Archives of Environmental Protection
Vol. 49 no. 4 pp. 27–36

PL ISSN 2083-4772
DOI 10.24425/aep.2023.148683



© 2023. The Author(s). This is an open-access article distributed under the terms of the Creative Commons Attribution-ShareAlike 4.0 International Public License (CC BY SA 4.0, https://creativecommons.org/licenses/by-sa/4.0/legalcode), which permits use, distribution, and reproduction in any medium, provided that the article is properly cited.

Electricity generation in a microbial fuel cell with a ceramic separator utilizing a bacterial consortium for penicillin removal

Pimprapa Chaijak*, Alisa Kongthong, Junjira Thipraksa, Panisa Michu

Thaksin University, Thailand

* Corresponding author's e-mail: chaijak.pimprapa@gmail.com

Keywords: bioelectricity generation, biodegradation, laccase, microbial fuel cell, penicillin, swine wastewater

Abstract: The contamination of the environment by antibiotics has become a serious problem, supported by abundant scientific evidence of its negative impact on both aquatic ecosystems and human health. Therefore, it is crucial to intensify research efforts towards developing effective and efficient processes for removing antibiotics from the aquatic environment. In this study, a bacterial consortium capable of breaking down penicillin was employed in a ceramic separator microbial fuel cell (MFC) to generate electricity. The consortium's properties such as laccase activity, penicillin removal and microbial structure were studied. The SF11 bacterial consortium, with a laccase activity of 6.16±0.04 U/mL, was found to be effective in breaking down penicillin. The highest rate of penicillin removal (92.15±0.27%) was achieved when the SF11 consortium was incubated at 30 °C for 48 hours. Furthermore, when used as a whole-cell biocatalyst in a low-cost upflow MFC, the Morganella morganii-rich SF11 consortium demonstrated the highest voltage and power density of 964.93±1.86 mV and 0.56±0.00 W/m3, respectively. These results suggest that the SF11 bacterial consortium has the potential for use in ceramic separator MFCs for the removal of penicillin and electricity generation.

Introduction

The escalating global population growth poses a significant challenge in addressing the mounting demands for both food security and sustainable practices (Prosekov and Ivanoa 2018, Jahan et al. 2022). Primary protein sources for meat-consuming populations come from feedstock products, with pork and poultry standing out as the most prominent choices (Tsai, 2018). Consequently, this scenario has led to an increase in swine farms to meet these demands. However, a pressing environmental concern arises due to the generation of substantial amounts of steroidal hormones and veterinary antibiotics in swine excreta, which subsequently find their way into the environment (Zhang et al. 2017). In light of this situation, prior research has indicated that each pig produces approximately 4-8 liters of swine wastewater per day (Garcia et al. 2017).

The widespread use of swine wastewater in pork production has rendered it a significant source of hormones and antibiotics in the environment. This raises serious concerns about the direct discharge of hormone- and antibiotic-contaminated swine wastewater into the environment, given its potential adverse effects on human and aquatic organism health (Cheng et al. 2020). In pig farms, antibiotics play a crucial role in preventing and treating microbial infections, as they possess the ability to kill microbes and inhibit their growth (Sekyere 2014). Additionally, in swine production, certain antibiotics, such as tetracycline, sulfonamide, and penicillin are extensively utilized as growth promoters (He et al. 2016, Kim et al. 2013).

Penicillin, a widely used β-lactam antibiotic, plays a crucial role in treating infectious diseases caused by penicillin-sensitive bacteria, including upper respiratory infections and bacterial pneumonia in feedstock animals such as cattle, sheep, and swine (Portis et al. 2012, Vogel et al. 2001). Despite it efficacy, a concerning observation from a previous study reveals that approximately 50–90% of antibiotics are released in their active form through animal excretion (Feng et al. 2017). Addressing the issue of antibiotic-contaminated swine excreta, anaerobic digestion has been a conventional approach due to its environmentally friendly attributes (Sun et al. 2019). Nevertheless, the presence of penicillin contamination has been shown to diminish the excreta digesting potential by up to 35% (Masse et al. 2000). As an alternative solution, enzyme-based bioremediation emerges as a promising technology for removing antibiotics from wastewater, with laccase being a particularly notable microbial enzyme belonging to the oxidoreductase group. Laccase exhibits the ability to reduce oxygen molecules, leading to the one-electron oxidation of antibiotics (Piontek et al. 2002). Moreover, recent investigations have demonstrated the efficacy of microbial

laccase in degrading β-lactam drugs, attributing this capability to its potential for β-lactam ring deformation (Mukhopadhyay et al. 2022).

In recent decades, the steady rise in global energy consumption has led to an impending energy crisis, prompting the urgent exploration of alternative and renewable energy sources (Rahman et al. 2022). Among these alternatives, the microbial fuel cell (MFC) has emerged as a promising contender, garnering significant interest for its unique ability to utilize diverse organic matter as fuel (Rahimnejad et al. 2015). The MFC operates by employing exoelectrogenic bacteria that act as whole-cell biocatalysts on the anodic electrode, facilitating the degradation of organic substrates while generating electrons and protons in the process (Guang et al. 2020, Rahimnejad et al. 2011). To function effectively, the MFC incorporates a proton exchange membrane (PEM) that physically separates the cathodic part from the anodic part (Ghasemi et al. 2013). However, despite the promising potential of MFC technology, certain limitations have impeded its practical application. High structural costs, particularly associated with the expensive PEM, have been a significant obstacle (Das et al. 2020).

The use of earth clay-based membranes as cost-effective and durable proton exchange membranes (PEMs) in microbial fuel cells (MFCs) has sparked significant interest, driven by their exceptional chemical stability, ability to withstand high pressure, and straightforward production process. These ceramic separators have emerged as the most promising option for industrial-scale MFC applications (Ahilan et al. 2019, Ghadge et al. 2016, Rossi et al. 2019, Yousefi et al. 2017). Various clay materials have been explored in the development of ceramic separators, including terracotta, black soil (rich in calcium, iron, and magnesium) (Ajayi and Weigele 2012), red soil (containing aluminum and silica) (Ghadge et al. 2014), and composite clay (comprising kaolinite and montmorillonite) (Ghadge and Ghangrekar 2015). The distinctive properties of these clay-based membranes, including their abundance, accessibility, and low-cost nature, make them highly appealing for practical implementation in MFC systems.

In the quest for suitable materials for proton exchange membranes (PEMs) in microbial fuel cells (MFCs), akadama clay, a metal-containing red soil, has emerged as a compelling candidate. Its remarkable characteristics, including high osmotic ability, strong water impounding, and efficient drainage, have made it extensively utilized in the fields of agriculture and horticulture (Chen et al. 2010). The composition of akadama clay typically consists of 21.83-78.58% silicon dioxide (SiO_2), 4.13-38.00% aluminum oxide (Al_2O_3), and 0.84-7.70% ferrous oxide (Fe_2O_3) (Bhakta and Munekage 2013). In addition to its agricultural applications, akadama soil has displayed intriguing potential in the removal of hazardous metals from aqueous solutions. Previous studies have highlighted its effectiveness in capturing cesium (Ding et al. 2014), as well as other metals such as cadmium, zinc, copper, arsenic (Nguyen et al. 2021), mercury (Bhakta and Munekage 2011), and chromium (Ji et al. 2015).

The upflow microbial fuel cell (UMFC) has emerged as a promising design, drawing significant interest for its potential to achieve efficient power output while simultaneously addressing wastewater treatment needs (Bhakta and Munekage 2011). Key to the success of UMFCs is the selection of an appropriate proton separator, which plays a critical role in facilitating proton transfer within the system. Researchers have explored various materials for use as proton separators in UMFCs, each offering distinct advantages. Nafion 117 has been studied as a potential separator, showcasing its unique characteristics in enhancing UMFC performance (Subha et al. 2019). Additionally, gravel has been considered as an alternative material, demonstrating its suitability for facilitating proton transport in UMFC setups (Li et al. 2019). Quartz sand has also been investigated for its potential as a proton separator, contributing valuable insights into its application in UMFC technology (Ge et al. 2020). Furthermore, ceramsite has been evaluated as a promising candidate, shedding light on its performance and viability in UMFCs (Xu et al. 2019).

