD₅ was determined according to the standard BOD method 5210 B, as outlined by the United States Environmental Protection Agency [26]. Cr(VI) concentrations were measured using the colorimetric 1,5-diphenylcarbazide (DPC) assay, following the procedure described by Lace et al. (2019) [27].

2.8. Statistical Analysis

The data in this study were subjected to one-way analysis of variance (ANOVA), followed by Duncan's multiple range test for post hoc comparisons. Results are presented as mean $\pm$ standard deviation (SD), based on a minimum of three replicates. Statistical significance was defined as a *p*-value less than 0.05. All statistical analyses were conducted using IBM SPSS software, version 28 (IBM, Armonk, NY, USA).

3. Results and Discussion

3.1. Effects of Cryogenic Temperature and Storage Duration on the Continuous Monitoring of Cr(VI) in Artificial Wastewater

The calibration curves for Cr(VI) concentration versus voltage response of the single-chamber MFC-based biosensor inoculated with freshly prepared biosensing elements are described by the equations $y = -8.9158x + 490.84$ within the concentration range of 0.0075–0.5 mg/L and $y = -0.4062x + 486.76$ for the range of 0.5–200 mg/L [23]. As shown in Figure 3A, both precision, expressed as standard deviation (SD), and accuracy, indicated by relative error (RE), of the biosensor using thawed *ChrA–ChB–E. coli* cells stored at -80°C as the biosensing element decrease with increasing storage duration during Cr(VI) detection. Specifically, at a Cr(VI) concentration of 5 mg/L, accuracy declined to $98.2\% \pm 0.18\%$ after 450 days of storage and further decreased to $92.4\% \pm 0.32\%$ after 630 days. Notably, only thawed cells stored for 270 days or less exhibited activity statistically comparable to freshly prepared cells ($p > 0.05$). For detecting 50 mg/L Cr(VI), accuracy reduced to $98.6\% \pm 0.25\%$, while precision deteriorated, as evidenced by an increased SD of 0.1263 after 630 days of storage, indicating substantial variability in measurements. Cells thawed after storage periods of 450 days or less maintained activity levels not significantly different from freshly prepared cells ($p > 0.05$).

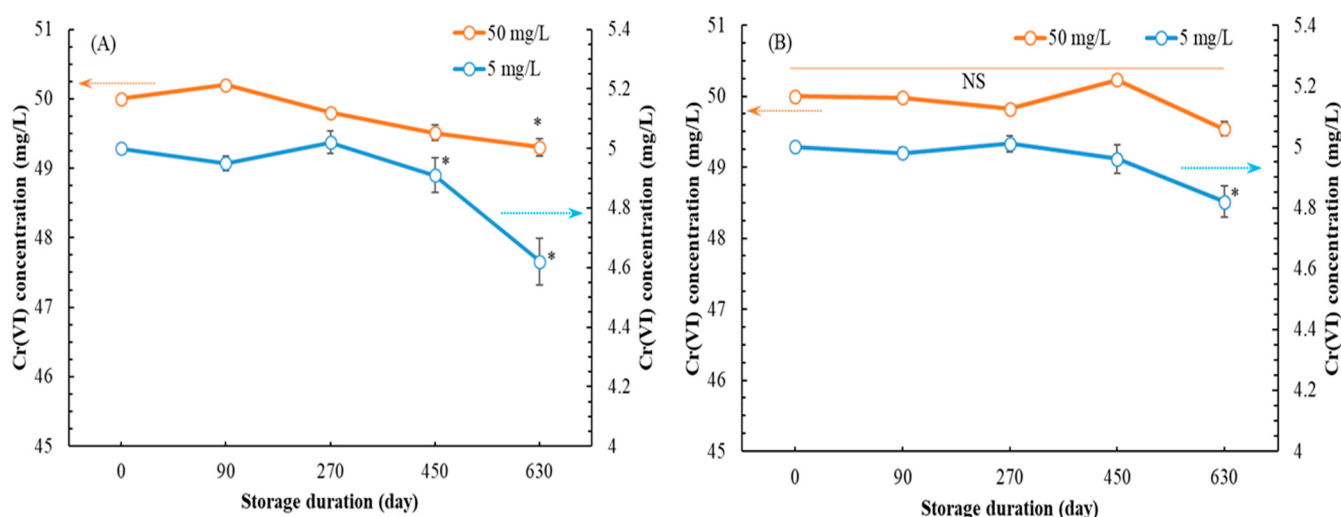


Figure 3. Effects of cryogenic temperature and storage duration on the continuous monitoring of Cr(VI) in artificial wastewater. The biosensing element was stored at (A) -80°C and (B) -20°C . Data are expressed as the means and standard deviations of three independent experiments. * indicates a statistically significant difference compared to the results at $t = 0$ ($p < 0.05$). NS indicates statistical non-significance compared to the results at $t = 0$ ($p > 0.05$).

Figure 3B illustrates that the precision and accuracy of the single-chamber MFC-based biosensor using thawed *ChrA–ChB–E. coli* cells stored at -20°C as the biosensing element, decline with prolonged storage duration. However, this reduction is less pronounced compared to that observed under -80°C cryogenic conditions, indicating that storage at -20°C better preserves the activity of frozen cells [28]. This difference may be attributed to DNA damage caused by freeze–thaw stress [29] or to cell inactivation and membrane damage induced by extremely low temperatures, which can lead to cell death [30]. Specifically, the biosensor’s accuracy in detecting 5 mg/L Cr(VI) decreased to 96.4% when using thawed *ChrA–ChB–E. coli* cells stored for 630 days. In contrast, cells stored for 450 days or less retained activity comparable to freshly prepared cells ($99.2\% \pm 0.47\%$, $p > 0.05$). For the detection of 50 mg/L Cr(VI), the biosensor’s accuracy using thawed cells stored for

630 days remained high at $99.08\% \pm 0.91\%$, with no statistically significant difference from freshly prepared cells ($p > 0.05$).

These findings suggest that cells stored at $-20\text{ }^{\circ}\text{C}$ for up to 450 days retain their original activity after thawing and can be effectively used as biosensing elements. This capability reduces the technical challenges associated with production and minimizes the need for frequent preparation of fresh biosensing elements, which is critically important for practical applications and industrial scalability. Wang et al. (2021) reported that recombinant *E. coli* strains, regardless of the promoter gene used, containing the *ChrB* gene as the chromate-sensing regulator and the *luxAB* gene as the reporter, maintained activity for only 90 days when stored at $-80\text{ }^{\circ}\text{C}$ [31]. In contrast, Wang et al. (2023) demonstrated that recombinant *SP6-lux-E. coli*, incorporating the *SP6* promoter, *ChrB* biosensing element, and *luxAB* reporter gene, exhibited no significant loss of activity compared to freshly prepared cells after 270 days of storage at $-20\text{ }^{\circ}\text{C}$ [28]. Collectively, these results indicate that the shelf life of biosensors depends on multiple factors, including cryogenic temperature, storage duration, composition of the biosensing element, and the nature of the output element.

