Electricity Generation from Hospital Wastewater in Microbial Fuel Cell using Radiation Tolerant Bacteria

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Abstract: Hospital waste is a type of hazardous waste that contains a wide range of dangerous substances, including radioactive materials. Radiation-tolerant microbes have shown an interest in treating this liquid waste. Radiation-resistant microorganisms were chosen from irradiated fermented sausage in this investigation. The activity of enzymes such as protease, lipase, and laccase was studied. For hospital wastewater treatment, a single chamber microbial fuel cell (sMFC) with a radiation-tolerant bacterial consortium was deployed. The microbial structure analysis showed the selected consortium was similar to Acinetobacter sp. The COD was removed at a rate of 90.10±0.30%, and the power density (PD) was 168.91±3.89 mW/m². This was the first study to use the radiation-resistant Acinetobacter sp. bacterial consortia to treat hospital waste and generate power simultaneously.

Keywords: hospital wastewater; gamma radiation; Laccase; microbial fuel cell

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1. Introduction

Hospital waste is a source of high concentrations of complicated micro-contaminants for which no specialized treatment has been investigated yet. This liquid waste and its impurities endanger the unit operation of the hospital’s wastewater treatment plant [1]. Hospital waste is released at a rate of 400-1,200 L/day/bed in developed countries than 200-400 L/day/bed in developing countries [2]. Drug residues, chemicals, pathogenic microbes, and poisons are all found in hospital waste [3]. DNA damage, ecotoxicity, toxic impact, and drug-resistance pathogens are some of the unfavorable health effects [4-5]. Furthermore, radioactive chemicals such as technetium-99m (Tc-99m), Iodine-131 (I-131), Iodine-125 (I-125), Iodine-123 (I-123), Flourine-18 (F-18), Tritium (H-3), and Carbon-14 (C-14) are found in hospital effluent, which has a severe impact on human health [6]. By producing DNA damage, radioactive waste poses a threat to living organisms. Chemical, physical, and biological treatment methods are required to manage radioactive leftovers. The radioactive residue can be degraded and converted to renewable energy, such as electricity. These methods are cost-effective, viable, and environmentally friendly [7].

Bacteria that are resistant to radiation are often resistant to DNA and protein damage. They’ve been discovered in hot springs [8], red mud [9], cow dung [10], mining sites [11], marine sediment [12], and other places. Radiation-resistant microorganisms are commonly
used in the bioremediation of toxic compounds. However, there was no mention of bioremediation and simultaneous power generation from hospital effluent.

A microbial fuel cell (MFC) is a bioreactor that uses electron-releasing microorganisms (exoelectrogen) to degrade contaminants and generate power from waste [13]. Pharmaceutical waste [13], high-salinity waste [14], dairy [15], household [16], and other forms of waste have all been used.

Radiation-resistant bacteria were gathered and chosen from irradiated fermented pork sausages in this study. The activities of enzymes (lipase, protease, and laccase) were studied. Finally, it was used in a single-chamber MFC to treat hospital waste while also generating power.

2. Materials and Methods

2.1. Screening of gamma-radiation tolerant bacteria.

The 50 samples of irradiated fermented pork sausages were collected from the local market in Phatthalung province, Southern Thailand. The gamma-radiation tolerant bacteria were enriched according to the modified method of Lee et al. [17]. To avoid fungal growth, 1.0 g of irradiated samples were suspended in 10 mL sterilized nutrient broth (NB, Himedia) with 50 mg/L nystatin and incubated at 30°C for 3 days. The 10% (v/v) of growing bacteria was transferred to 90% (v/v) new media 5 times to ensure they could be cultured in a laboratory. The mixed cultures were grown under facultative anaerobic conditions.

2.2. Enzyme activity.

Hospital waste is a high chemical oxygen demand (COD) liquid waste that mainly contains biodegradable organic materials (protein and lipid) and antimicrobial agents [3, 18]. Bacteria with protease, lipase, and laccase activities were chosen to treat hospital waste. The 10% (v/v) mixed cultures (1.0 x 10⁷ cell/mL) were inoculated into 90% (v/v) sterilized NB and incubated at 30°C for 3 days. The cultured mixed cultures were centrifuged at 12,000 rpm for 5 mins at 4°C. The supernatants were collected and stored at 4°C until they were used. The protease activity was determined by the modified method of Sharmin et al. [19]. The lipase activity was determined according to the study of Sirisha et al. [20]. The laccase activity was measured using the method described by Chaijak et al. [21].

