

Chapter 7

Gamma Ray Mutagenesis on Bacteria Isolated from Shrimp Farm Mud for Microbial Fuel Cell Enhancement and Degradation of Organic Waste



Ayoub Ahmed Ali, Azura Amid , and Azhar Muhamad

Abstract This chapter discusses the use of prokaryotic microorganisms to produce electrical bioenergy from a wide range of organic substrates in microbial fuel cells (MFCs). MFCs offer promising sustainable energy production and at the same time, simultaneous degradation of organic waste in the substrate. Active microorganisms capable of producing electricity bypassing the electron to the electrode are called electrochemically active bacteria. The study identified a method to obtain an optimum dose to increase the bacterial potential using the one factor at time (OFAT) method. The 63 Gy gamma dose irradiation increased the cell voltage to 280 mV with 33% of chemical oxygen demand (COD) removal while the maximum voltage of the wild strain was 154 mV with 55.7% of COD removal. The successful effect of the gamma radiation dose on the increase of the MFC's bioelectricity and organic matter removal indicates that gamma rays are a way to boost the ability of the electrically active bacteria.

Keywords Gamma ray · Microbial fuel cell · Mutagenesis

7.1 Introduction

All living organisms need energy, and there are two main sources of energy: chemical and light. Organisms that use light as a source of energy are called phototrophs, while others are chemotropic. Microbial fuel cell (MFC), as the French claim, “living

A. A. Ali
Kulliyah of Engineering, International Islamic University Malaysia (IIUM),
Kuala Lumpur, Malaysia

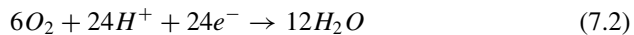
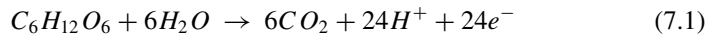
A. Amid (✉)
International Institute for Halal Research and Training, International Islamic University Malaysia,
Jalan Gombak, Kuala Lumpur, Malaysia
e-mail: azuraamid@iium.edu.my

A. Muhamad
Agensi Nuklear Malaysia, Kajang, Selangor, Malaysia

battery” generates electricity from a chemotrophic bacterium that transforms chemical energy (organic matter present in wastewater) into electrical energy. An organism that drives electrical current is called electrochemically active bacteria, electricigens, and anode respiring bacteria (Logan, 2009).

7.1.1 Principle: Microbial Fuel Cell (MFC)

The standard configuration of the MFC system consists of two interconnected chambers. The anode chamber is filled with a medium in which the living catalyst breaks down the organic or inorganic materials of the media and produces an electron from its metabolism on the surface of the electrode. On the other hand, the cathode chamber contains electron acceptors where several electron acceptors have been used, e.g. oxygen (O_2), phosphate buffer solution (PBS), ferricyanide ($K_3Fe[CN]_6$) and nitrate (NO_3) (Wei et al., 2012; Wen et al., 2010; Zhao et al., 2006). The two chambers are separated by a membrane which not only separates the solutions of each compartment but preserves the electro-neutrality of the system by diffusing the proton from the anode to the cathode. The reactions in anode and cathode are described from the following equations.



7.1.2 Principle: Mutation

A mutation is a modification of the genomic arrangement that can alter or disable the functionality of a cell. The disorder may occur at the nucleotide level (nucleotide deletion, alteration, and replacement) as well as at the chromosomal level (chromosome rearrangement or duplication) or other levels. Traditionally, mutations are classified into three categories (single gene disorder, chromosome disorder, and multifactorial disorder). Single gene disorder is an error that occurred in the DNA sequence of the gene. It is categorized as somatic (non-reproductive cell) and germline (reproductive cell). The somatic disorder may be so-called autosomal recessive in the recessive allele or maybe on the autosomal dominant. Where the germline is either on the X chromosome (X-linked recessive and dominant disorder) or Y chromosome (holandric disorder) (Mahdieh & Rabbani, 2013). Thus, the basis of the category may or may not appear in the phenotype of the offspring. The mutation occurs spontaneously or is induced by an agent named mutagens such as chemical, radiation, viruses, diet, or lifestyle. A spontaneous mutation is natural; it appears without any induction agent

and is the result of a mispairing nucleotide (added or omitted) during the DNA replication phase that is contrary to the mutation induced by a mutagen that can damage the DNA and result in nucleotide mispairing (Watford & Warrington, 2017).