The activity of thawed cells stored at $-20\text{ }^{\circ}\text{C}$ for 450 days exhibited minimal changes in their ability to detect Cr(VI) concentrations at 5 mg/L and 50 mg/L compared to freshly prepared cells. However, subtle biochemical responses may vary across different Cr(VI) concentrations. Consequently, a calibration curve correlating Cr(VI) concentration with voltage output was re-established for a single-chamber MFC-based biosensor using these thawed cells as the biosensing element. Within the Cr(VI) concentration range of 0.0075–200 mg/L, the calibration curve was described by the equation $y = -0.4011x + 486.13$, with an R^2 of 0.9872. This curve is suitable for general concentration analysis but lacks sufficient sensitivity at lower concentrations. Moreover, as illustrated in Figure 4A, the calibration curve can be divided into three distinct linear regions. Stepwise regression analysis revealed strong linear correlations between Cr(VI) concentrations and biosensor voltage output within the ranges of 0.015–1 mg/L, 1–50 mg/L, and 50–200 mg/L, each exhibiting R^2 values exceeding 0.999. The corresponding linear equations for these intervals were $y = -10.064x + 490.88$, $y = -0.2999x + 481.11$, and $y = -0.4037x + 486.66$, respectively (Figure 4B–D). These calibration models will be employed for continuous monitoring of Cr(VI) concentrations in actual wastewater samples to evaluate their practical applicability. Although the LOD for Cr(VI) using thawed bacterial cells increased from 0.0075 mg/L to 0.015 mg/L, the measurement accuracy was substantially improved. Notably, this LOD of 0.015 mg/L falls below the maximum allowable levels for surface water (0.05 mg/L) and current regulatory standards for wastewater discharge (0.02–0.25 mg/L). Compared to MFC biosensors utilizing *E. aestuarii* (LOD of 0.5 mg/L) [19], *Pseudomonas veronii* 2E (LOD of 4 mg/L) [20], and *Geobacter* spp. (LOD of 0.1 mg/L) [21] as biosensing elements, the present biosensor incorporating thawed *ChA-ChB-E. coli* cells demonstrates a competitive advantage in detection sensitivity.

3.2. Analysis of Actual Wastewater

3.2.1. Efficacy of Cr(VI) Detection Methods

In a previous study, we demonstrated the feasibility of using a single-chamber MFC-based biosensor to detect Cr(VI) in artificial wastewater [23]. However, certain variables inherent to actual wastewater matrices were not examined. For Cr(VI)-containing wastewater, critical water quality parameters include DO, BOD, and the presence of coexisting cations. Accordingly, the present study extends the application of this biosensor to monitor Cr(VI) concentrations in actual wastewater samples, aiming to assess its accuracy under diverse water quality conditions and to propose pretreatment protocols for samples that yield inaccurate results. Figure 5 presents continuous monitoring results of Cr(VI) in wastew-

ater from an electroplating facility using the single-chamber MFC-based biosensor. A distinctive characteristic of electroplating plant wastewater is its elevated concentration of metal ions, which may interfere with biosensor performance. To mitigate such interference, comparative analyses were conducted following sample pretreatment with EDTA. Using the MFC-based biosensor for continuous Cr(VI) detection, measured concentrations ranged from 150.7 ± 2.4 mg/L to 193.6 ± 2.2 mg/L, exhibiting relative errors between -1.49% and -2.33% compared to values obtained via the standard colorimetric method, indicating a slight negative bias. After pretreatment, the relative error was reduced to between -0.43% and -0.69% , differences that were not statistically significant compared to the standard colorimetric method ($p > 0.05$). Additionally, the relative standard deviation (RSD) for the biosensor ranged from 0.73% to 1.24% . This level of accuracy surpasses that observed in our previous system when analyzing artificial wastewater containing 150 mg/L Cr(VI) using freshly prepared *ChrA-ChB-E. coli* biosensors without pretreatment [23]. Furthermore, the error margin is smaller than that reported for dual-chamber MFCs employing *E. aestuarii* as the biosensing element, which ranged from -6.1% to 2.2% [17]. Wang et al. (2023) utilized dilution techniques to alleviate conductivity-related interference in Cr(VI) analysis, thereby enhancing the accuracy of batch analyses using the *SP6-lux-E. coli* biosensor; however, this approach is unsuitable for detecting low Cr(VI) concentrations [28].

Table 1 summarizes the mean values obtained from a 5 min continuous analysis of leather manufacturing wastewater using a single-chamber MFC-based biosensor, alongside batch analysis results derived from a colorimetric method. The wastewater samples originate from four distinct effluent units within the leather production process: tanning effluent (Unit A), chrome tanning rinse water (Unit B), coloring and fatliquoring effluents (Unit C), and finishing wastewater (Unit D). While these effluents exhibit comparable DO levels, their BOD contents vary. The results demonstrate that the system accurately quantifies Cr(VI) concentrations across the different effluent units, with relative errors ranging from -0.83% to 0.98% compared to measurements obtained via the standard colorimetric batch method. Furthermore, a strong positive correlation was observed between the continuous biosensor measurements and the batch colorimetric results ($y = 1.0073x - 0.0657$, $R^2 = 1$), thereby validating the potential of this biosensor approach as a viable alternative for continuous monitoring of Cr(VI) in wastewater.

Following the initial validation of the biosensor's fundamental performance in detecting Cr(VI) across various leather manufacturing wastewater samples, tanning wastewater (unit A) was selected as the target matrix for extended, continuous monitoring of Cr(VI) levels. Figure 6 presents the results of a one-hour continuous measurement of Cr(VI) concentration in tanning wastewater using the single-chamber MFC-based biosensor, conducted without any sample pretreatment. The Cr(VI) concentration in tanning wastewater measured by the biosensor ranged from 59.74 ± 2.58 mg/L to 70.56 ± 2.31 mg/L, exhibiting relative errors between 0.49% and 0.62% compared to concentrations obtained via the colorimetric method with batch sampling at 20 min intervals. Statistical analysis revealed no significant difference between the two measurement methods ($p > 0.05$). Furthermore, the RSD of the Cr(VI) concentrations measured by the biosensor in tanning wastewater ranged from 1.16% to 1.32% . Notably, the observed relative error is substantially lower than that reported for measurements using a three-stage single-chamber MFC biosensor inoculated with *E. aestuarii* under comparable water quality conditions, which ranged from 1.9% to 6.2% [19]. These findings substantiate the capability of the single-chamber MFC-based biosensor to provide continuous, real-time, and precise monitoring of Cr(VI) concentrations.