The primary objective of this study was to isolate and characterize a bacterial consortium capable of efficiently degrading penicillin extracted from swine wastewater sediment. Subsequently, the selected highly penicillin-degrading consortium was employed in an upflow microbial fuel cell (UMFC) setup, with the innovative utilization of an Akamada clay separator for electricity generation. The aim was to investigate the performance and effectiveness of this penicillin-degrading consortium in the UMFC system, with a focus on its potential application in sustainable wastewater treatment and electricity production. The flowchart of this process is depicted in Figure 1, illustrating the sequential steps involved in isolating the consortium, integrating it into the UMFC, and monitoring its penicillin degradation capabilities and electricity generation efficiency. By addressing the efficient removal of penicillin from wastewater and harnessing it for electricity production, this study seeks to contribute to the advancement of eco-friendly technologies with implications for both environmental protection and renewable energy generation.

Materials and Methods

Sampling

One hundred sediment samples were collected from swine wastewater in Phatthalung Province, Southern Thailand using aseptic technique. The samples were then transported to the

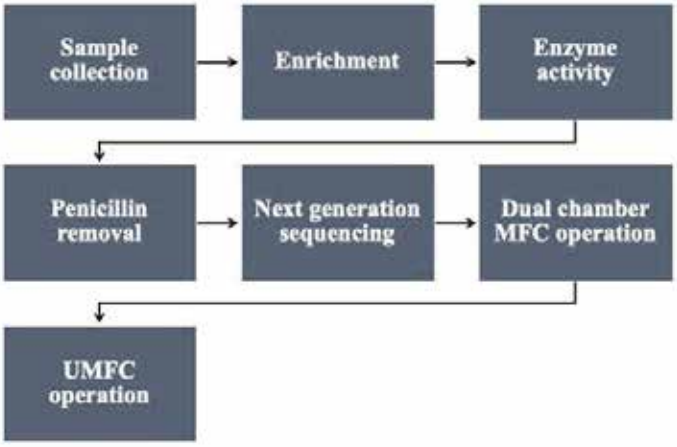


Figure 1. The flowchart of study process.

Microbial Fuel Cell and Bioremediation Laboratory at the Faculty of Science, Thaksin University, for further analysis. All samples were carefully maintained at a temperature of 4 °C until they were used for bacterial enrichment.

Enrichment

A modified formula was used to prepare an enrichment buffer for cultivating a group of bacteria capable of degrading penicillin. The buffer contained 1.0 g/L of yeast extract, 0.03 g/L of $CaCl_2$, 0.08 g/L of $MgCl_2 \times 6H_2O$, 0.05 g/L of KH_2PO_4, 0.11 g/L of $(NH_4)_2SO_4$, and 0.01 g/L of $NaHCO_3$ (Thipraksa and Chaijak, 2022). Sterilization was achieved by adding 100 mg/L of penicillin to the buffer.

To initiate the bacterial enrichment process, 10 g of sediment was added to 100 mL of the enrichment buffer and incubated at 30 °C for 48 hr without shaking. Subsequently, 10 mL of the growing liquid culture was transferred into 90 mL of fresh enrichment buffer and incubated at 30 °C for 48 hr without shaking. This process was repeated ten times to ensure that the bacterial culture was capable of utilizing penicillin as the sole carbon source.

Selection of bacterial consortium

The selection of bacterial consortia was based on their laccase activity and their efficient ability to remove penicillin. The inoculation process involved the addition of 4 mL of bacterial consortium (1 x 10^8 cell/mL) into 36 mL of buffer containing 100 mg/L of penicillin, followed by incubation for 48 hr without shaking. After incubation, the buffer was centrifuged at 12,000 rpm for 10 min at 4 °C, and the resulting supernatant was collected for laccase activity determination.

The laccase activity was measured using spectrophotometry at a wavelength of 420 nm. The measurement was carried out using 950 µL of reaction reagent containing 0.5 mM 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) in a 0.1 M sodium acetate buffer with pH 4.5 and 5 µL of supernatant. The laccase activity was calculated based on the oxidation of ABTS and defined as 1.0 mmol of ABTS oxidized per minute (More et al. 2011).

To evaluate the bacterial culture's ability to remove penicillin, the penicillin removal rate (%) was monitored using UV-Vis spectroscopy at 325 nm (Krasnikova and Iozep, 2003). The experiment was conducted in triplicate, and the bacterial consortium that demonstrated a penicillin removal rate greater than 80% was chosen.

Penicillin removal in swine wastewater

A modified formula was used to prepare synthetic swine wastewater (Cheng et al. 2020), which included 3 g/L of glucose, 0.45 g/L of NH_4Cl, 0.13 g/L of KH_2PO_4, 0.05 g/L of $MgSO_4$, and 0.01 g/L of $CaCl_2$. The wastewater was sterilized at 121 °C for 15 minutes, and penicillin was subsequently added to adjust the final concentration to 100 mg/L.

4 mL of enriched mixed culture (1 x 10^8 cell/mL) was inoculated into the 36 mL of synthetic swine wastewater filled in the 50 mL centrifuge tube. The reactions were incubated at 30 °C for 48 hr without shaking. The experiment was carried out in triplicate. The penicillin removal rate (%) was monitored, and the bacterial consortium that demonstrated the highest potential for penicillin removal was selected.

Next-generation sequencing

The selected bacterial consortium was cultured in nutrient broth containing 100 mg/mL of penicillin. Following 48 hr of incubation, the cells were harvested by centrifugation at 12,000 rpm for 10 minutes at 4 °C. 1 g of cell pellet was utilized to extract genomic DNA using the QIAwave DNA Blood and Tissue Kit (Qiagen, Germany). The quality of the extracted genomic DNA was examined using a nanodrop spectrophotometer, and only high-quality DNA was utilized for next-generation sequencing analysis.

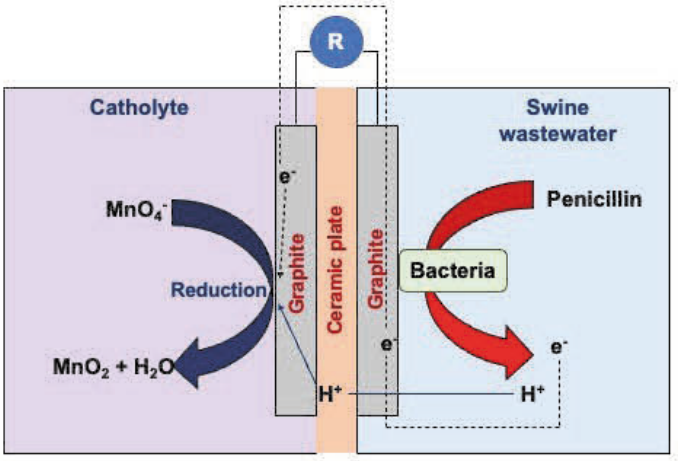


Figure 2. The diagram of the dual chamber MFC.

Dual-chamber MFC operation

The construction of the microbial fuel cell (MFC) was carried out according to the design depicted in Figure 2. To serve as the electrode, a 25 cm^2 graphite plate was utilized, and it was activated using a microwave (Kim et al. 2022). The activation process involved submerging the graphite plate into 10 mL H_2O_2 and gently stirring it for 60 min at room temperature. Next, 100 mL H_2SO_4 was slowly added and stirred for 60 min at 40 °C. The graphite plate was then washed thrice using deionized water and dried for 24 hr at 80 °C. Finally, the graphite plate was activated using a microwave for 60 min. A 2 mm thick 30% (w/v) silica-ceramic plate prepared at 680 °C was used as a low-cost proton exchange membrane (Michu and Chaijak, 2022). The anode and cathode chambers were created using 40 mL cell culture bottles, and the electrodes were linked with copper wire.