7.1.2.1 Chemical Mutagens

Due to the incapability of a cell to produce its thymidine or the presence of thymidine inhibitor in the media because it is replaced by 5-bromodeoxyuridine (BUdR), an unstable molecule converted enzymatically by 5-bromouracil (Fishbein, 1970). It makes the same mistake when pairing with guanine instead of adenine during DNA replication. The transition of A-T to G-C is thought to be due to a higher electronegativity of the bromine atom compared to the methyl group of thymidine, rendering the ring weaker in electrons and favoring guanine pairing. Additionally, Fishbein (1970) emphasizes that the transition does not stabilize in G-C pairing, but an occasional error leads the pairing BUdR to adenine. Moreover, proflavin, acridine orange, and ethidium bromide are mutagens that can slip between the stacked nitrogen bases at the core of the DNA double helix. In this position, these agents can add or delete nucleotides in the sequence (Habibi & Pezeshki, 2013). Furthermore, some chemicals, such as nitrous acid, can chemically modify the base so that it appears like a different base (Jena, 2012).

7.1.2.2 Ultraviolet Irradiation

Ultraviolet in a wavelength between 200 and 280 nm incorporates the formation of the bond between adjacent pyrimidine amino acids, commonly referred to as thymine dimers for DNA and uracil dimers for RNA (Cutler & Zimmerman, 2011). They also reported that these dimers set up the blockade of DNA replication as well as the transcription.

7.1.2.3 Radioactive Particle

There are five forms of ionizing radiation: alpha, beta, neutron, gamma, and X-rays. They caused an unstable atom with an excess of mass and/or energy. For the atom to stabilize, it must free that extra energy and/or mass in the form of radiation. Alpha and beta rays are positively and negatively charged, while gamma rays are the result of the Compton effect or Compton scattering (Parks, 2015). Compton scattering is the quantum theory where a scattering photon collides with an atom. Basically, the energy of the incoming photon is partly absorbed by an outer shell electron, where the electron is not only knocked out from the atom, but a scattering photon is also released (Parks, 2015). Generally, radiation exposure causes a change in the DNA structure and the formation of a free radical that cause the excitation, ionization, and breakage of molecules.

Henceforth, irradiation can inhibit the growth of the living organism or lead to the apparition of mutation that can potentially change the metabolism pathways and morphology of the organism. The irradiation effect depends on the type of radiation, duration of the exposure, the distance between the sample and the source, and the species type (Min et al., 2003; Quillardet et al., 1989). Moreover, the hormesis hypothesis suggests a beneficial effect of low-dose radiation on the microorganism, increases interest in low-dose radiation (Bolsunovsky et al., 2016). Besides, a considerable amount of literature has been published on the effects of ionizing radiation on the viability of bacterial cells, inducing an SOS response; response to DNA damage in which the cell cycle is stopped and initiates DNA repair and mutagenesis in bacterial cells. The comparison of three types of ionizing radiation (alpha, gamma, and neutron radiation) using SOS Chromo test showed a minimum inducing doses (MID) of 2.5 Gy for alpha and neutrons ionizing, and around 5 Gy for gamma rays, with the highest inducing dose, was 100 Gy for neutrons and 200 Gy for gamma and alpha particles (Quillardet et al., 1989). Since the minimum inducing dose were approximately similar for the 3 types of radiation Quillardet, the authors of that investigation stated that the standard Ames test procedure measured 1–2 Gy dose as the lowest mutation doubling dose for gamma rays and concluded that the value of SOS chromotest and Ames test were logically comparable.

