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Effects of arsenate on electricity generation and microbial communities in single-chamber microbial fuel cells

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Abstract

In this study, the removal of arsenate, an important environmental pollutant found in wastewater, and simultaneous electricity generation were investigated using microbial fuel cells. Single-chamber air cathode microbial fuel cells were used to examine the effects of synthetic wastewater prepared using sodium arsenate at a concentration range of 0-300 mg/L on electricity production. Arsenate removal percentages were investigated, and changes in microbial ecology were also examined. According to the results, 0.179 V electricity was produced in microbial fuel cells up to 200 mg/L sodium arsenate concentration. However, when the concentration was increased to 300 mg/L, the voltage production decreased significantly ($p = 0.005$). A significant difference ($p < 0.0001$) between lower concentrations (0–15 mg/L) and 300 mg/L arsenate was confirmed by one-way ANOVA analysis, suggesting a strong inhibitory response. 11.5% of sodium arsenate was removed from synthetic wastewater during batch operations. The microbial ecology results indicated that *Geobacter*, *Azospirillum*, and *Xanthobacter* genera significantly increased following arsenate treatment. In conclusion, arsenate-contaminated wastewater can be biologically treated with single-chamber microbial fuel cells, and electricity can be produced simultaneously.

Introduction

Embracing green energy obtained from wastewater reflects responsible use of resources, reduction of environmental impact, and a move towards cleaner and more sustainable energy production (Guo et al., 2019; Akagunduz et al., 2025). Microbial electrochemical systems are promising systems for green energy and biodegradation, such as microbial electrolysis cells (MECs) or microbial fuel cells (MFCs) (Kilinc et al., 2023; Sukkasem, 2024). MFCs have applications not only in power generation but also as biosensors (Kilinc & Catal, 2023). They have unique properties associated with the biological activity and metabolism of microbial communities used in research. They are sensitive to changes in both the external and internal environment, which makes it possible to adjust the appropriate parameters of the influence on them and record current changes. Electrical signals arising in the reactor correlate with the presence or absence of certain substances in the system, as well as their concentrations. Expansion and rapid growth of industrial corporations and many other human activities more often lead to unscrupulous use and regulation of recycling and the utilization of dangerous, toxic, and carcinogenic substances. Heavy metals and metalloids have a huge negative impact on the environment and population globally. Lead, arsenic, cadmium, nickel, and other elements and their compounds pose significant risks to ecosystems and human health due to their high

bioaccumulation and harmful, persistent effects (Tripathi & Ranjan, 2015; Madhu et al., 2017; Wang et al., 2022; Catal & Liu, 2025). Undoubtedly, careful monitoring and management are required to diminish the adverse effects. This involves implementing effective wastewater treatment methods to minimize the release of these hazardous elements into water, air, and soil. An MFC bioreactor has the capacity to produce electrical energy by passing electrons from the anode to the cathode in the external circuit (Du et al., 2007). A single MFC's voltage generation tends to be less than 0.8 V, and frequently less than 0.3 V at maximum power output, limiting MFC applications significantly (Fan et al., 2024).

Microbial communities present in MFCs can generate electrical energy in the process of biodegrading organic or inorganic matter or waste (Park & Zeikus, 2000). The resilience of microbial communities to toxic substances in MFCs, as previously shown in earlier paper (Zhang et al., 2022), highlights their adaptability to challenging environmental conditions. A wide range of microbes can be used as biocatalysts, as pure as mixed cultures. Bacteria *Escherichia coli* and *Geoalkalibacter spp.* are some of the most well-known examples (Badalamenti et al., 2013). *Pseudomonas aeruginosa* is known for its ability to synthesize redox mediators to improve the electron transport process. Additionally, *P. aeruginosa* strains can undergo genetic manipulations (Chong & Li, 2017). Depending on electron transfer mechanisms, MFCs use different microbes. Mediator-based MFCs frequently use species like *Pseudomonas*, *E. coli*, and *Clostridium*. Mediator-less MFCs use *Geobacter* and *Shewanella*, which can transfer electrons directly to the anode. Both types of MFCs typically use glucose and acetate as their main substrates to support microbial metabolism and electricity production. Lactase, pyruvate primarily used as substrates by mediator-less MFCs (Kumar et al., 2020; Obileke et al., 2021). Microorganisms forming biofilm on the anode are involved in the oxidation of organic matter, which results in the generation of free electrons and is part of an energy-producing process (Malekmohammadi & Ahmad Mirbaghari, 2021). These exoelectrogenic microorganisms can play an important role in the bioremediation of various environmental pollutants, such as heavy metals, including arsenate. In MFCs, heavy metals such as silver, selenium, gold, chromium, cobalt, copper, mercury, and vanadium are efficiently reduced in the cathode chamber (Almatouq et al., 2022; Catal et al., 2009; Choi & Hu, 2013; Rikame et al., 2020; Qiu et al., 2017). Prior studies have identified over 20 electrochemically active bacterial species in MFCs, including *Pseudomonas*, *Shewanella*, *Geobacter*, *Clostridium*, *Rhodopseudomonas* and other (Fang & Achal, 2019).