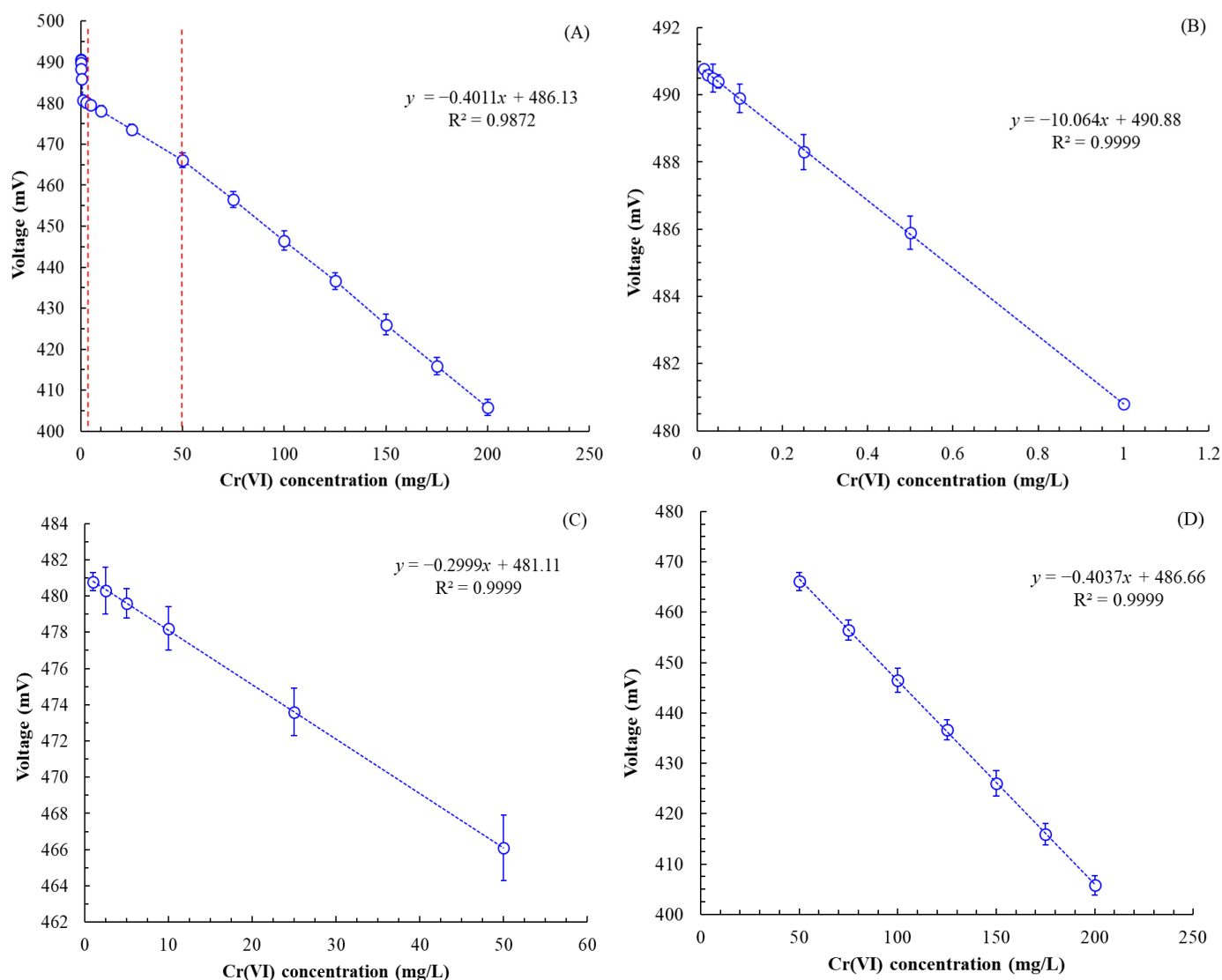


Figure 4. Relationship between Cr(VI) concentration and the voltage output of the single-chamber MFC-based biosensor during continuous operation: Cr(VI) concentration ranges (A) 0.0075–200 mg/L; (B) 0.015–1 mg/L; (C) 1–50 mg/L; (D) 50–200 mg/L. Operational temperature: 35 °C; external resistance: 800 Ω ; LRT: 2 min. Data are expressed as the means and standard deviations of five independent experiments.

Table 1. Cr(VI) concentrations in leather manufacturing wastewater measured using a single-chamber MFC-based biosensor in continuous mode and a colorimetric method in batch mode.

	Unit			
	A	B	C	D
BOD (mg/L)	195 $\pm$ 23 *	32 $\pm$ 6	64 $\pm$ 13	15 $\pm$ 2
DO (mg/L)	2.31 $\pm$ 0.22	2.63 $\pm$ 0.31	1.82 $\pm$ 0.15	2.87 $\pm$ 0.24
Cr(VI) conc. by colorimetric method	64.12 $\pm$ 1.95	18.54 $\pm$ 1.02	9.62 $\pm$ 0.85	5.12 $\pm$ 0.84
Cr(VI) conc. by MFC biosensor	64.53 $\pm$ 2.24	18.61 $\pm$ 1.31	9.54 $\pm$ 1.01	5.17 $\pm$ 0.92
Deviation (%)	0.64	0.38	−0.83	0.98

* Data are expressed as the means and standard deviations of five independent experiments.

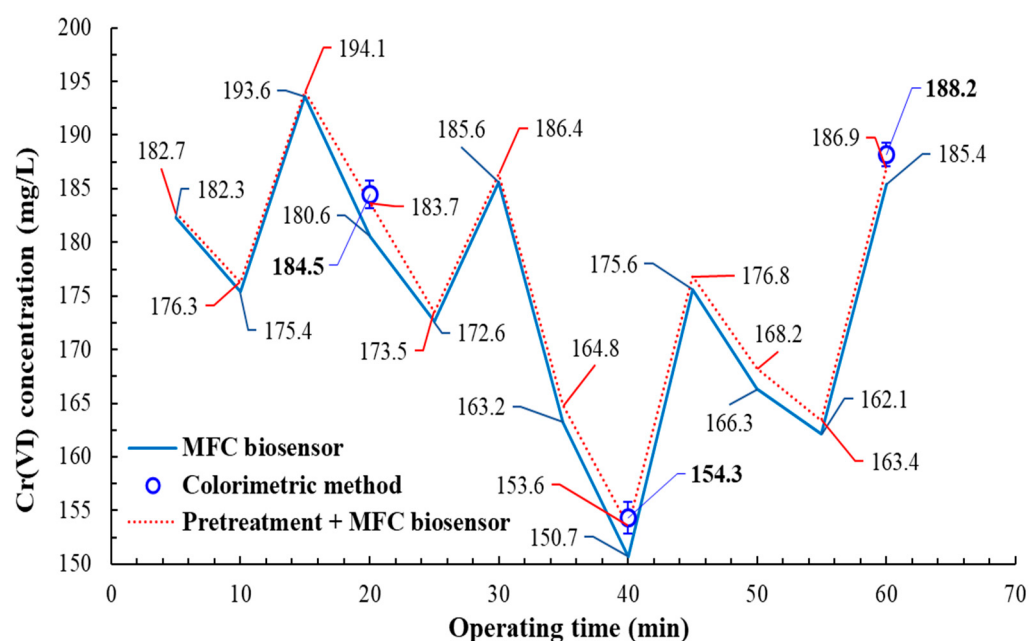


Figure 5. Measurement of Cr(VI) concentrations in electroplating wastewater using a single-chamber MFC-based biosensor, both with and without pretreatment (EDTA addition), during continuous operation. The values obtained through the colorimetric method in batch mode were considered the true measurements. Data are expressed as the means of three independent experiments.