Moreover, Min and co-workers (Min et al., 2003) stated that the minimum dose detects by *Escherichia coli* is 1.5 Gy, whereas the maximum response was 200 Gy. The amount of emitted bioluminescence increased proportionally with the gamma-ray doses, which caused DNA damage response in a range of “1–50 Gy”. Since the maximum gamma irradiation dose that inhibits bacteria is 200 Gy and the minimum detectable DNA damage dose is 1.5 Gy, the gamma irradiation dose of this study was 25, 63, and 100 Gy.

7.1.3 Objective of Experiment

The objective of this procedure is to increase the MFC performance through gamma irradiation on isolated bacteria.

7.2 Materials

Tables 7.1, 7.2 and 7.3 show the materials used in this experiment.

Table 7.1 Consumable used in this experiment

No.	Consumable	Usage
1	Petri dishes	Bacteria growth
2	Inoculation tube	Bacteria growth
3	Cuvette	Optical density reading
4	COD digestion vial	COD measurement
5	Pipette tips	Liquid handling
6	Micro centrifuge tube	Storage

Table 7.2 Equipment used in this experiment

No.	Equipment	Usage
1	U-tube MFC	Isolation of electrochemically active bacteria
2	Multimeter	Electricity production measurement
3	Spectrophotometer	Bacteria growth measurement
4	Digital multimeter	Resistance and voltage measurement
5	COD reactor	Chemical Oxygen Demand measurement

Table 7.3 Chemicals used in this experiment

No.	Chemicals	Usage
1	LB broth	Bacteria growth

7.3 Methodology

7.3.1 Bacterial Radiation

The identified bacteria were sent to the Malaysian Nuclear Agency, Bangi, Selangor for mutation by gamma radiation. Serial dilution of the LB broth containing the wild-type strain was performed. The reason for the dilution was to cultivate ten colonies in each petri dish to which the radiation was to be applied. Since the high dose will cause inhibition of the bacterium as well as the low dose, the mutation is caused. Also, gamma doses greater than or equal to 200 Gy cause bacterial inhibition (Bolsunovsky et al., 2016). Therefore, the dose of gamma radiation in this study was 25, 63, and 100 Gy.

7.3.1.1 One Factor at a Time (OFAT) Optimization of Gamma Radiation

The isolated *Bacillus cereus* strain cc-1 was irradiated with gamma radiation by the Malaysian nuclear agency. Electrochemically active bacteria were exposed to three different doses (25, 63, 100 Gy) of gamma rays. Tenfold dilution series

were performed in order to get a solid plate containing approximately ten colonies of *Bacillus cereus* strain cc-1. Optimization studies on the increase in electricity production of electrochemically active bacteria of MFC using gamma radiation were conducted based on one factor (gamma radiation dose) that has a positive significant effect on the enhancement of cell potential.

Optimization was carried out using One-Factor at Time (OFAT) in order to determine the relationship between the factor and responses (voltage, mV; time, h). The high and low levels of the values of the actual variables were 100 and 25 Gy, respectively. Cell voltage and time were taken as the response to the experimental design. The experiment was conducted in the same reactor (U-tube MFC) with the wild strain containing all its physical properties, and then 1 mL of LB broth containing the mutant strain of 0.1 OD was added. Electricity production was recorded in 3 days using a multimeter (Fluke 179) and the analysis of variance (ANOVA) was conducted using the Design Expert Software.

7.3.2 *Bacterial Growth*

Bacterial growth was measured by using a spectrophotometer as the selected wavelength of the isolated bacteria was not known to be 600 nm (Fakhiruddin et al., 2018). After the cell was inoculated in Luria broth, 1 mL of the cell-containing nutrient broth was deducted from the inoculation tube and poured into a 1 mL cuvette for reading the optical density of the sample. This process continued until 14 h after inoculation, with an interval of 1 h for the first 6 h and 30 min for the remaining 8 h.