To operate the MFC, 4 mL of the selected bacterial consortium aged 48 hr, along with 36 mL of synthetic swine wastewater, were introduced into the anode chamber. The cathode chamber was filled with 40 mL of 400 µM KMnO4, which serves as an effective electron acceptor (Eliato et al. 2016). The consortium was allowed to accumulate in the anode chamber for 5 days to immobilize the bacterial cells onto the anode electrode surface. Subsequently, the anolyte was removed, and 40 mL of synthetic swine wastewater was added.

The open-circuit voltage (OCV) was monitored hourly using a digital multimeter, while the closed-circuit voltage (CCV) was measured at an external resistance of 100-3,000 Ω. Current (I), power (P), current density (CD), and power density were calculated according to Ohm's law.

$$I = V/R \quad (1)$$

$$P = IV \quad (2)$$

$$CD = I/A \quad (3)$$

$$PD = P/A \quad (4)$$

Where the V is the CCV (V), I is the current (A), R is the external resistance (Ω), P is the power (W), CD is the current density (A/m^3), A is the working volume (m^3), and PD is the power density (W/m^3).

Ceramsite preparation

A modified method was utilized to produce ceramsite, which is a lightweight ceramic ball using Akadama clay (Shang et al. 2022). The clay has a pH of 6.9, an electroconductivity (EC) of 0.052 ms/cm, and a water absorption rate of 20%. The composition of the clay is as follows: 42.7% SiO_2, 0.98% CaO, 2.5% MgO, 0.15% MnO, 8.4% Fe_2O_3, and 25.1% Al_2O_3.

The process of creating ceramsite began with the drying of Akadama clay at 105 °C until it reached a constant weight. Subsequently, the dried clay was ground and passed through a 0.12-mm sieve. The next step involved adding 30% (w/w) of deionized water to the sieved clay and mixing it thoroughly. The resulting mixture was then molded into 1 cm sizes and dried overnight at 105 °C until it attained a constant weight. The final step involved sintering the dried ceramsite at 1100 °C for 30 min.

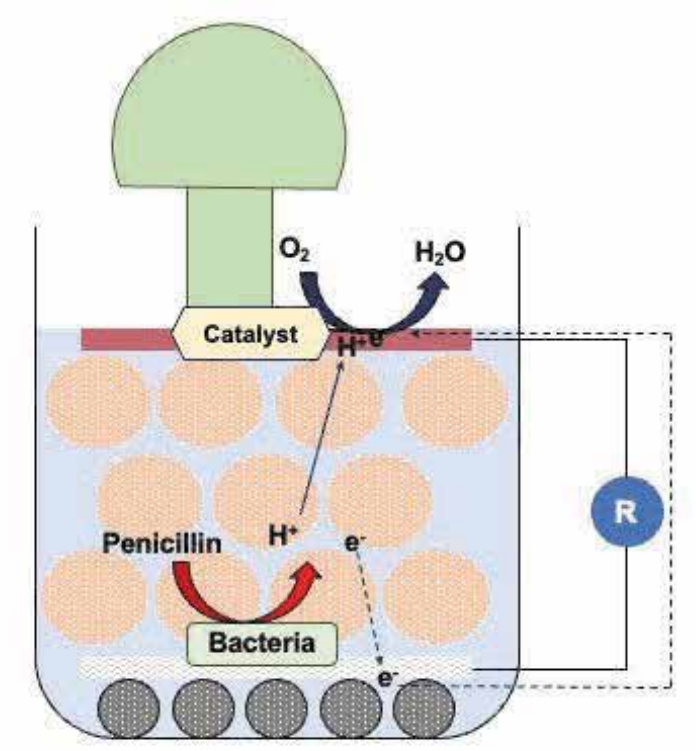


Figure 3. The diagram of the UMFC.

UMFC operation

In this experiment, the UMFC (Upflow Microbial Fuel Cell) system as shown in Figure 3, was utilized. The MFC chamber had a working volume of 1,000 mL and was constructed from plastic containers. The anodic electrode was fabricated from water hyacinth biochar (WHB) and stainless-steel mesh, according to the previous procedure (Chaijak and Michu, 2022). Specifically, the WHB was pyrolyzed at 350 °C for 30 minutes, and subsequently immersed in 500 mL of 0.5 M HNO_3 at room temperature for 12 hours. The WHB was then washed with deionized water thrice and dried at 80 °C for 24 hours. A copper-plated electrode with dimensions of 1 cm x 5 cm was used as the cathodic electrode. Akadama clay ceramsite (500 g) was added between the two electrodes. To degrade penicillin, 100 mL of penicillin-degrading bacteria consortium was mixed with the 900 mL of synthetic swine wastewater, which was used as the anolyte. The UMFC was operated under batch conditions.

Furthermore, the edible mushroom *Pleurotus pulmonarius* was employed as a whole-cell biocatalyst and placed on the cathodic surface (Chaijak and Thipraksa, 2022). Specifically, the *P. pulmonarius* was cultured in a sucrose-yeast medium containing 10% (w/v) of sucrose and 0.1% (w/v) of yeast extract and it was incubated at room temperature for 7 days. The fungal cells were then placed on the surface of the cathodic electrode and incubated for another 7 days. The open-circuit voltage (OCV) was monitored hourly. Additionally, the polarization curve of the bacterial consortium was plotted.

Results and Discussion

Selection

In order to select a bacterial consortium that could utilize penicillin as its sole source of carbon, several samples were inoculated into a penicillin solution for 10 consecutive batches. The activity of laccase, an enzyme that breaks down certain organic compounds, was monitored and only 16 of the tested consortia showed laccase activity. Among these consortia, consortium SF11 exhibited the highest laccase activity, with a value of 6.16±0.04 U/mL, as shown in Figure 4. Ambika et al. (2022) previously selected a laccase-producing consortium with maximal laccase activity of 0.031 U/mL from soil samples of decaying wood, plant rhizosphere, and lake sediment of the Himalayan region. Additionally, a previous study showed that a bacterial consortium selected from forest soil had maximal laccase activity of 22.00 U/mL (Thipraksa et al. 2022).

The ability of all laccase-producing bacterial consortia to degrade penicillin in a solution was measured, and only the consortium with a penicillin removal ability exceeding 80%

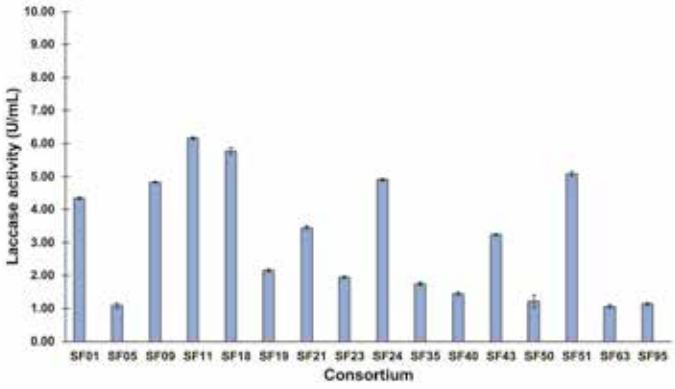


Figure 4. Laccase activity of the enriched consortia.

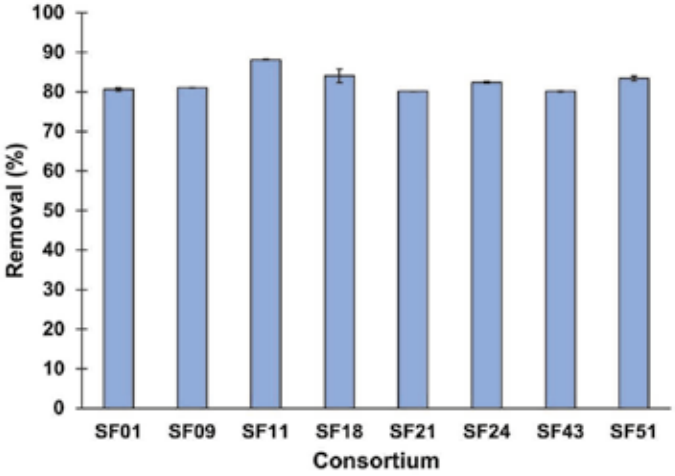


Figure 5. Penicillin removal in the 100 mg/L penicillin-containing buffer.

was selected for further study, as shown in Figure 5. The results indicated that consortium SF11 had the highest penicillin removal ability of 88.08±0.11%, followed by consortium SF18 with 84.07±1.69%, without any shaking or adjustment of pH.