Arsenic belongs to the class of metalloids and expresses the inherent properties of metals, such as being a semiconductor of electricity. It has the ability to accumulate in specific environments (Kaur et al., 2011; Bjorklund et al., 2020; Sandhi et al., 2022). Arsenic, recognized as a widespread environmental contaminant, is found ubiquitously, and thus arises global concern regarding toxicity associated with arsenic exposure and accumulation across diverse geographical regions and environments (Bjorklund et al., 2020). Arsenic (As) enters aquatic ecosystems through the disposal of byproducts from coal combustion, waste generated from metallurgical processes, and the use of pesticides that contain arsenic (Kim et al., 2011). Previous reports have highlighted arsenic poisoning as a significant public health issue (Tchounwou et al., 2004). Various chemical, physical, and biological techniques have been developed so far for the biological remediation of arsenic-contaminated wastewater (Altowayti et al., 2022). A previous study on a dual-chamber MFC-B with 1000 Ω external resistance reported a decline in voltage output as copper (1 – 10 mg/L) and arsenic (0.5–5 mg/L) concentrations increased. These findings highlight MFCs' potential for arsenic detection and remediation (Do et al., 2022). However, there is a need for research on the potential use of microbial fuel cells in arsenic pollution.

In this study, microbial communities forming a biofilm on anodes of single-chamber MFC experimental reactors are examined both before and after treatment with different concentrations of arsenate. In order to understand the potential of MFC biosensors and bioremediation, voltage production, power density, current density, arsenate removal efficiency, and microbial dynamics have been assessed in this study. The current research offers novel insights on how sodium arsenate dibasic heptahydrate affects ecological shifts, microbial diversity, and electrochemical performance. This research aims to contribute to improving MFC stability, power generation, and arsenic remediation; optimizing bioelectrochemical systems; and developing more efficient wastewater treatment methods.

Experimental Procedures

Construction of MFCs and electricity generation

HP 20% Platinum on Vulcan XC-72 (Fuel Cell Store, USA) with Nafion (Sigma-Aldrich, CAS: 31175-20-9, USA) coating was applied to one side of the carbon cloth cathode electrode. Single-chamber MFCs were designed and constructed as in a recent publication (Akagunduz et al., 2023). One bioreactor was disassembled and autoclaved (121 °C, 15 min) to be used as an abiotic control. Three MFCs (two planktonic and one abiotic) were exposed to different concentrations (mg/L) of sodium arsenate dibasic heptahydrate ($Na_2HAsO_4 \cdot 7H_2O$, Sigma-Aldrich, CAS: 10048-95-0, India) at the same external resistance of 1000 Ω. External resistance was connected to the carbon cloth cathode, which is an effective conductive material (Ozer, 2025), and electrical circuit to record power generation by operating microbial fuel cell reactors. Anode and

cathode were placed on parallel sides of plexiglass microbial reactors (Sonmez et al., 2024; Kilinc et al., 2024).

Inoculation and operation of MFCs

Inoculum containing mixed bacterial culture was collected from the Paşaköy Advanced Biological Wastewater Treatment Plant (Istanbul, Türkiye). Synthetic wastewater was inoculated into experimental MFC reactors and operated for 42 days prior to the experiment to obtain stable power output. The media contained a freshly prepared 20 mM acetate solution and was replaced at the end of each batch prior to the experiment. The medium composition, including supplements, contained the following: 8.19 g/L of Na_2HPO_4; 6.6 g/L of $NaH_2PO_4 \cdot 2H_2O$; 1.25 mL/L of vitamin solution; and 12.5 mL/L of trace element solution taken from stock (Lovley & Phillips, 1988). The temperature during all experimental operations was maintained in the range of 30 ± 2 °C. The concentration of sodium arsenate gradually increased during batch operations (0-300 mg/L).

Analyses and calculations

MFC performance and concentrations of arsenate inhibition ratio percentage (I%) were calculated by following formula (Wu et al., 2014):

$$I=((A_{M1}-A_{M2})/A_{M1}) \times 100\%, \quad [1]$$

where A_{M1} is the maximum current value recorded before the introduction of sodium arsenate and A_{M2} is the maximum current obtained for each concentration after the addition of sodium arsenate into the operational bioreactor (Wu et al., 2014; Catal et al., 2018). Power density was calculated by multiplying the mean value of the current (I) obtained in all operations for the chosen dose and voltage (V) and dividing the result by the area of the anode (Table 1) (Catal et al., 2009):

$$P\ (mW/m^2) = IV/A, \text{ where } A = 0.0007\ m^2 \quad [2]$$

Chemical oxygen demand removal was measured according to the standard method (APHA, 1992). The arsenic amount before and after the operation was measured and analyzed by an external laboratory Biyotar Co. (Ankara, Türkiye). The method employed was Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES). Analysis parameter was As (arsenic), results reported in parts per million (ppm) units and expressed in weight/weight (w/w) format.