7.3.3 *MFC Power Measurement*

After constructing an MFC device with an external resistance of 1000 Ohms (refer to Sect. 7.2.1), the voltage generation of the MFC was measured with a digital multimeter. As described in Sect. 7.2.1, the voltage was measured directly in the function of time where the current and the power density were calculated using Eqs. (7.1–7.3)

$$A = V/R \quad (7.1)$$

where A is the intensity of the current, V is the voltage, and R is the resistance.

The equation of the current density and the power density is written as follows:

$$Am = A/(a) \quad (7.2)$$

$$Pd = V * Am \quad (7.3)$$

where A_m is the current density (mA/cm^{-2}), P_d is the power density (mW/cm^{-2}), and a is the surface area of the anodic electrode (cm^2).

7.3.4 Total Chemical Oxygen Demand (COD)

Chemical Oxygen demand (COD) is a test that determines the amount of organic compounds in wastewater. If the COD value is very high, this means that there is a high amount of organic matter in the sample. A box of Hach® high range (HR) COD digestion vials was used for the COD test in this research. The vials were all labeled and all TCODs were measured at the same time by using one blank.

The sample was well mixed and 1 mL was pipetted in a 100 mL tube, which was then diluted to 99 mL with distilled water. A 2 mL diluted sample was transferred to a COD vial and a blank was made using 2 mL of distilled water instead of a sample. The COD vials were put and left for 2 h in the COD reactor (Hach DRB200 Digital Reactor Block for TNTplus, Thomas Scientific, US) preheated at 150 °C for 20 min. After 2 h heating, the vials were placed on a wooden rack for 10 min to cool down in the ambient temperature. Finally, the reading was done by using DR 5000™ UV-Vis Laboratory Spectrophotometer (Hach company, US).

7.4 Results and Discussion

7.4.1 Suitable Gamma Rays Dose by OFAT

Design Expert 7.0. (2021 East Hennepin Ave., Suite 480 Minneapolis, MN 55413) was used for the design, analysis, and optimization, where the variable was the gamma radiation dose (25, 63, and 100 Gy). It was the only factor used as a parameter in order to study its effect in response to an increase in electricity performance by mutating the electrochemically active bacteria. The responses were voltage (Y1) and time (Y2).

A total of five treatments was designed to search for the optimum dose (Gy) using the statistical software of DOE applying OFAT, Table 7.4 illustrates the experimental data of OFAT with the two responses. The highest voltage generation with the shortest time was 280 mV in 20 h achieved by the dose of 63 Gy, while the lowest voltage potential was 29.1 mV in 55 h produced by the strain radiated with the lowest dose (25 Gy) (Table 7.4).

The corresponding model graph for the voltage (a) and the time (b) was used in Fig. 7.1 to examine and clearly understand the relationship between the factor (gamma radiation dose) and the two responses. The objective of this model is to increase the voltage generation and reduce time. Therefore, in Fig. 7.1a, a parabolic concave curve is seen towards the maximum point, where the lowest radiation dose

Table 7.4 Experimental data of OFAT with the result

Run	Factor 1: Dose (Gy)	Response 1: Voltage (mV)	Response 2: Time (h)
1	100	44.8	72
2	25	-29.1	55
3	100	44.8	72
4	25	-29.1	55
5	63	280	20