In another study, *Bacillus velezensis*, a bacterium that produces oxidoreductase, demonstrated the ability to degrade 89 mg/L of antibiotic from synthetic wastewater after 10 days of incubation (Al-Dhabi et al. 2021). Additionally, Copete-Pertuz et al. (2018) reported that a native fungus, *Leptosphaerulina* sp. significantly removed penicillin from synthetic hospital wastewater after a 15-day incubation period at 28 °C with shaking at 160 rpm and pH 5.6.

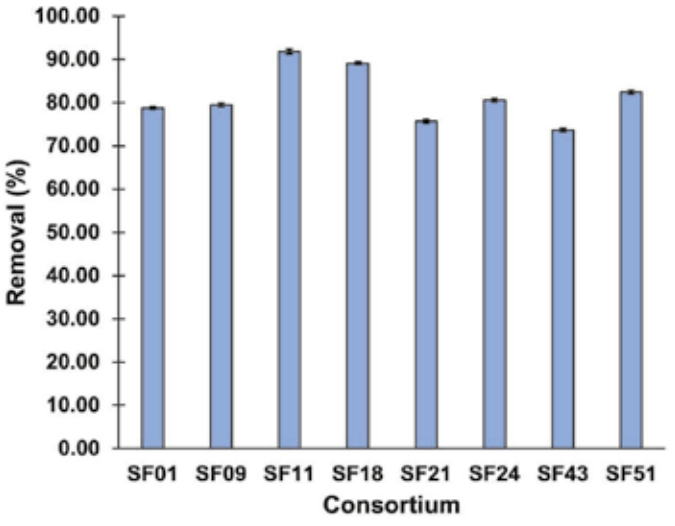


Figure 6. Penicillin removal in the 100 mg/L penicillin-containing synthetic swine wastewater.

Figure 6 shows the penicillin removal ability of the laccase-producing consortium from synthetic swine wastewater. The highest penicillin removal ability of 91.82±0.58% was achieved by the SF11 consortium, which was incubated at 30 °C for 48 hours without shaking. According to the study conducted by Wang et al. (2019), antibiotic-contaminated swine wastewater was treated using aerobic granular sludge that was selected from a combination of raw swine wastewater and domestic sewage. The researchers found that the highest level of antibiotic removal, amounting to 89.40%, was achieved when the treatment process was carried out in a bioreactor. In contrast, a study utilized the bacterial community present in the rhizosphere of the common reed *Phragmites australis* (Cav.) to remove antibiotics from swine wastewater. The results showed that after 20 weeks of operation, the maximum level of antibiotic removal exceeded 90.00% (Santos et al. 2019).

Microbial diversity

The utilization of 16S rDNA amplicon sequencing on the Illumina Miseq platform allowed us to comprehensively explore the bacterial consortium SF11 at the taxonomic level. This next-generation sequencing (NGS) approach provided a rich dataset, encompassing 175,000 reads with a total of 52.7M base pairs. The analysis of the sequencing data using bioinformatic tools revealed key characteristics of SF11. The evaluation of GC content (%) and Q30 quality score demonstrated the robustness of the sequencing data, with the values of 53.17% and 87.41%, respectively, ensuring the reliability of subsequent analyses. The investigation of diversity and richness in the bacterial consortium SF11, as presented in Table 1, provided valuable insights into the microbial community's composition. Figure 7 further illustrated the diversity observed in SF11.

Table 1. Community richness and diversity of the consortium SF11.

Consortium	OTUs	Chao1	Shannon	Gini-Simpson
SF11	11	11	1.81	0.65

At the phylum level, the bacterial community in SF11 was dominated by Proteobacteria, representing 80.95% of the total, followed by Firmicutes at 19.04% and Actinobacteria at a marginal 0.01%. The class-level analysis reaffirmed the predominance of Gammaproteobacteria, accounting for 80.95% of the bacterial community. Clostridia constituted 13.88%, Bacilli made up 5.16%, and Actinomycetia was present at a negligible 0.01%.

Species-level analysis allowed for the identification of specific bacterial constituents within SF11. *Morganella morganii* and *Pseudomonas multiresinivorans* were the most prevalent species, comprising 45.58% and 35.25% of the consortium, respectively. Other notable species included *Clostridium senegalense* (11.04%), *Lysinibacillus sphaericus* (4.83%), *Clostridium tepidum* (2.84%), *Aneurinibacillus aneurinilyticus* (0.27%), *Pseudomonas jinjuensis* (0.11%), *Paenibacillus timonensis* (0.05%), and *Leucobacter chromiireducens* (0.01%).

In another study, a bacterial consortium capable of degrading antibiotics was successfully created by enriching soil contaminated with penicillin. The consortium comprised diverse bacterial species, including *Sphingomonas* sp., *Diaphorobacter* sp., *Acidovorax* sp., *Stenotrophomonas* sp., and *Mycobacterium* sp. (Kusada et al. 2019). This achievement highlights the potential of harnessing natural