Microbial community analysis

Microbial genomic DNA isolation of all samples was performed with the QIAamp PowerFecal DNA Isolation Kit (Qiagen, Hilden, Germany) in accordance with the manufacturer's instructions. Genomic DNA quality and quantity were measured with gel electrophoresis, fluorometric, and spectrophotometric techniques. DNA samples were stored at -20°C until downstream analysis was performed. For 16S rRNA amplicon sequencing and bioinformatics, universal primers of 341F (CCTACGGGNGGCWGCAG) and 805R (GACTACHVGGGTATCTAATCC) were used to amplify the V3-V4 region of 16S rRNA genes. KAPA HiFi HotStart ReadyMix (KAPA Biosystems, Wilmington, MA, USA) was used for amplification, and PCR was performed with a T100 thermal cycler (Bio-Rad, USA). Amplicon and Index PCR reactions and purification of PCR products were performed as described in 16S Metagenomic Sequencing Library Preparation (https://support.illumina.com/downloads/16s_metagenomic_sequencing_library_preparation.html). The resulting 16S library was sequenced by paired-end sequencing (2x300 bp) on the MiSeq platform using the MiSeq Reagent Kit v3 (600 cycles) (Illumina, San Diego, CA, USA).

Results and Discussion

Voltage results

Arsenate-free medium produced an average voltage of 0.228 ± 0.007 V with a power density of 74.53 ± 4.9 mW/m^2, voltage curves of tested batches are shown in Figure 1. The experimental batches (biological replicates) were performed in duplicate. All samples were tested across two independent reactor batches, allowing us to verify reproducibility and observe consistent electrochemical trends. In select conditions, additional replicates were conducted to account for unexpected increases in voltage generation. Since voltage was expected to decrease progressively with higher arsenate concentrations, replication for some concentrations was expanded. For 2 mg/mL concentration, quadruplicates were performed to investigate the voltage surge in the first batch of one of the experimental reactors to ensure reliability of the results. Triplicates were conducted for the 50 mg/mL concentration, and quadruplicates were again used for the 300 mg/mL concentration, as it was the final tested concentration before recovery process was initiated. The results of the voltage generation graph indicate a decrease in voltage with an increase in arsenate concentration. It is also shown that the duration of operations increased, starting at 10 mg/L.

The effect of sodium arsenate concentration on bioelectricity generation was evaluated. Our MFC reactor generated 0.156 – 0.233 V on average, depending on sodium arsenate concentration, which falls within acceptable range. Recent study on wetland plant-sediment microbial fuel cells (P-SMFCs) and SMFCs indicated that the presence of arsenic affected power generation with an average output voltage of 0.24 V – 0.32 V (Zhu et al., 2019). Another double-chamber MFCs study for arsenic removal reported a maximum output voltage of 0.388 V (Guo et al., 2021). Despite the more advanced bioelectrochemical interactions in plant-sediment and double-chamber MFC systems, the slightly lower power generation efficiency of our single-chamber MFC remains within an acceptable range. Further optimization of the system,

along with microbial analysis and composition adjustments, could positively impact voltage output.

Voltage output significantly decreased with increasing arsenate levels, demonstrating a concentration-dependent microbial response. Statistical analysis using one-way ANOVA confirmed that these changes were highly significant ($p < 0.0001$) across different arsenate concentrations. Multiple comparisons with 300 mg/L arsenate showed statistically significant differences at various concentrations ($p = 0.0007$ at 20 – 30 mg/L, $p = 0.0023$ at 40 mg/L, $p = 0.0011$ at 50 mg/L, and $p \leq 0.0050$ for 100 – 200 mg/L). These findings highlight arsenate's inhibitory effects on electrogenic bacterial activity, impacting COD removal efficiency and voltage output. Increasing the As concentration led to changes in the internal environment and affected the composition of biofilm, resulting in a reduction in voltage generation at a significant level at the change of concentration from 200 to 300 mg/L (on average 0.179 ± 0.00 to 0.093 ± 0.05 V). The sterile abiotic control did not contribute relevant data for microbial analysis or electricity generation and was not shown as reported in previous studies (Abourached et al., 2014; Yang et al., 2009). Due to variations in reactor design, electrode materials, microbial communities and substrate composition maximum power density data reported in MFC research can differ significantly, as shown in Table 2. The optimization of MFC systems remains challenging, requiring further investigation to enable industrial scale. Previous approaches indicated higher voltage outputs using microbial fuel cells at 1000 Ω external resistance with several carbohydrates as substrates; the maximum voltage reported was 0.59 V (Catal et al., 2011). Moreover, in a 2019 study investigating the removal of COOH-THC, a cannabis metabolite, 50% removal was achieved at different concentrations, and a maximum electricity production of 0.305 V was detected (Ozdemir et al., 2019). Maximum current density decreased from 0.033 to 0.022 mA/cm^2 (0-300 mg/L). Compared to the results, a maximum of 300 mWm^{-2} power density and a maximum of 1.2 Am^{-2} current density were reported with different concentrations of platinum (Pt) loading in the cathode region (Sonmez et al., 2024). Furthermore, various configurations of microbial fuel cells (MFCs) were examined, resulting in the generation of a peak power density of 3.5 W/m^{-2}, achieved particularly with carbon fabric cathodes integrated with activated carbon as a catalyst (Fan et al., 2024). Extracellular polymeric substances (EPS) play a crucial role in power density generation within microbial fuel cells (MFCs). Thus, the power density can be influenced by the structure of the anodic EPS. A power density of 6840 mW/m^2 was documented as a result (Catal et al., 2024). In groups with the same concentration of the metalloid, no significant voltage surges were detected when the medium solution was replaced at concentrations of 1-6 mg/L As. Figure 1 shows a gradual decrease in voltage up to a concentration of 200 mg/L. A significant decrease was observed in a range of 10–20 mg/mL. Voltage dramatically dropped by 23.73%, 59.74%, and 76.4% on the second, third, and fourth batches compared to the first batch at a range of 300 mg/L. After completing 4 batches of 300 mg/L operation, electricity generation was recovered using a 20 mM As-free sodium acetate substrate. It is known that microorganisms can adapt to media, containing toxic compounds and heavy metals and develop resistance (Norberg et al., 1983). A further increase in voltage indicates a successful recovery and adaptation of the mixed-culture bacteria to the changes.