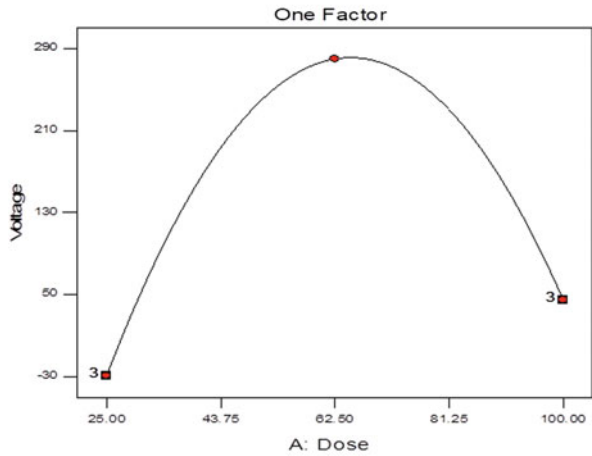
a

Design-Expert® Software

Voltage

● Design Points

X1 = A: Dose



b

Design-Expert® Software

Time

● Design Points

X1 = A: Dose

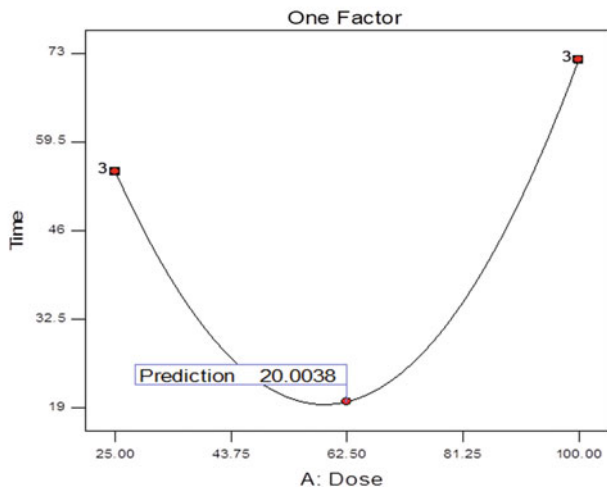


Fig. 7.1 Response curve, **a** voltage over dose; **b** time over dose

and the highest gamma dose caused a considerable drop in cell voltage generation. Thus, the optimal dose that increased the potential voltage was 63 Gy with a voltage of 280 mV. Moreover, Fig. 7.1b demonstrates that both the highest dose and the lowest dose of 100 and 25 Gy, respectively, increased the time to reach the maximum electricity generation, when a convex curve towards the minimum point was observed. Similar to the first response—voltage, the optimum dose that minimized the time of MFC performance was 63 Gy.

7.4.2 Formation of Biofilm in the Anolyte

Figure 7.2 presents the scanning electron microscopy (SEM) images, which show the results of the biofilm formation on the anode electrode. The sample used for the analysis was a carbon cloth, which was obtained from the electrochemical bacteria after being exposed by the optimization dose of the gamma radiation. An electrode sample of 10 × 10 mm was taken from each experiment using a radiated strain and a wild strain, where an unused sterile carbon cloth was used as a reference. All samples undergo the same preparation procedure and the image shown in Fig. 7.2 was taken with a magnification of ×1500.

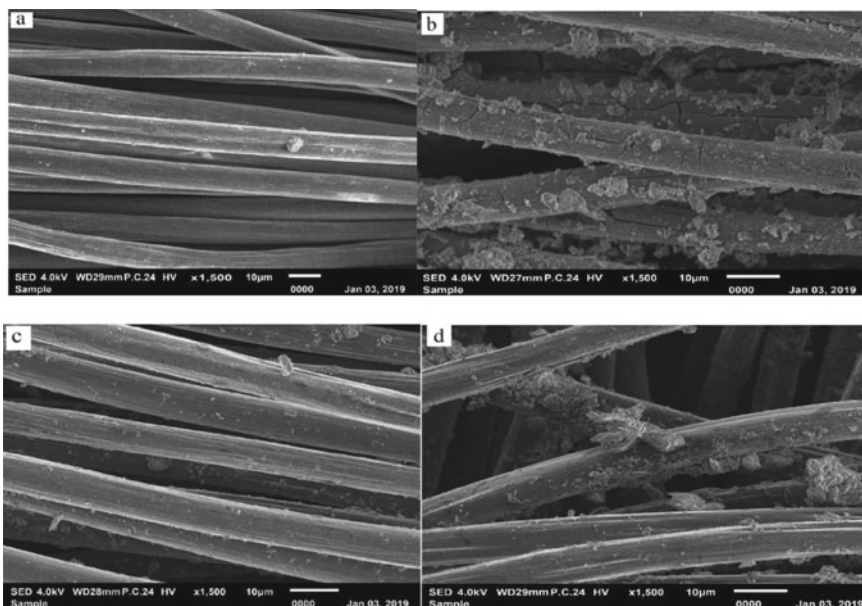


Fig. 7.2 SEM image of carbon cloth anode after 3 days electricity production in U-tube MFC; **a** control ×1500, **b** *Bacillus cereus* strain cc-1, **c** *Bacillus cereus* mutant 25 Gy, **d** *Bacillus cereus* mutant 63 Gy, **e** *Bacillus cereus* mutant 100 Gy