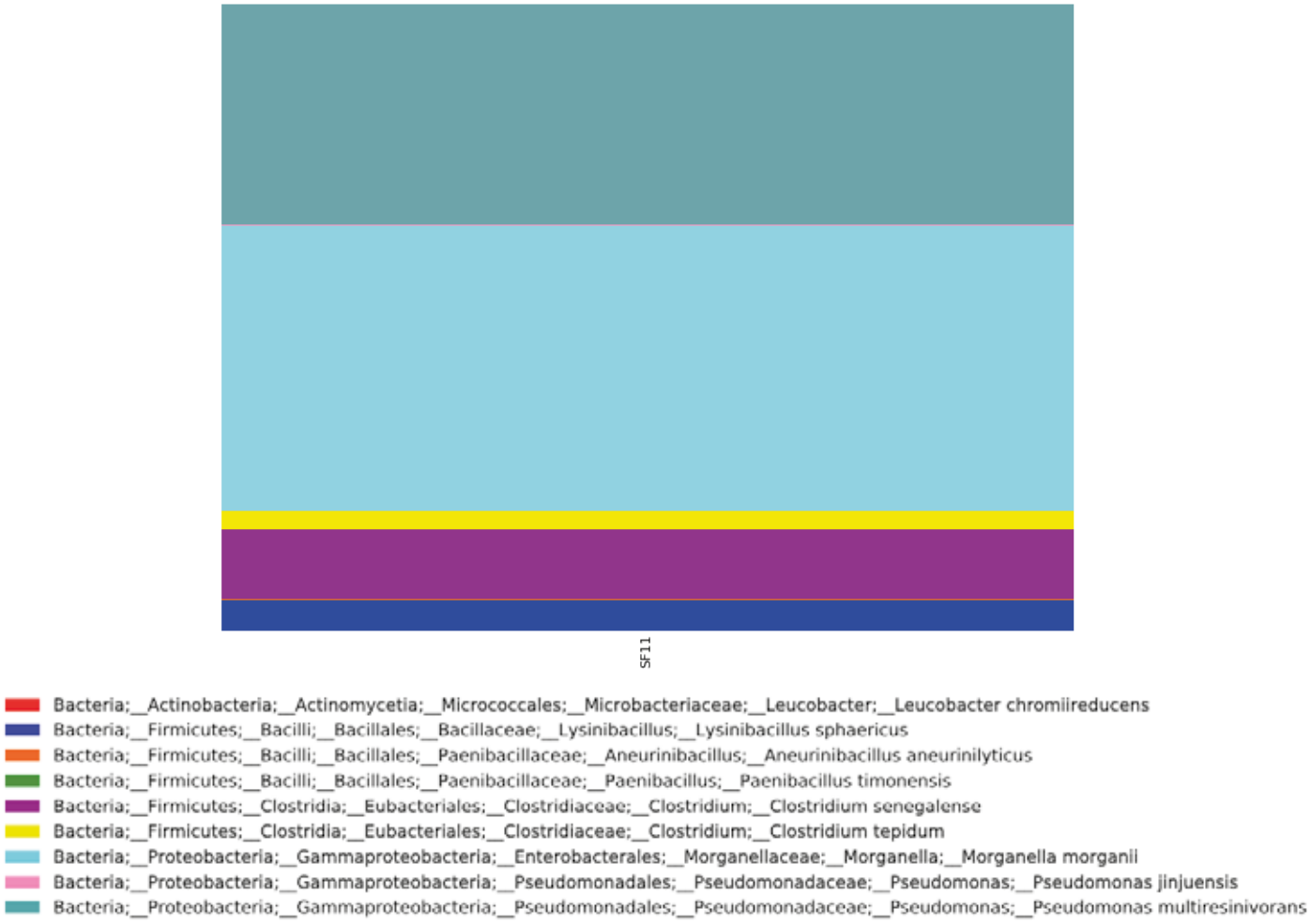


Figure 7. Microbial structure of the consortium SF11.

microbial communities for the remediation of antibiotic-contaminated environments. Another noteworthy finding from the literature is the successful use of *Pseudomonas aeruginosa* for antibiotic degradation (Sunder et al. 2017). This particular species has demonstrated its ability to contribute significantly to the breakdown of antibiotics, emphasizing the importance of exploring and utilizing different bacterial strains for their biodegradation capabilities. Furthermore, Yan et al. (2022) discovered a novel approach to enhance antibiotic resistance by utilizing a co-culture of heterotrophic and nitrifying bacteria. This unique co-culture demonstrated increased resistance to antibiotic biotoxicity, showcasing the potential benefits of integrating diverse microbial communities to improve antibiotic degradation efficiency.

Electrochemical properties of dual-chamber MFC

The results obtained from this study demonstrate the successful utilization of the penicillin-degrading bacterial consortium SF11 as a biocatalyst in the anodic chamber of the microbial fuel cell (MFC) setup. The highest open circuit voltage (OCV) of 895.00 ± 4.71 mV was achieved during the stationary phase, as depicted in Figure 8. Furthermore, the current density (CD) and power density (PD) generated by the CMFC were determined by analyzing the polarization curve, shown in Figure 9. The maximum CD reached 16.25 ± 0.01 A/m^3, while the maximum PD attained a value of 3.12 ± 0.00 W/m^3. Comparing our findings with other relevant studies, it is evident that MFC technology offers considerable potential for electricity generation from swine wastewater. In a study conducted by Ni et al. (2020), the H-type microbial fuel cell was utilized to produce electricity from swine wastewater.

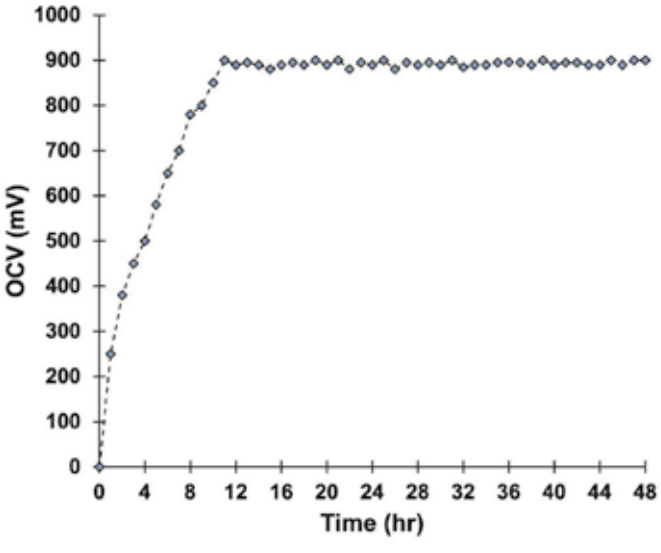


Figure 8. Open circuit voltage of the conventional MFC with consortium SF11.

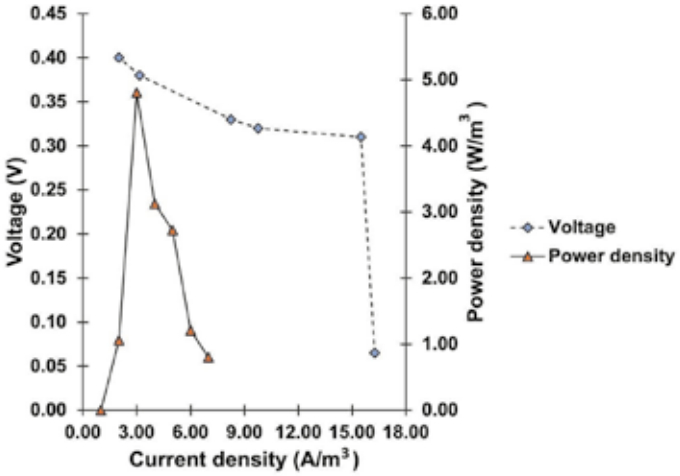


Figure 9. Polarization curve of the conventional MFC with consortium SF11.

The anodic biofilm primarily comprised Proteobacteria, Bacteroidetes, Firmicutes, Chloroflexi, and Spirochaetae, leading to a maximum voltage output of 610 V. Moreover, Ding et al. (2014) employed a combination of MFC and flocculation for untreated swine wastewater treatment, achieving the highest power density of 37.5 W/m^3. Different MFC configurations have also been explored for swine wastewater treatment and electricity generation. For instance, the single-chamber MFC demonstrated a maximum power output of 0.6-2.2 W/m^3 with a hydraulic retention time (HRT) of 3-5 days (Goto and Yoshida, 2019). Additionally, Liu et al. (2020) employed a constructed wetland-MFC with the presence of the macrophyte Canna indica for swine wastewater treatment, resulting in a peak voltage output of 715 mV. Overall, the outcomes of this study highlight the feasibility and potential of the penicillin-degrading bacterial consortium SF11 in the context of microbial fuel cells. The obtained OCV, CD, and PD values demonstrate promising electricity generation capabilities using swine wastewater as a substrate. These findings contribute to the expanding knowledge base surrounding MFC technology and its applications in sustainable wastewater treatment and renewable energy production. As further research is conducted in this field, the optimization and implementation of MFC systems for practical applications can pave the way for more efficient and eco-friendly solutions to address the challenges of wastewater management and energy sustainability.

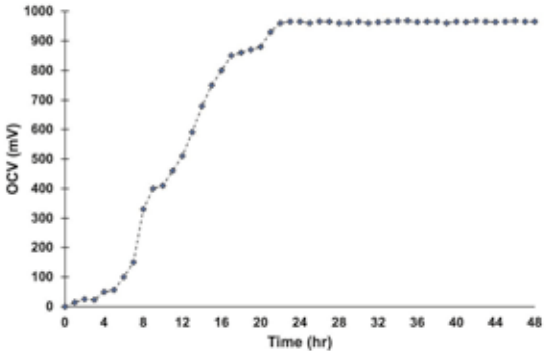


Figure 10. Open circuit voltage of the UMFC with consortium SF11.

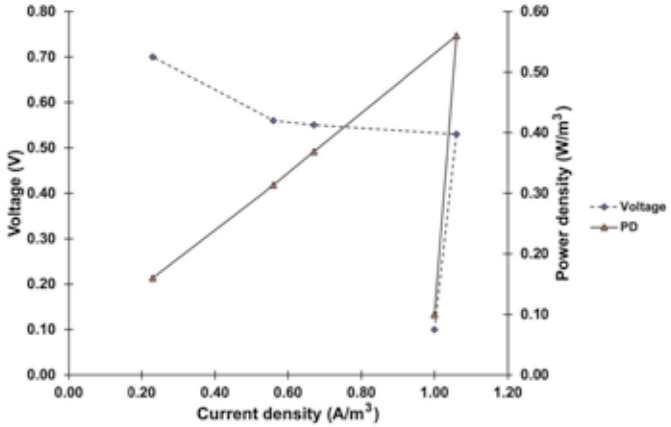


Figure 11. Polarization curve of the UMFC with consortium SF11.