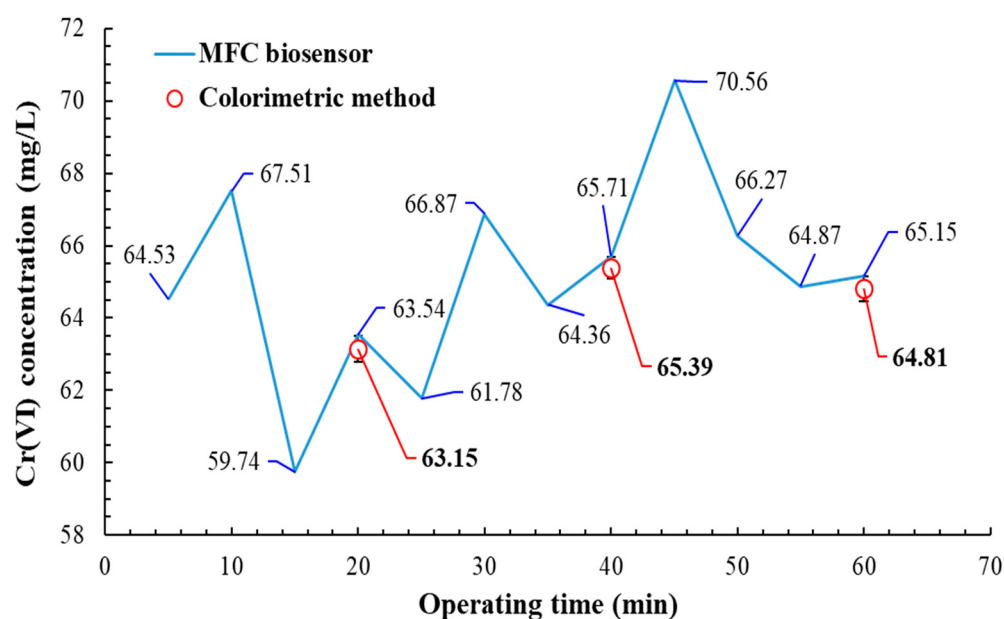


Figure 6. Measurement of Cr(VI) concentrations in tanning wastewater using a single-chamber MFC-based biosensor during continuous operation. The values obtained through the colorimetric method in batch mode were considered the true measurements. Data are expressed as the means of three independent experiments.

In addition to examining electroplating and leather manufacturing wastewaters—two prototypical sources of Cr(VI)-contaminated effluents—this study also assessed various other water samples potentially containing Cr(VI) to evaluate the applicability of continuous Cr(VI) monitoring across diverse environmental matrices. Table 2 shows that the relative errors between Cr(VI) concentrations obtained from a 5 min continuous analysis using the single-chamber MFC-based biosensor and those obtained from batch analysis

via the colorimetric method ranged from -7.12% to 0.85% . The highest relative error was observed in fisheries water; excluding this sample, the analytical errors for other samples remained within $\pm 1.52\%$. Further investigation identified that DO as the primary factor influencing analytical accuracy, given that the anaerobic environment within the MFC anode chamber enhances voltage generation [32]. The Cr(VI) concentration measured by the MFC exhibited a strong dependence on this voltage output. Notably, pretreatment of fisheries water through mechanical agitation to remove oxygen reduced the analytical error from -7.12% to -0.75% . While degassing all water samples did not markedly affect measurement errors in most cases, it substantially improved analytical precision for fisheries water. Moreover, the Cr(VI) concentrations obtained via continuous and batch methods demonstrated a robust positive correlation. Following degassing, the coefficient of determination (R^2) for the regression analysis increased from 0.9983 to 0.9999, indicating enhanced agreement between the two measurement approaches.

Table 2. Cr(VI) concentrations in actual water samples measured using a single-chamber MFC-based biosensor in continuous mode and a colorimetric method in batch mode.

	Fisheries Water	Domestic Effluents	River Water	Chemical Industrial Wastewater	Ground Water
BOD (mg/L)	92 ± 12 *	21 ± 5	11 ± 2	36 ± 4	8 ± 1
DO (mg/L)	5.32 ± 0.47	4.23 ± 0.25	4.53 ± 0.18	3.26 ± 0.25	2.68 ± 0.21
Cr(VI) conc. by colorimetric method	2.67 ± 0.28	1.18 ± 0.05	0.0462 ± 0.011	0.574 ± 0.009	0.814 ± 0.015
Cr(VI) conc. by MFC biosensor	2.48 ± 0.31	1.19 ± 0.07	0.0455 ± 0.008	0.571 ± 0.012	0.802 ± 0.017
Deviation (%)	−7.12	0.85	−1.52	−0.52	−1.47

* Data are expressed as the means and standard deviations of five independent experiments.

To evaluate the effectiveness of mechanical agitation in detecting Cr(VI) concentrations in fisheries water, we continuously monitored Cr(VI) levels using our system while simultaneously conducting colorimetric tests by sampling every 20 min. As shown in Figure 7, the Cr(VI) concentration measured by the single-chamber MFC-based biosensor ranged from 2.51 ± 0.28 mg/L to 3.04 ± 0.33 mg/L. Following mechanical agitation, the measured Cr(VI) concentrations improved and closely matched those obtained through batch colorimetric analysis. For example, the initial relative errors for samples taken at 20, 40, and 60 min were -5.82% , -6.41% , and -7.87% , respectively; however, these errors significantly decreased to -0.34% , -0.36% , and -0.33% . The differences between the two measurement methods were no longer statistically significant ($p > 0.05$). Moreover, the biosensor's RSD ranged from 0.28% to 0.56%. These low RSD values for the single-chamber MFC-based biosensor indicate operational stability. Additionally, this confirms that mechanical agitation is effective for treating wastewater with high DO levels, such as fisheries water. In our setup, we installed a wastewater storage tank upstream of the monitoring system, equipped with a submersible mixer that reduces DO by gently stirring the wastewater at a low speed (e.g., 50 rpm). These results demonstrate much smaller errors (ranging from -13% to 2.3%) compared to a dual-chamber MFC biosensor using *O. anthropi* for Cr(VI) detection in domestic wastewater and groundwater [11]. They also show notably lower deviations (-1.7% to 18.4%) than the *T7-lux-E. coli* biosensor applied to domestic wastewater and river water [28]. Furthermore, these deviations are less than those observed with the *SP6-lux-E. coli* biosensor, which uses bioluminescence as the detection signal in batch analyses of Cr(VI) in fisheries water, domestic wastewater, and groundwater [28]. Compared to continuous analysis results from this system, these findings highlight a clear

competitive advantage. Table 3 presents a comparative analysis of different biosensors used for Cr(VI) detection.

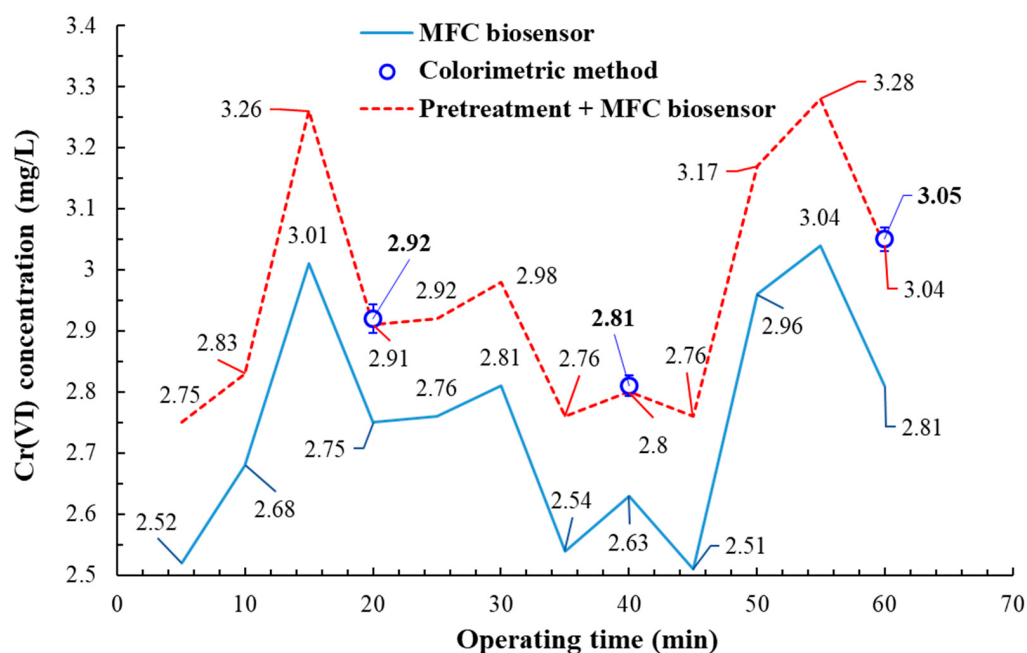


Figure 7. Measurement of Cr(VI) concentrations in fisheries water using a single-chamber MFC-based biosensor, with and without pretreatment (mechanical stirring), during continuous operation. The values obtained through the colorimetric method in batch mode were considered to the true measurements. Data are expressed as the means of three independent experiments.