The surface of the control carbon cloth was uncovered of bacteria or biofilm, whereas the surface of the electrode sample from the other test had a deposition with different magnitude of layers. The magnitude of deposition was great on the sample from gamma radiated strain of 63 Gy and the wild strain, and moderate on the sample from 100 Gy. Besides, the surface of the carbon cloth had an insignificant deposition of bacteria for the assay sample that used the strain radiated with 25 Gy dose. This deposition shows the colonization of biofilm cells and their exopolysaccharide (EPS) on the surface of the carbon cloth and in fact, these images are consistent with the result obtained from the optimization process.

7.4.3 Electricity Performance

All experiments have been carried out in triplicates. The electrochemically active bacterium identified was subjected to gamma radiation. It had previously been reported that ionizing radiation induces mutagenic damage to cellular DNA, while 200 Gy induced the maximum gamma dose, causing a 90% bacterial inhibition (Min et al., 2003). Also, the hypothesis of hormesis (Bolsunovsky et al., 2016) suggested that low ionizing radiation (less than 200 Gy) induces a beneficial mutation. This experiment was aimed to increase the electricity production of the wild strain (*Bacillus cereus* cc-1) and hence, *Bacillus cereus* cc-1 was mutated using three different doses of gamma radiation (25, 63, and 100 Gy). Therefore, the following information in Fig. 7.3 indicates the generation of bacterial voltage after gamma radiation. Bacteria emitted with 63 Gy radiation produced the highest voltage (280

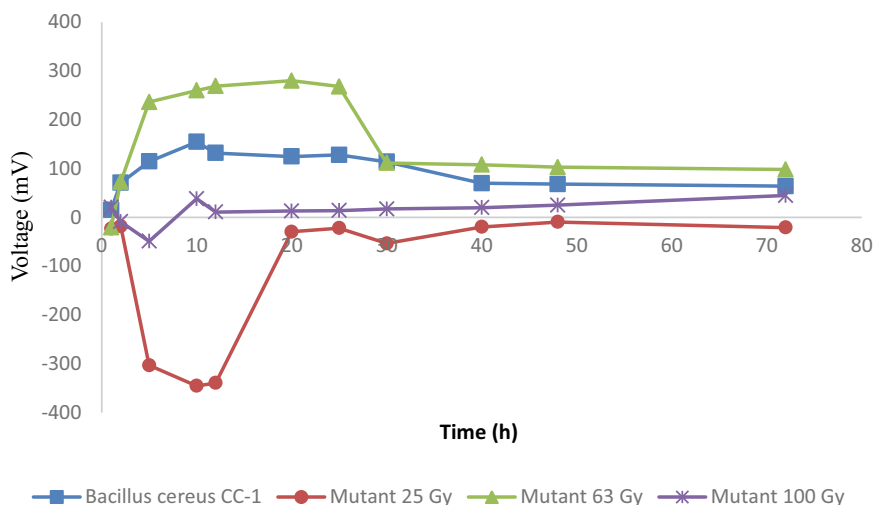


Fig. 7.3 Comparison of cell potential between the three mutant strain and the wild strain of *Bacillus cereus* cc-1

mV) in 20 h and the 25 Gy-irradiated strain showed reverse polarity whereas the highest dose of the experiment (100 Gy) induced a current production loss.