Electrochemical properties of UMFC

The findings of this study demonstrate the impressive electricity generation capabilities of the penicillin-degrading bacterial consortium SF11 in the upflow microbial fuel cell (UMFC) setup. Over a monitoring period of 48 hours, the maximal open circuit voltage (OCV) generated by SF11 was consistently high, averaging 964.93±1.86 mV, as depicted in Figure 10. The polarization curve of the UMFC revealed that SF11 achieved a maximal current density (CD) of 1.06±0.01 A/m^3 and a maximal power density (PD) of 0.56±0.00 W/m^3, as shown in Figure 11. Comparing these results with previous studies, it is evident that the performance of SF11 in the UMFC system is noteworthy. For instance, in a dual-chamber MFC employed

Table 2. Electricity generation performance of MFC.

MFC type	Microbe/ Consortium	Power output (mV)	Reference
UMFC	SF11	964.93±1.86	This study
Dual chamber	Anaerobic sludge	610.00	Gota et al. (2019)
CW-MFC	Soil bacteria	440.00	Ren et al. (2021)
Dual chamber	Anaerobic sludge	747.00	Zhang et al. (2019)
CWMFC	Geobacter sp. Desulfuromonas sp.	715.00	Liu et al. (2020)
UMFC	Methanogenic archea Fermentative bacteria	110.00	Kim et al. (2020)

for treating antibiotic-contaminated wastewater, a maximum PD of 5.78 W/m^3 was achieved. However, it is important to highlight that this system utilized a costly cation exchange membrane as a proton separator, which can be a limiting factor for practical application due to its expenses and operational considerations. Table 2 presents a comprehensive overview of various studies exploring the electricity generation potential from swine wastewater using different MFC configurations and materials. The outcomes from this study contribute to the growing body of research in this field, showcasing the viability of the penicillin-degrading bacterial consortium SF11 as a potent biocatalyst for electricity production in the UMFC. The consistently high OCV values over the monitoring period demonstrate the stability and efficiency of SF11 in electron transfer processes, while the polarization curve illustrates its CD and PD performance. The utilization of SF11 in the UMFC represents a promising step towards sustainable wastewater treatment and renewable energy generation. The achieved PD values, though relatively lower than those in some other studies, demonstrate the potential for further optimization and enhancement in future research. Additionally, the UMFC's use of an Akamada clay separator is noteworthy, as it offers a low-cost and environmentally friendly alternative to expensive proton exchange membranes.

Conclusion

The present study focused on the enrichment and characterization of a bacterial consortium (SF11) that was able to degrade penicillin isolated from swine wastewater sediment. The consortium was identified by next-generation sequencing and was primarily composed of Gammaproteobacteria, Clostridia, Bacilli, and Actinomycetia. The penicillin removal efficiency of the consortium was determined to be 92.15±0.27%. The consortium was utilized as a whole-cell biocatalyst in a microbial fuel cell (MFC) and was able to generate a maximum voltage of 895.00±4.71 mV and a maximum PD of 3.12±0.00 W/m^3. In addition, the UMFC with SF11 produced a maximum voltage of 964.93±1.86 mV and a maximum PD of 0.56±0.00 W/m^3. The findings demonstrate the potential of the *M. morganii*-rich bacterial consortium for biodegrading penicillin in swine wastewater. However, further studies are required to develop an MFC model that can be scaled up for industrial applications.

Funding

This work was financially supported by the Office of the Permanent Secretary, Ministry of Higher Education, Science, Research and Innovation (Grant No. RGNS 64-087).

References

Ahilan, V., Bhowmick, G.D., Ghangrekar, M.M., Wilhelm, M. & Rezwan, K. (2019). Tailoring hydrophilic and porous nature of polysiloxane derived ceramer and ceramic membranes for enhanced bioelectricity generation in microbial fuel cell, *Inonics,* 25, pp. 5907-5918. DOI:10.1007/s11581-019-03083-5

Ajayi, F.F. & Weigele, P.R. (2012). A terracotta bio-battery, *Bioresource Technology*, 116, pp. 86-91. DOI:10.1016/j.biortech.2012.04.019

Al-Dhabi, N.A., Esmail, G.A. & Arasu, M.V. (2021). Effective degradation of tetracycline by manganese peroxidase producing *Bacillus velezensis* strain Al-Dhabi 140 from Saudi Arabia using fibrous-bed reactor, *Chemosphere,* 268, pp. 128726. DOI:10.1016/j.chemosphere.2020.128726

Ambika, A., Kumar, V., Jamwal, A., Kumar, V. & Singh, D. (2022). Green bioprocess for degradation of synthetic dyes mixture using consortium of laccase-producing bacteria from Himalayan niches, *Journal of Environmental Management*, 310, pp. 114764. DOI:10.1016/j.jenvman.2022.114764

Bhakta, J. & Munekage, Y. (2011). Mercury(II) Adsorption onto the magnesium oxide impregnated volcanic ash soil derived ceramic from aqueous phase, *International Journal of Environmental Research,* 5, pp. 585-594. DOI:10.22059/ijer.2011.365

Bhakta, J.N. & Munekage, Y. (2013). Identification of potential soil adsorbent for the removal of hazardous metals from aqueous phase, *International Journal of Environmental Science and Technology*, 10, pp. 315-324. DOI:10.1007/s13762-012-0116-9

Chaijak, P. & Michu, P. (2022). Modified water hyacinth biochar as a low-cost supercapacitor electrode for electricity generation from pharmaceutical wastewater, *Polish Journal of Environmental Studies*, 31, 6, pp. 5471-5475. DOI:10.15244/pjoes/150463

Chaijak, P. & Thipraksa, J. (2022). Improved performance of a novel-model laccase based microbial fuel cell (LB-MFC) with edible mushroom as a whole-cell biocatalyst, *Polish Journal of Environmental Studies*, 31, 5, pp. 4481-4485. DOI:10.15244/pjoes/147196

Chen, R., Zhang, Z., Feng, C., Lei, Z., Li, Y., Li, M., Shimizu, K. & Sugiura, N. (2010). Batch study of arsenate (V) adsorption using Akadama mud: Effect of water mineralization, *Applied Surface Science*, 256, 9, pp. 2961-2967. DOI:10.1016/j.apsusc.2009.11.058

Cheng, D., Ngo, H.H., Guo, W., Lee, D., Nghiem, D.L., Zhang, J., Liang, S., Varjani, S. & Wang, J. (2020). Performance of microbial fuel cell for treating swine wastewater containing sulfonamide antibiotics, *Bioresource Technology*, 311, pp. 123588. DOI:10.1016/j.biortech.2020.123588

Copete-Pertuz, L.S., Placido, J., Serna-Galvis, E.A., Torres-Palma, R.A. & Mora, A. (2018). Elimination of Isoxazolyl-Penicillins antibiotics in waters by the ligninolytic native Colombian strain *Leptosphaerulina* sp. considerations on biodegradation process and antimicrobial activity removal, *The Science of The Total Environment*, 630, pp. 1195-1204. DOI:10.1016/j.scitotenv.2018.02.244

Das, I., Das, S., Dixit, R. & Ghangrekar, M.M. (2020). Goethite supplemented natural clay ceramic as an alternative proton exchange membrane and its application in microbial fuel cell, *Ionics*, 26, pp. 3061-3072. DOI:10.1007/s11581-020-03472-1

Ding, D., Lei, Z., Yang, Y. & Zhang, Z. (2014). Efficiency of transition metal modified akadama clay on cesium removal from aqueous solutions, *Chemical Engineering Journal*, 236, pp. 17-28. DOI:10.1016/j.cej.2013.09.075