Table 3. Comparative analysis of different biosensors used for Cr(VI) detection.

Biosensor	Detection Range (mg/L)	Response Time (min)	LOD (mg/L)	Analytical Error (%)	Operating Mode	References
Dual-chamber MFC inoculated with <i>O. anthropi</i>	0.0125–5	45	0.0125	<10	batch	[11]
Flat microliter membrane-based MFC	5–20	6–180	—	—	continuous flow	[15]
Dual-chamber MFC inoculated with sludge	0.1–15	15–20	—	—	batch	[16]
Dual-chamber MFC inoculated with <i>E. aestuarii</i>	2.5–60	15–30	2.5	≤6.1	batch	[17]
Dual-chamber MFC inoculated with native microbes	0.2–0.7	18.3	0.2	<8	batch	[18]
Three-stage single-chamber MFC	5–90	3.3–6.6	—	<7	continuous flow	[19]
Single-chamber MFC inoculated with <i>P. veronii</i>	4–18.5	20–30	2.4	—	batch	[20]
Microfluidic MFC	0.1–10	—	0.1	—	batch	[21]
MFC inoculated with <i>S. oneidensis</i>	1–5	3	—	—	batch	[22]

Table 3. Cont.

Biosensor	Detection Range (mg/L)	Response Time (min)	LOD (mg/L)	Analytical Error (%)	Operating Mode	References
Recombinant <i>lux E. coli</i>						
T7-biosensor	0.0005–0.5	90	0.0005	<8	batch	[30]
T3-biosensor	0.001–0.5	60	0.001	≤14.6		
SP6-biosensor	0.005–0.5	30	0.005	≤18.4		
Single-chamber MFC inoculated with <i>ChrA-ChrB-E. coli</i>	0.015–200	0.33–4	0.015	<1	continuous flow	This study

3.2.2. Bacterial Community Profiling

A major concern when treating actual wastewater is that the diverse bacterial communities present may replace the original bacteria in the biosensor, leading to a loss of the system's initial performance. Several factors influence the distribution of bacterial communities in the biosensor, including the quality of the incoming water, the competitive ability of wastewater strains to attach to the anode, their adaptability within the biosystem, interactions of competition and coexistence among bacteria, and the dominance of the originally inoculated species in the system [33]. Testing each of these factors separately is practically challenging. Therefore, a more feasible approach is to study changes in the biofilm bacterial community and the bacterial composition in the effluent by introducing actual wastewater. Figure 8A shows the bacterial communities on the anode biofilm and in the MFC effluent after one hour and six months of continuous exposure to electroplating wastewater. Figure 8B presents similar data for tanning wastewater. In Figure 8A, at the start (one hour), the MFC effluent contained at least eight bacterial species, with *Exiguobacterium* and *Shewanella* being the most abundant, accounting for 64.28% and 15.86%, respectively. Other species such as *Bacillus*, *Lysinibacillus*, *Geothrix*, *Acinetobacter*, and *Escherichia* each accounted for less than 5.5%. At this point, only the originally inoculated strain (*Escherichia*) was found on the MFC anode biofilm. After six months of continuous operation, the effluent's bacterial community remained similar, with *Exiguobacterium* and *Shewanella* still dominant at 57.52% and 16.15%, respectively. However, the proportion of minor bacteria increased from 0.54% to 4.61%, indicating a more complex bacterial community in the effluent. Three bacterial genera appeared on the anode biofilm, but the originally inoculated strain remained overwhelmingly dominant at 99.84%. The bacterial strains found in electroplating wastewater were mainly chromate-reducing bacteria (*Exiguobacterium*, *Bacillus*, *Lysinibacillus*, *Acinetobacter*) [19,34–36] and metal-reducing bacteria (*Shewanella*, *Geothrix*) [37,38]. This bacterial community structure aligns with previous reports on electroplating wastewater [39]. Similar findings were observed in a dual-chamber MFC system inoculated with *E. aestuarii* as the biosensing element after continuous treatment of electroplating wastewater. In that system, the inoculated strains remained the most dominant in the biofilm, accounting for 95.3% of all bacterial sequences, with chromate-reducing and metal-reducing bacteria being the primary dominant strains [17].