Inhibition ratio

The relationship between arsenate concentration and inhibition is represented by a polynomial equation showing a dependence on arsenate concentration at low and high doses, $R^2 = 0.9156$ (Figure 2). As previously reported, As(III), urease, and protease were more toxic than all enzymes tested. It has been reported that As(V) is more toxic on urease, while As(III) is more toxic on protease activity (Xu, 1991; Gebel, 1997). Inhibition ratios of MFCs increased with higher concentrations of arsenate with sodium acetate substrate. This indicates a positive correlation between the concentration of arsenate and inhibition. Moreover, when the inhibition ratio came to 50% at 300 mg/L As concentration, voltage generation gained downward acceleration. Figure 2 demonstrates that microbial biofilm is affected by exposure to As, which has a direct impact on the performance of MFCs.

Arsenate removal results

COD removal results of 0, 100, 200 and 300 mg/L arsenate treatment are summarized in Table 3. COD measurements were conducted in technical replicates (triplicate) on the same sample. It is used to verify the reliability and reproducibility of the results in scientific experiments. Analysis showed that despite the uniform concentration of organic content (20 mM sodium acetate) in samples 0, 100, 200, and 300 mg/L, varied COD percentage (62 ± 3, 53 ± 2, 69 ± 2, and 48 ± 1, respectively). This indicates variations in the efficiency of organic matter degradation at different concentrations of arsenate. Although all samples contained same substrate, arsenate toxicity affected microbial activity. At 200 mg/L, microbial adaptation may have contributed to the higher COD. At 300 mg/L, sodium arsenate inhibitory properties reduced efficiency to 48 ± 1%. The control shows baseline microbial degradation without arsenic stress, while 100 mg/L suggests initial suppression that is possibly overcome at 200 mg/L through microbial culture adaptation. These results suggest a concentration-dependent microbial response to arsenate exposure. Our COD removal data was analyzed using descriptive statistics, where mean ± SD values were calculated from multiple replicates within tested samples. The obtained mean values indicate potential

microbial performance shifts under different sodium arsenate concentrations. Descriptive statistics provide a reliable representation of COD removal efficiency. Future studies including replicate measurements could further validate these observations through statistical significance testing.

MFC technology, which used in the bioremoval of psychoactive drugs found in wastewater, provides COD removal of different drugs between 94% and 48% (Catal et al., 2024). The results suggest complex dependencies in interactions between bacterial species present in a system, organic content, and removal mechanisms.

The effectiveness of mixed bacterial cultures was observed. The removal results at a concentration of As 200 mg/L showed a successful reduction of 11.5% after the treatment process. The results indicate the potential of the MFC system for the bioremoval of heavy metals and metalloids from contaminated wastewater. The use of mixed bacterial cultures enables the development of inexpensive and environmentally friendly purification strategies. Current research helps to improve understanding of bacterial application for further optimization to enhance arsenic removal efficiency. Previously, removal of various heavy metals using MFCs has been reported. For example, copper plays an important role in soil pollution and has been the subject of MFC studies in realized approaches, and while it produces approximately 0.5 V electricity, this removal rate is between 87.62% and 43.41% in different solvents (Zhang et al., 2020). When using dual chamber MFC, As(III) concentration decreased from approximately 17 mg/L to approximately 8 mg/L. As(V) concentration increased from approximately 2 mg/L to approximately 4 mg/L. Additionally, the maximum voltage was noted as 0.388 V (Guo et al., 2021). In our study, arsenate removal was shown, indicating the potential of MFCs for bioremediation of arsenate-contaminated wastewater. Conventional arsenic removal methods such as chemical precipitation, adsorption, and membrane filtration often achieve higher removal efficiencies but require significant chemical inputs, energy, and infrastructure. In contrast, microbial fuel cells offer a sustainable alternative by enabling simultaneous pollutant removal and electricity generation, with minimal chemical use and waste production.