Eliato, T.R., Pazuki, G. & Majidian, N. (2016). Potassium permanganate as an electron receiver in a microbial fuel cell, *Energy Sources, Part A: Recovery, Utilization, and Environmental Effects,* 38, 5, pp. 644-651. DOI:10.1080/15567036.2013.818079

Feng, L., Casas, M.E., Ottosen, L.D.M., Moller, H.B. & Bester, K. (2017). Removal of antibiotics during the anaerobic digestion of pig manure, *Science of The Total Environment*, 603-604, pp. 219-225. DOI:10.1016/j.scitotenv.2017.05.280

Garcia, D., Posadas, E., Grajeda, C., Blanco, S., Martinez-Paramo, S., Acien, G., Garcia-Encina, P., Bolado, S. & Munoz, R. (2017). Comparative evaluation of piggery wastewater treatment in algal-bacterial photobioreactors under indoor and outdoor conditions, *Bioresource Technology*, 245, pp. 483-490. DOI:10.1016/j.biortech.2017.08.135

Ge, X., Cao, X., Song, X., Wang, Y., Si, Z., Zhao, Y., Wang, W. & Tesfahunegn, A.A. (2020). Bioenergy generation and simultaneous nitrate and phosphorus removal in a pyrite-based constructed wetland-microbial fuel cell. *Bioresource Technology*, 296, pp. 122350. DOI:10.1016/j.biortech.2019.122350

Ghadge, A.N. & Ghangrekar, M.M. (2015). Development of low cost ceramic separator using mineral cation exchanger to enhance performance of microbial fuel cells*, Electrochimica Acta*, 166, 1, pp. 320-328. DOI:10.1016/j.electacta.2015.03.105

Ghadge, A.N., Jadhav, D.A. & Ghangrekar, M.M. (2016). Wastewater treatment in pilot-scale microbial fuel cell using multielectrode assembly with ceramic separator suitable for field applications, *Environmental Progress & Sustainable Energy*, 35, 6, pp. 1809-1817. DOI:10.1002/ep.12403

Ghadge, A.N., Sreemannarayana, M., Duteanu, N. & Ghangrekar, M.M. (2014). Influence of ceramic separator's characteristics on microbial fuel cell performance, *Electrochemical Engineering*, 4, 4, pp. 315-326. DOI:10.5599/jese.2014.0047

Ghasemi, M., Daud, W.R.W., Ismail, M., Rahimnejad, M., Ismail, A.F., Leong, J.X., Miskan, M. & Liew, K.B. (2013). Effect of pre-treatment and biofouling of proton exchange membrane on microbial fuel cell performance, *International Journal of Hydrogen Energy*, 38, 13, pp. 5480-5484. DOI:10.1016/j.ijhydene.2012.09.148

Goto, Y. & Yoshida, N. (2019). Scaling up microbial fuel cells for treating swine wastewater. *Water*, 11, 9, pp. 1803. DOI:10.3390/w11091803

Guang, L., Koomson, D.A., Jingyu, H., Ewusi-Mensah, D. & Miwornunyuie, N. (2020). Performance of exoelectrogenic bacteria used in microbial desalination cell technology, *International Journal of Environmental Research and Public Health*, 17, 3, 1, pp. 1121. DOI:10.3390/ijerph17031121

He, L.Y., Ying, G.G., Liu, Y.S., Su, H.C., Chen, J., Liu, S.S. & Zhao, J.L. (2016). Discharge of swine wastes risks water quality and food safety: Antibiotics and antibiotic resistance genes from swine sources to the receiving environments, *Environment International,* 92-93, pp. 210-219. DOI:10.1016/j.envint.2016.03.023

Jahan, N., Tahmid, M., Shoronika, A.Z., Fariha, A., Roy, H., Pervez, M.N., Cai, Y., Naddeo, V. & Islam, M.S. (2022). A comprehensive review on the sustainable treatment of textile wastewater: Zero liquid discharge and resource recovery perspectives, *Sustainability*, 14, pp. 15398. DOI:10.3390/su142215398

Ji, M., Su, X., Zhao, Y., Qi, W., Wang, Y., Chen, G. & Zhang, Z. (2015). Effective adsorption of Cr(VI) on mesoporous Fe-functionalized Akadama clay: optimization, selectivity, and mechanism, *Applied Surface Science*, 344, pp. 128-136. DOI:10.1016/j.apsusc.2015.03.006

Kim, D.P., Saegerman, C., Douny, C., Dinh, T.V., Xuan, B.H., Vu, B.D., Hong, N.P. & Scippo, M.L. (2013). First survey on the use of antibiotics in pig and poultry production in the Red River Delta Region of Vietnam, *Food and Public Health*, 3, 5, pp. 247-256. DOI:10.5923/j.fph.20130305.03

Kim, M., Song, Y.E., Li, S. & Kim, J.R. (2021). Microwave-treated expandable graphite granule for bioelectricity generation of microbial fuel cells, *Journal of Electrochemical Science and Technology*, 12, 3, pp. 297-301. DOI:10.33961/jecst.2020.01739

Kim, T., An, J., Jang, J.K. & Chang, I.S. (2020). Determination of optimum electricity connection mode for multi-electrode-embedded microbial fuel cells coupled with anaerobic digester for enhancement of swine wastewater treatment efficiency and electricity recover, *Bioresource Technology,* 297, pp. 122464. DOI:10.1016/j.biortech.2019.122464

Krasnikova, A.V. & Iozep, A.A. (2003). Structure of chemical compounds, Methods of analysis and process control, *Pharmaceutical Chemistry Journal*, 37, 9, pp. 504.

Kusada, H., Zhang, Y., Takami, H., Kimura, N. & Kamagata, Y. (2019). Novel N-Acyl Homoserine lactone-degrading bacteria isolated from penicillin-contaminated environments and their quorum-quenching activities, *Frontiers in Microbiology*, 10, pp. 445, 2019. DOI:10.3389/fmicb.2019.00455

Li, H., Xu, H., Yang, Y.L., Yang, X.L., Wu, Y., Zhang, S. & Song, H.L. (2019). Effects of graphite and Mn ore media on electro-active bacteria enrichment and fate of antibiotic and corresponding resistance gene in up flow microbial fuel cell constructed wetland, *Water Research*, 165, pp. 114988. DOI:10.1016/j.watres.2019.114988

Liu, F., Sun, L., Wan, J., Shen, L., Yu, Y., Hu, L. & Zhou, Y. (2020). Performance of different macrophytes in the decontamination of and electricity generation from swine wastewater via an integrated constructed wetland-microbial fuel cell process, *Journal of Environmental Science (China),* 89, pp. 252-263. DOI:10.1016/j.jes.2019.08.015

Masse, D.I., Lu, D., Masse, L. & Droste, R.L. (2000). Effect of antibiotics on psychrophilic anaerobic digestion of swine manure slurry in sequencing batch reactors*. Bioresource Technology*, 75, 3, pp. 205-211. DOI:10.1016/S0960-8524(00)00046-8

Michu, P. & Chaijak, P. (2022). Electricity generation and winery wastewater treatment using silica modified ceramic separator integrated with yeast-based microbial fuel cell, *Communication in Science and Technology,* 7, 1, pp. 98-102. DOI:10.21924/cst.7.1.2022.799

More, S.S., Renuka, P.S., Pruthvi, K., Swetha, M., Malini, S. & Veena, S.M. (2011). Isolation, purification, and characterization of fungal laccase from *Pleurotus* sp., *Enzyme Research*, 2011, pp. 248735. DOI:10.4061/2011/248735

Mukhopadhyay, D., Khan, N., Kamal, N., Varjani, S., Singh, S., Sindhu, R., Gupta, P. & Bhargava, P.C. (2022). Degradation of beta-lactam antibiotic ampicillin using sustainable microbial peroxide producing cell system, *Bioresource Technology*, 361, pp. 127605. DOI:10.1016/j.biortech.2022.127605

Nguyen, T.T., Soda, S., Kanayama, A. & Hamai, T. (2021). Effects of cattails and hydraulic loading on heavy metal removal from closed mine drainage by pilot-scale constructed wetlands, *Water*, 13, 14, pp. 1937. DOI:10.3390/w13141937