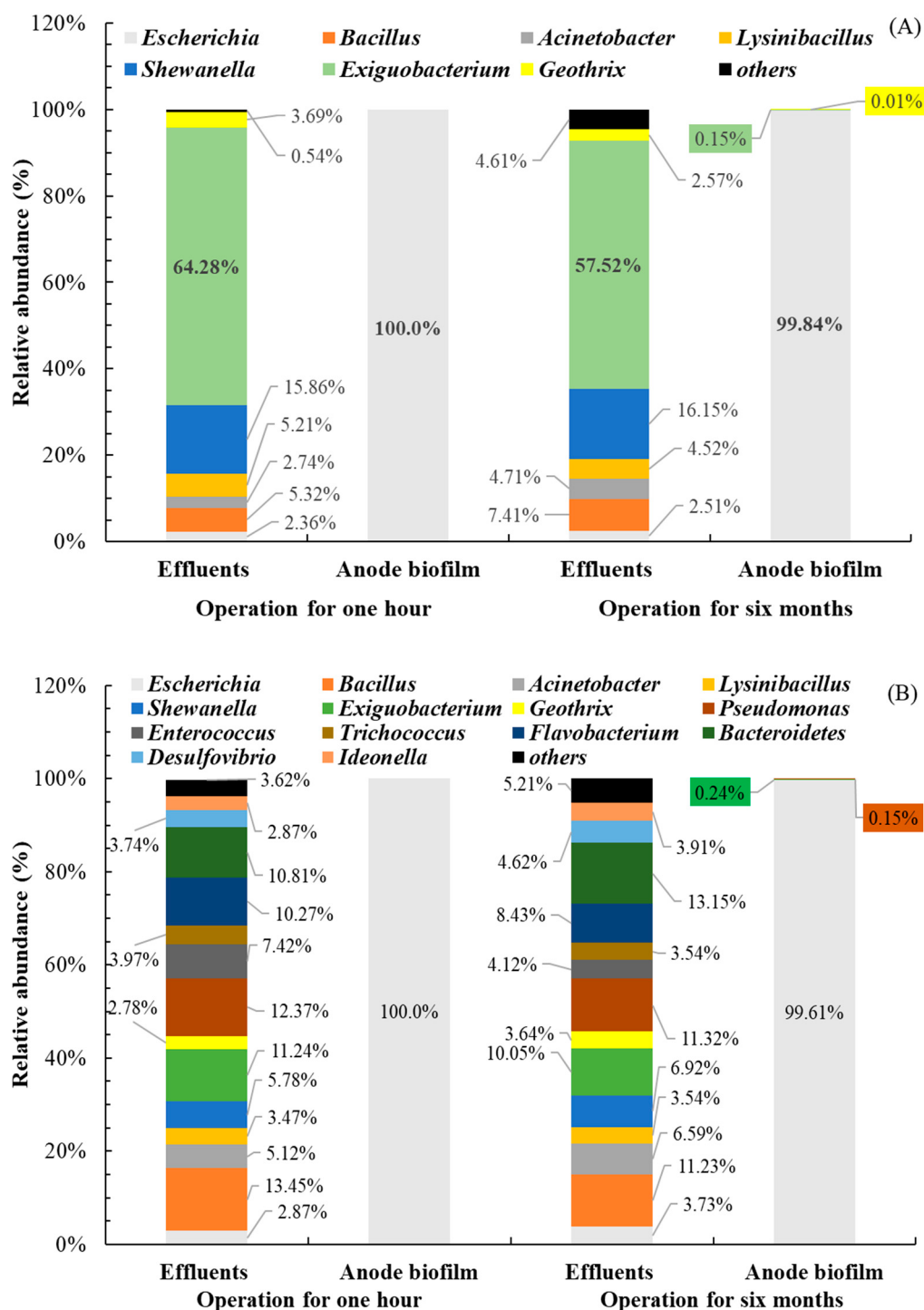


Figure 8. Relative abundances of the bacterial genera in the anode biofilm and MFC effluent after one hour and six months of continuous exposure to (A) electroplating wastewater and (B) tanning wastewater. Data are expressed as the means of five independent experiments.

Figure 8B illustrates that at the start of the operation (after one hour), the MFC effluent contained at least 15 bacterial species. The most prevalent were *Bacillus* (13.45%), *Pseudomonas* (12.37%), *Exiguobacterium* (11.24%), *Bacteroidetes* (10.81%), and *Flavobacterium* (10.27%). Other species each accounted for less than 7.5%, including *Enterococcus*, *Shewanella*, *Acinetobacter*, *Trichococcus*, *Desulfovibrio*, *Lysinibacillus*, *Escherichia*, *Ideonella*, and *Geothrix*. At this point, only the originally inoculated bacterium (*Escherichia*) was found on the MFC anode biofilm. After six months of continuous MFC operation, the bacterial community in the

effluent remained largely unchanged, with the five dominant genera—*Bacillus*, *Pseudomonas*, *Exiguobacterium*, *Bacteroidetes*, and *Flavobacterium*—still accounting for 10.05% to 13.15%. Other bacteria showed a slight increase to 5.21%, suggesting that the system did not retain microorganisms within the biofilm. Three genera appeared on the MFC anode biofilm, but the originally inoculated bacterium (*Escherichia*) remained overwhelmingly dominant at 99.61%. The bacterial strains identified in tanning wastewater primarily include chromate-reducing bacteria such as *Bacillus*, *Pseudomonas*, *Exiguobacterium*, *Enterococcus*, *Acinetobacter*, *Desulfovibrio*, and *Lysinibacillus* [12,19,35,36,40–42]; metal-reducing bacteria like *Shewanella* and *Geothrix* [37,38]; and bacteria involved in degrading persistent organic compounds, including *Bacteroidetes*, *Flavobacterium*, *Trichococcus*, and *Ideonella* [43–46]. This dominant bacterial community closely matches those previously reported in tannery wastewater [47]. Comparable findings were observed in a three-stage single-chamber MFC system using *E. aestuarii* as the biosensing element during continuous treatment of leather processing effluents, where the inoculated strains remained the most prevalent in the biofilm (92.54–97.50%) [19]. Tannery wastewater contains higher organic content than electroplating wastewater, leading to a more complex bacterial community. Nevertheless, in this system, the biofilm bacterial community serving as the biosensing element remained stable and uniform in both electroplating and tannery wastewater throughout continuous monitoring. This stability likely underpins the system's ability to provide accurate, ongoing monitoring of Cr(VI) concentrations in water. Consequently, the system can operate effectively in actual wastewater environments for at least six months of continuous use.

4. Conclusions

This study systematically evaluated an MFC-based biosensor utilizing *ChrA–ChB–E. coli* as the biosensing element for continuous detection of Cr(VI) in actual wastewater samples. It also investigated the effects of cryogenic storage temperature and duration on the biosensor's activity. The findings revealed that bacterial cells stored at -20°C for up to 450 days retained strong activity after thawing, reducing the technical challenges in biosensor production and minimizing the need for frequent preparation of fresh biosensing elements. These results have significant practical and industrial implications. During continuous monitoring of actual wastewater, the single-chamber MFC-based biosensor accurately measured Cr(VI) levels in electroplating and leather processing effluents, exhibiting minimal deviation from standard colorimetric methods. Pretreatment of samples with EDTA or application of mechanical stirring further enhanced measurement accuracy, suggesting that the biosensor could potentially replace traditional batch colorimetric assays. Furthermore, the system offers a broad detection range for Cr(VI), with a LOD of 0.015 mg/L, complying with current wastewater discharge regulations and outperforming many existing biosensors. Analysis of the bacterial community demonstrated that over long-term continuous operation—regardless of whether electroplating or leather wastewater was monitored—the initially inoculated *E. coli* remained the dominant species in the MFC anode biofilm. Although the wastewater contained diverse microbial populations, this did not disrupt the stability of the biofilm community, ensuring consistent and reliable long-term monitoring. In conclusion, this single-chamber MFC-based biosensor provides continuous, real-time, and precise monitoring, adapts well to various wastewater types, and shows strong potential as an efficient and scalable solution for detecting Cr(VI) in industrial wastewater.

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tion, G.-H.W.; funding acquisition, Y.-C.C. All authors have read and agreed to the published version of the manuscript.