Microbial ecology results

Proteobacteria and *Desulfobacteriota* were detected in high amounts in the anode samples, while *Proteobacteria* and *Firmicutes* were the most abundant phyla in planktonic samples (Figure 3A). As expected, the microbial community composition in anode and planktonic samples was different, and the community composition was altered after arsenate treatment (Figure 3B). *Geobacter* was very high in CF samples and very low in PR samples. *Azospirillum* and *Xanthobacter* were very high in PR samples. After arsenic treatment, an excessive increase in *Geobacter* in CF and *Azospirillum* and *Xanthobacter* genera in PR samples were observed (Figure 3). Figure 4 shows microbial ecology clusters, and Table 4 shows microbial ecology Shannon index values. *Azospirillum* species have been found to survive in heavy metal contaminated soils due to their metal resistance genes (Enebe & Babalola, 2018). In the genome analysis of 14 *Geobacter* species, also called electricigens, genes belonging to the arsenic detoxification system (ars operon) and arsenic respiration (arr operon) were identified (Dang et al., 2017). *Xanthobacter* species is also associated with arsenic metal resistance (Engel et al., 2013). The abundance of *Bacillus* species increased after arsenic treatment. These results demonstrate a significant impact on microbial dynamics following arsenate treatment. The quest for microorganisms exhibiting high capability in arsenic removal persists. For instance, *Herminiimonas arsenicoxidans* stands out as the pioneer bacterium engaged in arsenic redox reactions, with its arsenite oxidase operon and subsequently its entire genome sequenced (Muller et al., 2006). Following this breakthrough, the genomes of over 85 arsenic-metabolizing archaea and bacteria have been sequenced, albeit primarily consisting of *Proteobacteria*. The sequencing and analysis of these genomes have significantly contributed to the identification of genetic factors involved in arsenic-related processes (Andres & Bertin, 2016). *Bacillus selenitireducens*, which was discovered in the moderately hypersaline Mono Lake, California (US), exhibited the ability to thrive on lactate with arsenate serving as the electron acceptor. Moreover, it demonstrated growth even in the absence of arsenate, utilizing the anode as the electron acceptor. Notably, the presence of arsenate facilitated the generation of a power density of 3 $\mu W/m^{-2}$ by serving as an alternative electron acceptor to the anode (Miller et al., 2006). Also, it has been observed that the effect of arsenic on microbial communities decreases the dominance of prokaryotes and turns into a population dominated by eukaryotes (Landi et al., 2000). Previously, effective removal of zinc and cadmium using MFCs has also been shown (Abourached et al., 2014). Similarly, our results indicate that treatment with arsenate interestingly promotes the growth of exoelectrogenic microorganisms in MFCs. The proposed mechanism is hypothetical and should be validated in future studies using omics approaches.

Statistical analysis

GraphPad Prism (ver. 8.0.1.) was used to prepare the graphs and perform statistical analysis, including one-way ANOVA.

Conclusion

In this study, the operating performance of microbial fuel cells was examined using a synthetic wastewater model with arsenate. According to our study results, electricity production of up to 0.2 volts was achieved with an arsenate concentration of 200

mg/L. Due to the gradual increase in arsenate concentration, voltage production also gradually decreased. Power density and current density values were reached as 46.02 ± 0.36 mW m^{-2} and 0.026 ± 0.23 mA cm^{-2} at 200 mg/L arsenate concentration. In the 200 mg/L arsenate treatment, COD removal was found to be 69±2%, and arsenic removal was 11.5%. According to microbial ecology results, arsenic treatment caused a major shift in microbial composition. After arsenic treatment, there was a significant increase in the abundance of heavy metal (arsenic)-resistant *Geobacter*, *Azospirillum,* and *Xanthobacter* genera. The relatively modest arsenate removal efficiency of 11.5% could be related to the microbial community composition and distribution across the system and operational factors. The anode samples exhibited a high abundance of *Proteobacteria* and *Desulfobacteriota*. It suggests potential competition for electrons among populations. Moreover, the planktonic samples were dominated by *Proteobacteria* and *Firmicutes*. They may be involved in alternative nonproductive metabolic pathways, further affecting electron flow. The microbial community composition differed notably between anode and planktonic samples. Arsenate exposure triggered shifts in abundance, which reflected dynamic ecological restructuring and likely impacted overall outcome. In addition, the observed rise in *Bacillus* species after arsenate exposure suggests a possible adaptive or stress response, competing metabolically, which could be used in arsenate-reducing processes instead. These community-level changes are corroborated by the Shannon index values (Table 4). These microbial dynamics highlight that arsenate reduction was likely limited by electron donor allocation, competing metabolic pathways, and community structure changes. The system operated under anaerobic conditions, with acetate as the electron donor. While designed to operate in absence of oxygen, solution refreshment allowed trace oxygen get into the system, potentially disrupting redox balance. Oxygen knows as a high-affinity electron acceptor, which could pull electrons away from arsenate and inhibit the activity of anaerobic arsenate-reducing microbes (Ucar et al., 2017). It is necessary to further explore the performance optimization. Compared to traditional wastewater treatment methods, MFCs provide several benefits such as reduced energy consumption and less sludge generation. Additionally, previous studies have shown, both mixed-culture and pure single-culture MFCs can adapt and operate at low temperatures (<10°C) (Jadhav & Ghangrekar, 2009; Tkach et al., 2016). Our research introduces several novel aspects with main focus on pentavalent As(V), rather than As(III). It offers insight into microbial interactions with arsenate, which differs from arsenite. Single-chamber reactor design reduces operation costs and complexity, making MFCs more feasible for large-scale wastewater treatment applications. Further electron donor optimization could improve arsenic-reducing microbial activity. Modification of MFCs design, like double-chamber reactor could minimize oxygen in a system to promote more stable anaerobic conditions. Bioaugmentation approach may include better microbial selection based on obtained results. Introducing or enriching microbial strains with higher arsenic-reducing capability. Electrode material replacement can improve electron transfer efficiency and provide better baseline for microbial population growth. Retention time (RT) is one of the limitations in the overall performance of microbial fuel cells. A key challenge is a lack of standardized criteria for RT within MFCs applications. While shorter RTs provide enhanced power generation, it often lowers removal efficiency. In contrast, longer RTs may improve removal efficiency (Sumisha & Haribabu, 2020). Defining and optimizing retention time together with the choice of inoculum before starting MFC experiments can be a strategic solution. Metabolic activity, growth rate, and substrate utilization patterns of the inoculated microbial community determine how quickly and efficiently pollutants are degraded or transformed.