2.3. Bacterial community structure.

Total genomic DNA (gDNA) of selected gamma-radiation tolerant bacteria was extracted from 1.0 g of 24 hr-old cell pellets using a PCR-ready genomic DNA extraction kit (Bio-Rad, Thailand). The microbial community of the wastewater sludge was carried out by using the modified method of Denaturing gradient gel electrophoresis (DGGE) analysis according to de Lillo et al. [22] and Muyzer et al. [23]. The DGGE profile of the gamma-radiation tolerant bacteria was generated on an 8.0% polyacrylamide gel with 40-60% gradient denaturant solution for 16 hr at 70 V in 1x Tri-acetate ethylenediaminetetraacetic acid (EDTA) buffer at 60 ºC.
2.4. Hospital wastewater treatment.

The synthetic hospital waste was prepared according to Tiwari et al. [24] and Guerrero et al. [25]. Briefly, the working synthetic waste was freshly prepared (prepared day by day). Glucose was used as a carbon source. The yeast extract was desired protein 100 mg/L, and the sunflower oil was desired lipid 13 mg/L. In comparison, 50 mg/L of penicillin was used as an antimicrobial agent [26]. The final chemical oxygen demand (COD) of synthetic waste was 1,500 mg/L. The 10% (v/v) of the selected gamma-resistant consortium was inoculated into the 90% (v/v) synthetic hospital waste and incubated at 30 °C for 3 days without shaking. The COD, lipid, protein, and penicillin removal [27] were monitored and calculated.

2.5. Bio-electricity generation

The single ceramic-separator microbial fuel cell (CMFC) was constructed according to the study of Chaijak et al. [28], shown in Figure 1. The 15 cm² carbon cloth was used as an electrode. The stainless steel wire with a 10 mm diameter (TRUSCO, Thailand) was connected between the electrodes. The cathode and anode were separated by a 2 mm thick ceramic plate. To accumulate a selected culture on the electrode surface, the consortium's 10% (v/v) was inoculated into the anode chamber. The freshly synthesized hospital waste was then poured into a 100 mL working volume. The CMFC system operated at 30 °C for 3 days and fed all the liquids. After that, 100 mL of fresh synthetic waste was added to an anode chamber. As a control, fresh waste without a consortium was used. The open-circuit voltage (OCV) and close circuit voltage (CCV) at 1,000 Ω were determined. The current (mA), current density (mA/m²), power (mW) and power density (mW/m²) were calculated following Eq. (1) – (4).

\[
\begin{align*}
I &= \frac{V}{R} ~ (1) \\
CD &= \frac{I}{A} ~ (2) \\
P &= IV ~ (3) \\
PD &= \frac{P}{A} ~ (4)
\end{align*}
\]

where I is the current (mA), V is the CCV at 1,000 Ω (mV), R is the external resistance (Ω), CD is the current density (mA/m²), the A is the electrode area (m²), P is the power (mW), and PD is the power density (mW/m²).

![Figure 1. Schematic of CMFC used in this experiment.](https://biointerfaceresearch.com/)
3. Results and Discussion

3.1. Selection of gamma-tolerant bacteria.

Gamma radiation is commonly employed in various applications, including material enhancement and food preservation, including sterilization [29]. The archaea and bacteria are the most well-known gamma-tolerant microorganisms. Deep-sea silt and other sediment have been reported to have gamma-radiation resistant microorganisms that can tolerate high dose radiation of 3 – 30 KGray [30].

In Southern Thailand, 50 irradiated sausage samples were collected aseptically. The 11 samples were obtained after 3 days of incubation in basic bacterial media treated with fungicide. They were transferred five times to ensure that they could grow in the lab without utilizing the sample as a supplement. Only four mixed cultures (GR09, GR30, GR40, and GR50) demonstrated growing ability.