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References

1. Meaza, I.; Williams, A.R.; Wise, S.S.; Lu, H.; Wise, J.P., Sr. Carcinogenic mechanisms of hexavalent chromium: From DNA breaks to chromosome instability and neoplastic transformation. *Curr. Environ. Health Rep.* **2024**, *11*, 484–546. [\[CrossRef\]](#)
2. Zhigalenok, Y.; Tazhibayeva, A.; Kokhmetova, S.; Starodubtseva, A.; Kan, T.; Isbergenova, D.; Malchik, F. Hexavalent chromium at the crossroads of science, environment and public health. *RSC Adv.* **2025**, *15*, 21439–21464. [\[CrossRef\]](#)
3. Hui, C.Y.; Guo, Y.; Li, H.; Chen, Y.T.; Yi, J. Differential detection of bioavailable mercury and cadmium based on a robust dual-sensing bacterial biosensor. *Front. Microbiol.* **2022**, *13*, 846524. [\[CrossRef\]](#) [\[PubMed\]](#)
4. Cayron, J.; Effantin, G.; Prudent, E.; Rodrigue, A. Original sequence divergence among *Pseudomonas putida* CadRs drive specificity. *Res. Microbiol.* **2020**, *171*, 21–27. [\[CrossRef\]](#)
5. Huang, C.W.; Lin, C.; Nguyen, M.K.; Hussain, A.; Bui, X.T.; Ngo, H.H. A review of biosensor for environmental monitoring: Principle, application, and corresponding achievement of sustainable development goals. *Bioengineered* **2023**, *14*, 58–80. [\[CrossRef\]](#)
6. Tulin, G.; Figueroa, N.R.; Checa, S.K.; Soncini, F.C. The multifarious MerR family of transcriptional regulators. *Mol. Microbiol.* **2024**, *121*, 230–242. [\[CrossRef\]](#)
7. Qiu, Y.; Tong, Y.; Yang, H.; Feng, S. Molecular insights into a novel Cu(I)-sensitive ArsR/SmtB family repressor in extremophile *Acidithiobacillus caldus*. *Appl. Environ. Microbiol.* **2023**, *89*, e0126622. [\[CrossRef\]](#)
8. Liu, C.; Yu, H.; Zhang, B.; Liu, S.; Liu, C.G.; Li, F.; Song, H. Engineering whole-cell microbial biosensors: Design principles and applications in monitoring and treatment of heavy metals and organic pollutants. *Biotechnol. Adv.* **2022**, *60*, 108019. [\[CrossRef\]](#) [\[PubMed\]](#)
9. Ali, S.A.; Mittal, D.; Kaur, G. In-situ monitoring of xenobiotics using genetically engineered whole-cell-based microbial biosensors: Recent advances and outlook. *World J. Microbiol. Biotechnol.* **2021**, *37*, 81. [\[CrossRef\]](#) [\[PubMed\]](#)
10. Calabretta, M.M.; Lopreside, A.; Montali, L.; Zangheri, M.; Evangelisti, L.; D’Elia, M.; Michelini, E. Portable light detectors for bioluminescence biosensing applications: A comprehensive review from the analytical chemist’s perspective. *Anal. Chim. Acta* **2022**, *1200*, 339583. [\[CrossRef\]](#)
11. Wang, G.H.; Cheng, C.Y.; Liu, M.H.; Chen, T.Y.; Hsieh, M.C.; Chung, Y.C. Utility of *Ochrobactrum anthropi* YC152 in a microbial fuel cell as an early warning device for hexavalent chromium determination. *Sensors* **2016**, *16*, 1272. [\[CrossRef\]](#)
12. Chen, J.; Tian, Y. Hexavalent chromium reducing bacteria: Mechanism of reduction and characteristics. *Environ. Sci. Pollut. Res. Int.* **2021**, *28*, 20981–20997. [\[CrossRef\]](#)
13. Ebrahim, A.; Dey, P.; Samuel, J.; Jabez Osborne, W. Review on the robustness of technologies and strategies in removal of hexavalent chromium: A biological perspective. *Biometals* **2025**, *38*, 1049–1081. [\[CrossRef\]](#) [\[PubMed\]](#)
14. Dubey, P.; Farooqui, A.; Patel, A.; Srivastava, P.K. Microbial innovations in chromium remediation: Mechanistic insights and diverse applications. *World J. Microbiol. Biotechnol.* **2024**, *40*, 151. [\[CrossRef\]](#)
15. Xu, Z.; Liu, B.; Dong, Q.; Lei, Y.; Li, Y.; Ren, J. Flat microliter membrane-based microbial fuel cell as “on-line sticker sensor” for self-supported in situ monitoring of wastewater shocks. *Bioresour. Technol.* **2015**, *197*, 244–251. [\[CrossRef\]](#)
16. Chung, H.; Ju, W.J.; Jho, E.H.; Nam, K. Applicability of a submersible microbial fuel cell for Cr(VI) detection in water. *Environ. Monit. Assess.* **2016**, *188*, 613. [\[CrossRef\]](#) [\[PubMed\]](#)
17. Wu, L.C.; Tsai, T.H.; Liu, M.H.; Kuo, J.L.; Chang, Y.C.; Chung, Y.C. A green microbial fuel cell-based biosensor for in situ chromium (VI) measurement in electroplating wastewater. *Sensors* **2017**, *17*, 2461. [\[CrossRef\]](#)
18. Zhao, S.; Liu, P.; Niu, Y.; Chen, Z.; Khan, A.; Zhang, P.; Li, X. A novel early warning system based on a sediment microbial fuel cell for in situ and real time hexavalent chromium detection in industrial wastewater. *Sensors* **2018**, *18*, 642. [\[CrossRef\]](#)
19. Wu, L.C.; Wang, G.H.; Tsai, T.H.; Lo, S.Y.; Cheng, C.Y.; Chung, Y.C. Three-stage single-chambered microbial fuel cell biosensor inoculated with *Exiguobacterium aestuarii* YC211 for continuous chromium (VI) measurement. *Sensors* **2019**, *19*, 1418. [\[CrossRef\]](#)