In conclusion, arsenate-contaminated wastewater can be biologically treated with MFCs, and electricity can be produced simultaneously.

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Author Contributions

Aksana Kavaleuskaya: Designed, Performed, Analyzed, Writing -review and editing; Burak Kilinc: Designed, Performed, Analyzed, Writing -review and editing; Dilek Sever Kaya: Designed, Performed, Analyzed, Writing -review and editing;; Halil Kurt: Designed, Performed, Analyzed, Writing -review and editing; Tunc Catal: Designed, Writing -review and editing.

Conflict of Interest

The author(s) declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

Data Availability Statement

The data is available upon a reasonable request.

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Table 1. Average voltage values of all batches with a sample standard deviation, power density and maximum obtained current density.

Concentration (mg/L)	Power density (mW/m^2)	Current density $_{max}$ (mA/cm^2)
0	74.53 ± 4.9	0.033 ± 0.44
1	77.22 ± 0.46	0.033 ± 0.26
2	77.72 ± 1.47	0.033 ± 0.21
4	75.9 ± 0.46	0.033 ± 0.26
6	75.57 ± 1.85	0.033 ± 0.26
10	69.46 ± 1.33	0.032 ± 0.25
15	66.96 ± 2.18	0.031 ± 0.25
20	54.34 ± 3.15	0.028 ± 0.24
30	54.07 ± 3.53	0.028 ± 0.24
40	49.17 ± 2.62	0.027 ± 0.23
50	46.31 ± 2.34	0.026 ± 0.20
100	48.64 ± 2.61	0.026 ± 0.23
115	47.06 ± 0.36	0.026 ± 0.23
150	46.28 ± 0.72	0.026 ± 0.23
200	46.02 ± 0.36	0.026 ± 0.23
300	15.63 ± 15	0.013 ± 0.13

Table 2. Performance of microbial fuel cells (MFCs) in generating bioelectricity using mixed microbial cultures in a presence of heavy metals.

Source of inoculum/ substrate	Configuration of MFCs	Electrode material	Heavy metal	Maximum power density	Reference
Bacteria from Cr (VI)-contaminated site/ Acetate	Two-chamber MFCs	Graphite plate anode and cathode	Chromium Cr(VI)	2 400 mW/m^2	Huang et al., 2010
Mixed culture from domestic wastewater/ Acetate, glucose	Single-chamber air cathode MFCs	Carbon cloth anode and cathode	Selenium Se(IV)	2 900 mW/m^2	Catal et al., 2009
Anaerobic sludge from municipal wastewater/ Acetate	Double-chamber MFCs	Graphite plate anode and cathode	Chromium Cr(VI)	35.3 mW/m^2	Zhang et al., 2021
Raw sludge from an aeration tank/ Acetate	Double-chamber MFCs	Carbon bush anode and cathode	Arsenic As(III)	574.3 mW/m^3	Guo et al., 2021

**Surface power density is expressed in mW/m², representing power output per unit electrode area, while volume power density is expressed in mW/m³, indicating power generation per unit reactor volume.*

Table 3. Optical density and chemical oxygen demand results (0, 100, 200, 300 mg/L of As).

Concentration (mg/L)	Operation	OD*$_{600nm}$	COD* removal (%)	Arsenic Removal (%)
0	Before	0.008 ± 0.001	62 ± 3	*n.d.*
	After	0.055 ± 0.001		
100	Before	0.012 ± 0.00	53 ± 2	*n.d.*
	After	0.050 ± 0.00		
200	Before	0.016 ± 0.006	69 ± 2	*11.5%*
	After	0.046 ± 0.00		
300	Before	0.012 ± 0.00	48 ± 1	*n.d.*
	After	0.047 ± 0.00		

**OD$_{600nm}$: Optical density of the influent and effluent solutions at 600 nm*

**COD: Chemical oxygen demand*

n.d.: not determined

Table 4. *Microbial ecology Shannon index values.*

Samples	*shannon_entropy*	*faith_pd*	*pielou_evenness*	*observed_features*
CF1	*5.470652614*	*14.74054096*	*0.713027807*	*204*
CF2	*4.255257143*	*14.8505101*	*0.560458508*	*193*
PR1	*5.212502263*	*17.83239252*	*0.658735645*	*241*
PR2	*2.522235984*	*20.813822*	*0.352772754*	*142*
PR3	*4.536229881*	*13.65161243*	*0.616540648*	*164*