3.2. Enzyme activity.

Proteins, lipids, and antibacterial agents make for more than 70% of the organic matter in hospital and home wastewater [26, 31]. Lipase, protease, and laccase-producing microorganisms play a significant role in treating hazardous compounds in wastewater [32]. Fermented sausage is a well-known fermented food produced by proteolytic and lipolytic bacteria such as Lactobacillus plantarum and Staphylococcus xylosus [33]. Furthermore, Kanagaraj et al. [34] discovered that lactic acid bacteria isolated from fermented sausage could generate high laccase activity with 1.128 U/mL in prior work.

In this investigation, gamma-radiation-resistant microorganisms were isolated from irradiated fermented sausage. The enzyme activities of growing bacteria were assessed after the selection process. The protease, lipase, and laccase activities were determined using 10% (v/v) of enriched mixed cultures (GR09, GR30, GR40, and GR50). Figure 2 depicts the outcome of enzyme activity. The GR09 produced the highest protease and lipase activity of 1.96±0.06 and 1.32±0.03 U/mL, respectively. The consortium which yielded the laccase activity of 1.32±0.03 was the same.
3.3. Bacterial community structure.

The DGGE of a PCR amplified 16S rDNA fragment has traditionally been utilized for bacterial population fingerprinting. In the study of bacterial population structure, species identification is important [35]. Figure 3 shows the DGGE profiles of the bacterial population derived from DNA isolated directly from GR09. According to the findings, Acinetobacter sp. is the most common genus in the radiation-resistant bacterial consortia. According to a study by Xu et al., Escherichia coli isolated from irradiated chicken products can withstand gamma-radiation of 0.30 – 0.39 kGy [36].

![Figure 3. The DGGE profile of GR09.](image)

![Figure 4. The OCV of sMFC in this experiment.](image)

Table 1: The comparison of hospital wastewater treatment in this experiment with others.
3.4. Hospital wastewater treatment & electrochemical properties.

Due to their multi-benefits, such as power production, contaminant removal, and intrinsic low energy requirement, MFCs have recently attracted more interest in hospital wastewater treatment. They are employed as a therapeutic method for removing antibacterial agents [37].

The performance of MFCs in terms of COD elimination was evaluated in this study. The COD elimination was determined after the exoelectrogen's initial start-up acclimation time. The initial COD level was around 1,500 mg/L. COD elimination was obtained at a rate of 90.10±0.30% during the stationary phase. Moreover, 80.52±2.32% of penicillin removal was gained. The comparison of hospital wastewater treatment in this experiment with others was shown in Table 1. The electrochemical properties of this experiment revealed an open-circuit voltage (OCV) of 660.00±10.00 mV (Figure 4), a CD of 335.56±3.85 mA/m², and a PD of 168.91±3.89 mW/m², which is greater than the control rates of 77.27%, 92.05%, and 99.34%.

| Process                                             | Initial COD concentration (mg/L) | Initial antibiotic agent concentration (mg/L) | COD removal (%) | Power output (mW/m²) | Reference |
|-----------------------------------------------------|----------------------------------|---------------------------------------------|----------------|----------------------|-----------|
| Single chamber MFC (sMFC)                           | 1,500                            | 50                                         | 90.10±0.30     | 168.91±3.89         | This study|
| Dual chamber MFC                                    | 900                              | 4 - 128                                    | 90.50          | 700                  | [37]      |
| Constructed wastewater treatment system             | 307                              | NA                                         | 57.00          | 0.00                 | [38]      |
| Submerged membrane bioreactor (SMBR)                | 2,000                            | NA                                         | 85.00          | 0.00                 | [39]      |
| Aerobic fluidized bed Bioreactor                    | NA                               | NA                                         | 72.00          | 0.00                 | [40]      |

The results showed that the sMFC had a larger potential for COD removal from hospital wastewater than previous works, but it still had a lesser potential than the dual chamber MFC, as shown in Table 1. The sMFC, on the other hand, can work at a greater initial COD concentration.

4. Conclusions

The gamma radiation-resistant bacteria isolated from irradiated fermented pork sausage showed great potential for COD elimination in sMFC, with a maximum PD of 168.913.89 mW/m² and no energy consumption. The sMFC with radiation-resistant bacteria appears to be a promising approach for treating hospital wastewater while also generating power.

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Conflicts of Interest

The authors declare no conflict of interest.

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