Ni, H., Wang, K., Lv, S., Wang, X., Zhuo, L. & Zhang, J. (2020). Effects of Concentration Variations on the Performance and Microbial Community in Microbial Fuel Cell Using Swine Wastewater, *Energies*, 13, 9, pp. 2231. DOI:10.3390/en13092231

Piontek, K., Antorini, M. & Choinowski, T. (2002). Crystal structure of a laccase from the fungus *Trametes versicolor* at 1.90-A resolution containing a full complement of coppers, *Journal of Biological Chemistry*, 277, 40, pp. 37663. DOI:10.1074/jbc.M204571200

Portis, E., Lindeman, C., Johansen, L. & Stoltman, G. (2012). A ten-year (2000-2009) study of antimicrobial susceptibility of bacteria that cause bovine respiratory disease complex--*Mannheimia haemolytica*, *Pasteurella multocida*, and *Histophilus somni*--in the United States and Canada. *Journal of Veterinary Diagnostic Investigation*, 24, 5, pp. 932-944. DOI:10.1177/1040638712457559

Prosekov, A.Y. & Ivanova, S.A. (2018). Food security: The challenge of the present, *Geoforum,* 91, 1, pp. 73-77. DOI:10.1016/j.geoforum.2018.02.030

Rahimnejad, M., Adhami, A., Darvari, S., Zirepour, A. & Oh, S.E. (2015). Microbial fuel cell as new technology for bioelectricity generation: A review. *Alexandria Engineering Journal*, 54, 3, pp. 745-756. DOI:10.1016/j.aej.2015.03.031

Rahimnejad, M., Ghoreyshi, A.A., Najafpour, G. & Jafary, T. (2011). Power generation from organic substrate in batch and continuous flow microbial fuel cell operations, *Applied Energy*, 88, 11, pp. 3999-4004. DOI:10.1016/j.apenergy.2011.04.017

Rahman, T.U., Roy, H., Islam, M.R., Tahmid, M., Fariha, A., Mazumder, A., Tasnim, N., Pervez, M.N., Cai, Y., Naddeo, V. & Islam, M.S. (2022). The advancement in membrane reactor (MBR) technology toward sustainable industrial wastewater management, *Membranes,* 13, pp. 181. DOI:10.3390/membranes13020181

Ren, B., Wang, T. & Zhao, Y. (2021). Two-stage hybrid constructed wetland-microbial fuel cells for swine wastewater treatment and bioenergy generation, *Chemoshpere,* 268, pp. 128803. DOI:10.1016/j.chemosphere.2020.128803

Rossi, R., Jones, D., Myung, J., Zikmund, E., Yang, W., Gallego, Y.A., Pant, D., Evans, P.J., Page, M.A., Cropek, D.M. & Logan, B.E. Evaluating a multi-panel air cathode through electrochemical and biotic tests, *Water Research*, 148, pp. 51-59. DOI:10.1016/j.watres.2018.10.022

Santos, F., Almeida, C.M.R., Ribeiro, I. & Mucha, A.P. (2019). Potential of constructed wetland for the removal of antibiotics and antibiotic resistant bacteria from livestock wastewater, *Ecological Engineering*, 129, pp. 45-53. DOI:10.1016/j.ecoleng.2019.01.007

Shang, S., Fan, H., Li, Y., Li, L. & Li, Z. (2022). Preparation of lightweight ceramsite from solid waste using SiC as a foaming agent, *Materials (Basel),* 15, 1, pp. 325. DOI:10.3390/ma15010325

Sekyere, J.O. (2014). Antibiotic types and handling practices in disease management among pig farms in Ashanti region, Ghana, *Journal of Veterinary Medicine*, 2014, pp. 531952. DOI:10.1155/2014/531952

Subha, C., Kavitha, S., Abisheka, S., Tamilarasan, K., Arulazhagan, P. & Banu, J.R. (2019). Bioelectricity generation and effect studies from organic rich chocolaterie wastewater using continuous upflow anaerobic microbial fuel cell, *Fuel,* 251, pp. 224-232. DOI:10.1016/j.fuel.2019.04.052

Sun, W., Gu, J., Wang, X., Qian, X. &Peng, H. (2019). Solid-state anaerobic digestion facilitates the removal of antibiotic resistance genes and mobile genetic elements from cattle manure, *Bioresource Technology*, 274, pp. 287-295. DOI:10.1016/j.biortech.2018.09.013

Sunder, A.V., Utari, P.D., Ramasamy, S., Van Merkerk, R., Quax, W. & Pundle, A. (2017). Penicillin V acylases from gram-negative bacteria degrade N-acylhomoserine lactones and attenuate virulence in *Pseudomonas aeruginosa*, *Applied Microbiology and Biotechnology*, 101, 6, pp. 2383-2395. DOI:10.1007/s00253-016-8031-5

Thipraksa, J. & Chaijak, P. (2022). Improved the coconut shell biochar properties for bio-electricity generation of microbial fuel cells from synthetic wastewater, *Journal of Degraded and Mining Lands Management*, 9, 4, pp. 3613-3619.

Thipraksa, J., Chaijak, P., Michu, P. & Lertworapreecha, M. (2022). Biodegradation and electricity generation of melanoidin in palm oil mill effluent (POME) by laccase-producing bacterial consortium integrated with microbial fuel cell, *Biocatalysis and Agricultural Biotechnology*, 43, pp. 102444. DOI:10.1016/j.bcab.2022.102444

Tsai, W.T. (2018). Regulatory promotion and benefit analysis of biogas-power and biogas-digestate from anaerobic digestion in Taiwan's livestock industry, *Fermentation*, 4, 3, pp. 57. DOI:10.3390/fermentation4030057

Vogel, G., Nicolet, J., Martig, J., Tschudi, P. & Meylan, M. (2001). Pneumonia in calves: characterization of the bacterial spectrum and the resistance patterns to antimicrobial drugs, *Schweizer Archiv fur Tierheilkunde*, 143, 7, pp. 341-350.

Wang, S., Ma, X., Wang, Y., Du, G. &Tay, J.H. (2019). Piggery wastewater treatment by aerobic granular sludge: Granulation process and antibiotics and antibiotic-resistant bacteria removal and transport, *Bioresource Technology*, 273, pp. 350-357. DOI:10.1016/j.biortech.2018.11.023

Xu, F., Ouyang, D.L., Rene, E.R., Ng, H.Y., Guo, L.L., Zhu, Y.J., Zhou, L.L., Yuan, Q., Miao, M.S., Wang, Q. & Kong, Q. (2019). Electricity production enhancement in a constructed wetland-microbial fuel cell system for treating saline wastewater, *Bioresource Technology*, 288, pp. 121462. DOI:10.1016/j.biortech.2019.121462

Yan, R., Wang, Y., Li, J., Wang, X. & Wang, Y. (2022). Determination of the lower limits of antibiotic biodegradation and the fate of antibiotic resistant genes in activated sludge: Both nitrifying bacteria and heterotrophic bacteria matter, *Journal of Hazardous Materials*, 425, pp. 127764. DOI:10.1016/j.jhazmat.2021.127764

Yousefi, V., Mohebbi-Kalhori, D. & Samimi, A. (2017). Ceramic-based microbial fuel cells (MFCs): A review. *International Journal of Hydrogen Energy*, 42, 3, pp. 1672-1690. DOI:10.1016/j.ijhydene.2016.06.054

Zhang, D., Wang, X. & Zhou, Z. (2017). Impacts of small-scale industrialized swine farming on local soil, water and crop qualities in a hilly red soil region of subtropical China. *International Journal of Environmental Research and Public Health*, 14, 12, pp. 1524. DOI:10.3390/ijerph14121524

Zhang, Y., Zhao, Y. & Zhou, M. (2019). A photosynthetic algal microbial fuel cell for treating swine wastewater, *Environmental Science and Pollution Research*, 26, 6182-6190. DOI:10.1007/s11356-018-3960-4