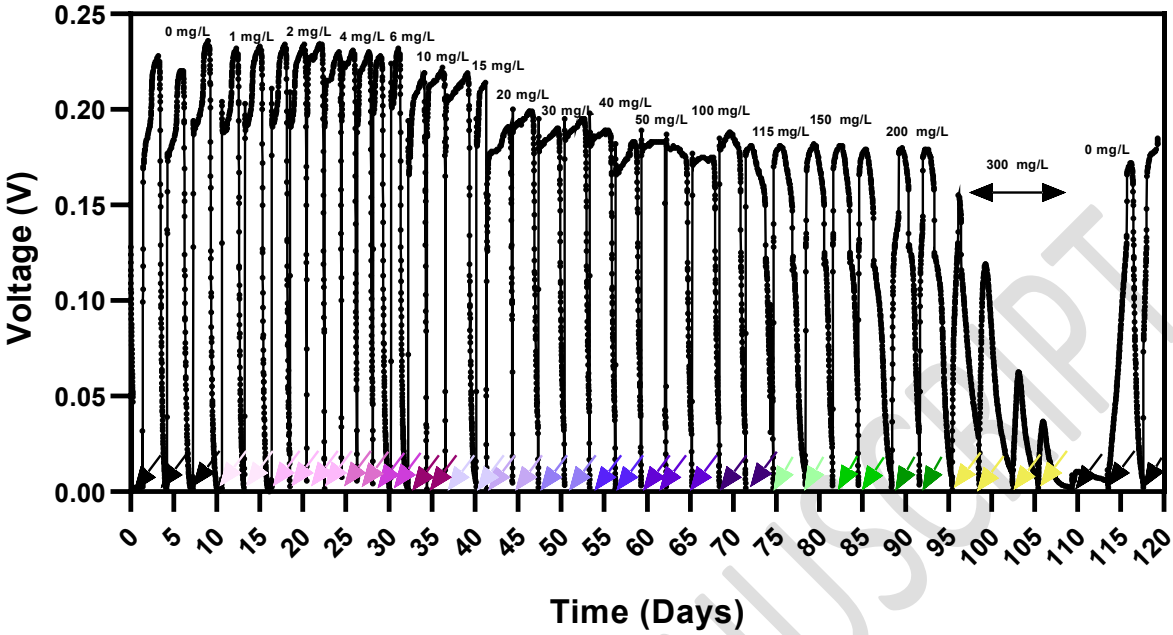

Figure 1. Output voltage generation at arsenate concentrations (0–300–0 mg/L) in 20 mM acetate solution (1000 Ω). Arrows indicate medium replacement. Voltage decline at 300 mg/L (marked by a double-headed arrow) suggests inhibition, followed by recovery upon removal of As from the operational system.

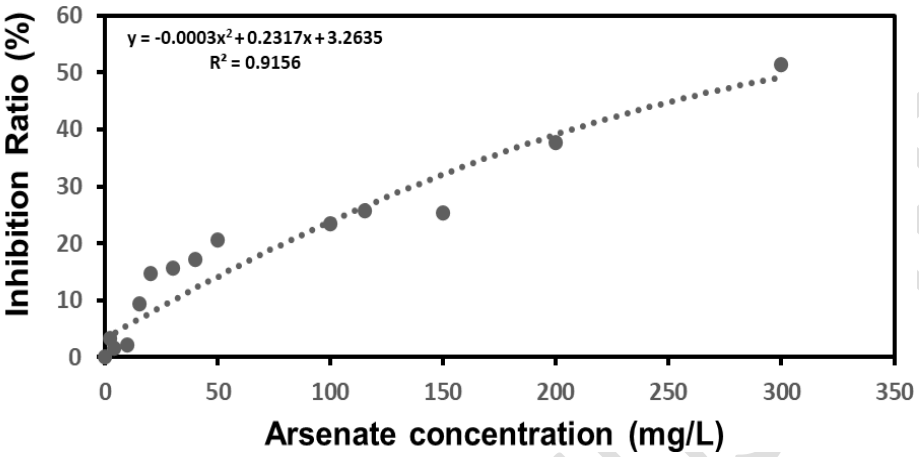

Figure 2. Inhibition ratio (%). Concentration of Arsenate (0 - 300 mg/L).