20. Lazzarini Behrmann, I.C.; Grattieri, M.; Minter, S.D.; Ramirez, S.A.; Vullo, D.L. Online self-powered Cr(VI) monitoring with autochthonous *Pseudomonas* and a bio-inspired redox polymer. *Anal. Bioanal. Chem.* **2020**, *412*, 6449–6457. [\[CrossRef\]](#) [\[PubMed\]](#)
21. Chang, C.C.; Li, S.L.; Wu, Z.X.; Yu, C.P. Developing a novel computer numerical control-fabricated laminar-flow microfluidic microbial fuel cells as the bioelectrochemical sensor and power source: Enrichment, operation, and Cr(VI) detection. *Biosens. Bioelectron.* **2023**, *226*, 115119. [\[CrossRef\]](#)
22. Liu, Z.; Liu, Y.; Teng, A.; Cao, B. Improving the response of microbial fuel cell-based biosensing through optogenetic enhancement of electroactive biofilms. *Environ. Sci. Technol.* **2025**, *59*, 12752–12762. [\[CrossRef\]](#) [\[PubMed\]](#)
23. Wang, G.H.; Kuo, J.T.; Cheng, C.Y.; Chung, Y.C. Single-chamber microbial fuel cell with an innovative sensing component for real-time continual monitoring of a wide range of Cr(VI) concentrations in wastewater. *Biosensors* **2025**, *15*, 158. [\[CrossRef\]](#)
24. He, Y.; Dong, L.; Zhou, S.; Jia, Y.; Gu, R.; Bai, Q.; Gao, J.; Li, Y.; Xiao, H. Chromium resistance characteristics of Cr(VI) resistance genes *ChrA* and *ChrB* in *Serratia* sp. S2. *Ecotoxicol. Environ. Saf.* **2018**, *157*, 7417–7423. [\[CrossRef\]](#)
25. Santoro, C.; Agrios, A.; Pasaogullari, U.; Li, B. Effects of gas diffusion layer (GDL) and micro porous layer (MPL) on cathode performance in microbial fuel cells (MFCs). *Int. J. Hydrogen Energy* **2011**, *36*, 13096–13104. [\[CrossRef\]](#)
26. USEPA. Method 5210B: 5-Day BOD Test in Standard Methods for the Examination of Water and Wastewater, Washington, DC, USA: APHA, AWWA, WEF, 2016. Available online: <https://www.scribd.com/document/443259537/BOD5-APHA-5210B-23rd> (accessed on 9 January 2026).
27. Lace, A.; Ryan, D.; Bowkett, M.; Cleary, J. Chromium monitoring in water by colorimetry using optimised 1,5- diphenylcarbazide method. *Int. J. Environ. Res. Public Health* **2019**, *16*, 1803. [\[CrossRef\]](#) [\[PubMed\]](#)
28. Wang, G.H.; Tang, C.H.; Cheng, C.Y.; Chung, Y.C. Improving the practicality of recombinant *Escherichia coli* biosensor in detecting trace Cr(VI) by modifying the cryogenic storage conditions of biosensors and applying simple pretreatment. *J. Environ. Sci. Health A Tox. Hazard Subst. Environ. Eng.* **2023**, *58*, 1028–1038. [\[CrossRef\]](#) [\[PubMed\]](#)
29. Taiwo, O.O.; Alaka, W.; Storey, K.B. Molecular mechanisms of DNA damage response and epigenetic regulation in cold-adapted species: Implications for genome stability and molecular network perspective. *Curr. Issues Mol. Biol.* **2025**, *47*, 923. [\[CrossRef\]](#)
30. Moussa, M.; Dumont, F.; Perrier-Cornet, J.M.; Gervais, P. Cell inactivation and membrane damage after long-term treatments at sub-zero temperature in the supercooled and frozen states. *Biotechnol. Bioeng.* **2008**, *101*, 1245–1255. [\[CrossRef\]](#)
31. Wang, G.H.; Cheng, C.Y.; Tsai, T.H.; Chiang, P.K.; Chung, Y.C. Highly sensitive luminescent bioassay using recombinant *Escherichia coli* biosensor for rapid detection of low Cr(VI) concentration in environmental water. *Biosensors* **2021**, *11*, 357. [\[CrossRef\]](#)
32. Varshney, A.; Sharma, L.; Pandit, C.; Gupta, P.K.; Mathuriya, A.S.; Pandit, S.; Lahiri, D.; Nag, M.; Upadhye, V.J. Microbial fuel cell-based biosensors and applications. *Appl. Biochem. Biotechnol.* **2023**, *195*, 3508–3531. [\[CrossRef\]](#)
33. Li, M.; Zhou, M.; Tian, X.; Tan, C.; McDaniel, C.T.; Hassett, D.J.; Gu, T. Microbial fuel cell (MFC) power performance improvement through enhanced microbial electrogenicity. *Biotechnol. Adv.* **2018**, *36*, 1316–1327. [\[CrossRef\]](#)
34. Chen, J.; Li, X.; Gan, L.; Jiang, G.; Zhang, R.; Xu, Z.; Tian, Y. Mechanism of Cr(VI) reduction by *Lysinibacillus* sp. HST-98, a newly isolated Cr(VI)-reducing strain. *Environ. Sci. Pollut. Res. Int.* **2021**, *28*, 66121–66132. [\[CrossRef\]](#)
35. Hu, L.; Liu, B.; Li, S.; Zhong, H.; He, Z. Study on the oxidative stress and transcriptional level in Cr(VI) and Hg(II) reducing strain *Acinetobacter indicus* yy-1 isolated from chromium-contaminated soil. *Chemosphere* **2021**, *269*, 128741. [\[CrossRef\]](#)
36. Li, T.; He, Y.; Wang, J.; Xiang, H.; Xu, X.; Li, C.; Wu, Z. Bioreduction of hexavalent chromium via *Bacillus subtilis* SL-44 enhanced by humic acid: An effective strategy for detoxification and immobilization of chromium. *Sci. Total Environ.* **2023**, *888*, 164246. [\[CrossRef\]](#)
37. Mehta-Kolte, M.G.; Bond, D.R. Geothrix fermentans secretes two different redox-active compounds to utilize electron acceptors across a wide range of redox potentials. *Appl. Environ. Microbiol.* **2012**, *78*, 6987–6995. [\[CrossRef\]](#) [\[PubMed\]](#)
38. Ding, J.; Guo, Y.; Tang, M.; Zhou, S. Effects of exogenous riboflavin or cytochrome addition on the cathodic reduction of Cr(VI) in microbial fuel cell with *Shewanella putrefaciens*. *Environ. Sci. Pollut. Res. Int.* **2024**, *31*, 29185–29198. [\[CrossRef\]](#)
39. Cai, X.; Zheng, X.; Zhang, D.; Iqbal, W.; Liu, C.; Yang, B.; Zhao, X.; Lu, X.; Mao, Y. Microbial characterization of heavy metal resistant bacterial strains isolated from an electroplating wastewater treatment plant. *Ecotoxicol. Environ. Saf.* **2019**, *181*, 472–480. [\[CrossRef\]](#) [\[PubMed\]](#)
40. Franco, L.C.; Steinbeisser, S.; Zane, G.M.; Wall, J.D.; Fields, M.W. Cr(VI) reduction and physiological toxicity are impacted by resource ratio in *Desulfovibrio vulgaris*. *Appl. Microbiol. Biotechnol.* **2018**, *102*, 2839–2850. [\[CrossRef\]](#)
41. Chen, S.; Wang, X.; Zhao, Q.; Xu, Q.; Zhang, Y. Dissecting the simultaneous extracellular/intracellular contributions to Cr(VI) reduction under aerobic and anaerobic conditions using the newly isolating Cr(VI)-reducing bacterium of *Pseudomonas* sp. HGB10. *Microorganisms* **2024**, *12*, 1958. [\[CrossRef\]](#) [\[PubMed\]](#)
42. Sarker, S.S.; Hasan Sarker, M.M.; Sharmin, S.A.; Tarannum, N.; Akter, T.; Alam, M.A.; Miah, M.I.; Ali Shaikh, M.A.; Parveen, S. Molecular characterization of chromium tolerant and gelatin hydrolyzing bacterial isolates from tannery wastes: Perspective on chrome-tanned leather waste biodegradation in Bangladesh. *J. Genet. Eng. Biotechnol.* **2025**, *23*, 100479. [\[CrossRef\]](#)

43. Strepis, N.; Naranjo, H.D.; Meier-Kolthoff, J.; Göker, M.; Shapiro, N.; Kyrpides, N.; Klenk, H.P.; Schaap, P.J.; Stams, A.J.M.; Sousa, D.Z. Genome-guided analysis allows the identification of novel physiological traits in *Trichococcus* species. *BMC Genom.* **2020**, *21*, 24. [[CrossRef](#)] [[PubMed](#)]
44. McKee, L.S.; La Rosa, S.L.; Westereng, B.; Eijsink, V.G.; Pope, P.B.; Larsbrink, J. Polysaccharide degradation by the Bacteroidetes: Mechanisms and nomenclature. *Environ. Microbiol. Rep.* **2021**, *13*, 559–581. [[CrossRef](#)]
45. Yoshida, S.; Hiraga, K.; Taniguchi, I.; Oda, K. *Ideonella sakaiensis*, PETase, and MHETase: From identification of microbial PET degradation to enzyme characterization. *Methods Enzymol.* **2021**, *648*, 187–205. [[PubMed](#)]
46. Kim, M.; Cha, I.T.; Lee, K.E.; Li, M.; Park, S.J. Pangenome analysis provides insights into the genetic diversity, metabolic versatility, and evolution of the genus *Flavobacterium*. *Microbiol. Spectr.* **2023**, *11*, e0100323. [[CrossRef](#)] [[PubMed](#)]
47. Abate, T.A.; Desta, A.F.; Love, N.G. Evaluating tannery wastewater treatment performance based on physicochemical and microbiological characteristics: An Ethiopian case study. *Water Environ. Res.* **2021**, *93*, 658–669. [[CrossRef](#)]

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