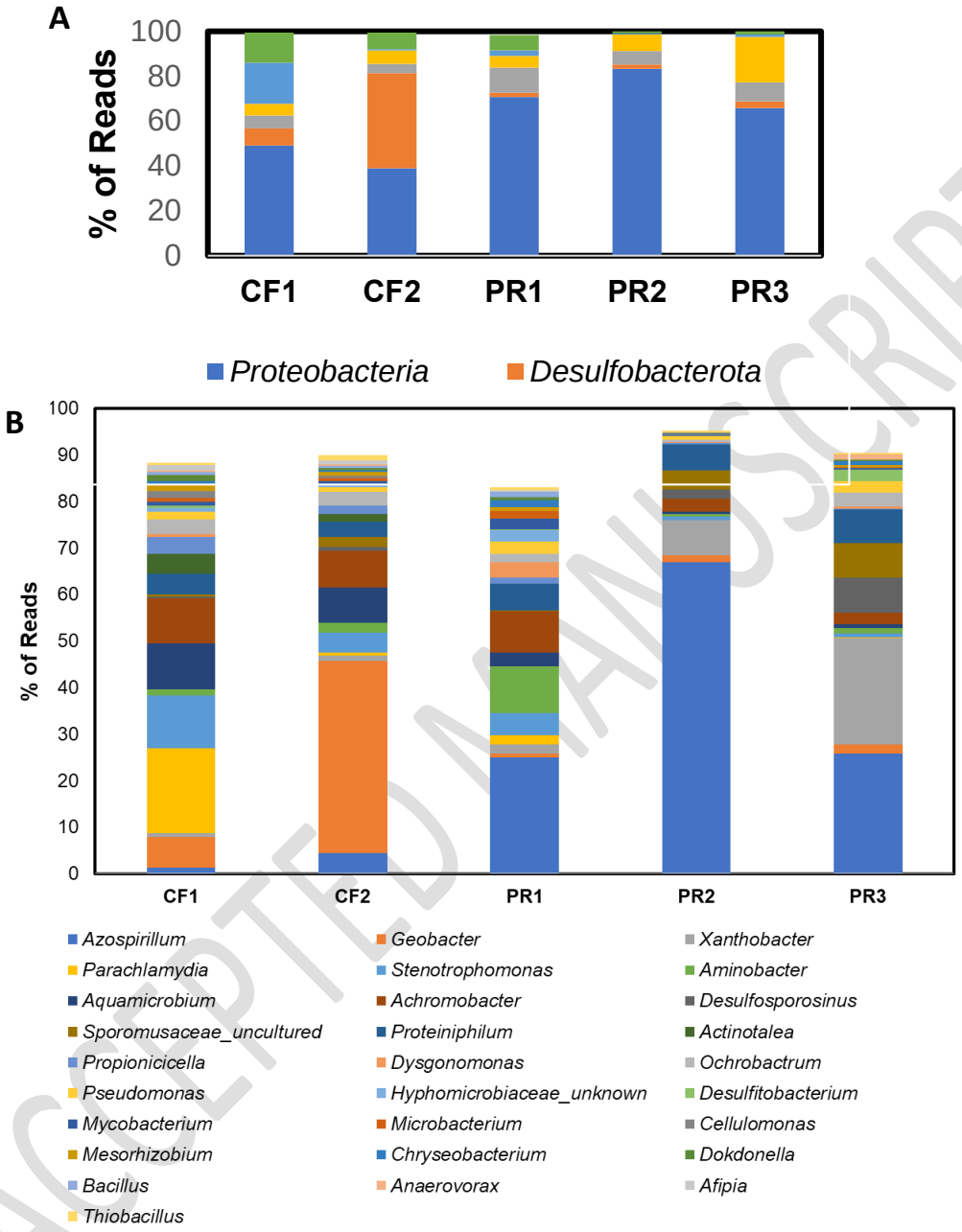

Figure 3. Microbial diversity at the phylum level (A) and genus level (B) in anode biofilm (CF1: before arsenate treatment; CF2: after arsenate treatment) and planktonic colonies (PR1: before arsenate treatment; PR2-3: after arsenate treatment at 15 mg/L and 40 mg/L, respectively).

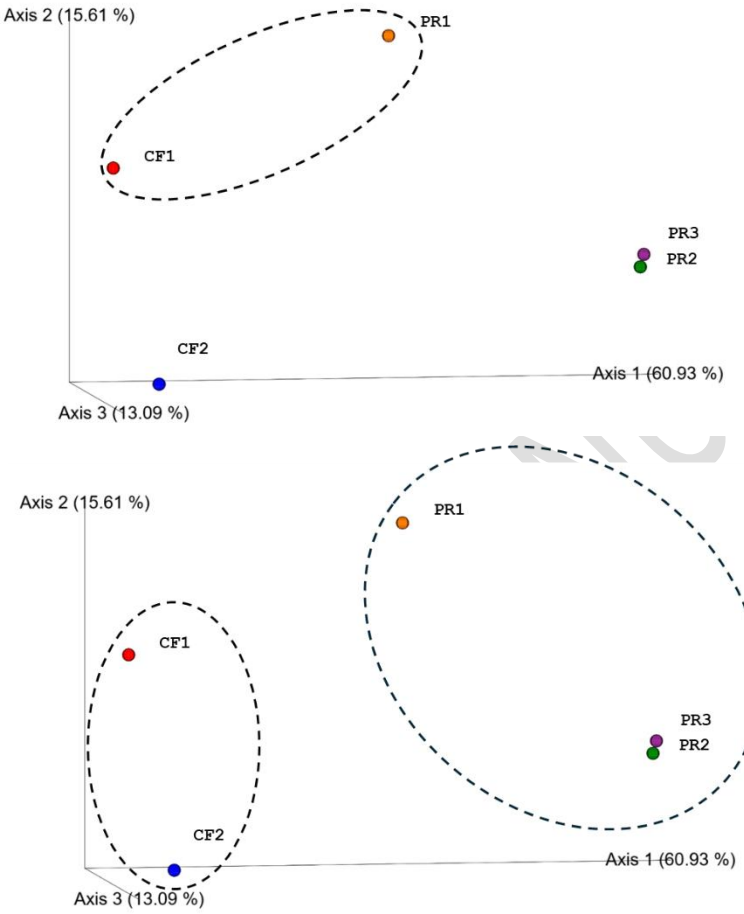

Figure 4. Microbial ecology clusters.