Reverse voltage phenomena are popular in hydrogen fuel cells when one cell of the fuel stack runs out of food (Lee et al., 2006). The investigation by Aelterman et al. (2006) is one of the pioneer studies that observed a reversal of polarization in MFCs, but they believed without experiment that it was due to food starvation. Therefore, Oh and Logan (2007) studied a series of three assays with two cells of MFC stack each where in the first experiment, both cells were inoculated with electrochemically active bacteria and fed perfectly, whereas in the second experiment, both cells were inoculated with electrochemical bacteria but one cell was intentionally deprived and fed the other cell, the third test was similar to the first experiment except one cell was free of the electrochemical bacteria. After monitoring the potential voltage of the stack MFC, the starved bacteria of the second assay and the bacteria-free MFC cell of the third experiment produced a potential reversal. Thus, the lack of food, as well as the lack of bacteria, may therefore cause a reversal voltage.

Moreover, the result from the SEM image of Fig. 7.2 shows that the sample from the test that used the strain irradiated with 25 Gy does not develop a biofilm formation, so either the radiation will cause a loss of metabolism pathway that used mud and water from the shrimp pond as a source of energy, or the bacteria have lost the ability to form a biofilm due to a mutation induced by gamma radiation. To prove these hypotheses, it was important to measure the removal of organic matter. Furthermore, the 100 Gy-dose mutant strain has developed a small layer of biofilm as well as a low electricity production compared to the 63 Gy-irradiated mutants that produced the maximum cell potential with a very thick layer of biofilm on the electrode surface. Henceforth, there is a positive direct correlation between biofilm formation and electricity generation in this study. Additionally, it was reported that the thicker biofilm-forming *Geobacter sulfurreducens* produces higher electricity generation (Sun et al., 2016). Moreover, some bacteria cannot produce electricity during the planktonic phase but can transfer electrons after biofilm formation (Watson & Logan, 2010). Therefore, based on the consequence of SEM and the literature of the reverse polarity induced by the radiation dose can be explained by the loss of bacterial biofilm formation.

7.4.4 The Effect of COD Removal and Strain Relations

COD is used to measure the amount of organic compounds present in the MFC substrate (water and mud from shrimp farm). Figure 7.4 shows the COD of the substrate containing bacteria inoculum (without culture, mix culture, pure culture, and mutants). The COD reading was observed after 3 days in the MFC operation. Mixed culture eliminates more COD than pure strain (55.7 and 65%, respectively). Similarly, Fatemi et al. (2012) found that mixed culture achieves a COD removal efficiency of 75%, whereas pure culture only reduces 54% of organic matter. Additionally, Nimje et al. (2012) also reported that the mixed culture removes more COD

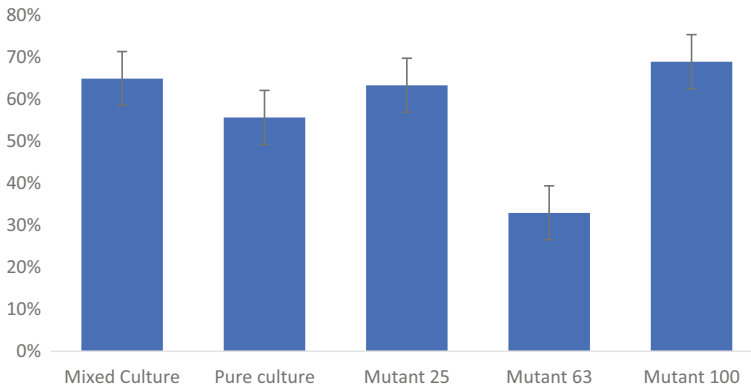


Fig. 7.4 Comparison of the Total COD removal between the mixed culture, the three mutant of strain CC-1 and the wild strain of CC-1

than pure oxygen. In both studies, they concluded that the methanogenic bacteria and denitrifying bacteria present in the mixed culture absorb the organic substrate without electricity generation.

Figure 7.4 depicts that the mutants treated with 25 and 100 Gy, despite their loss in electricity production, remove more COD compared to mutants treated with 63 Gy, 33, 63.4, and 69%, respectively. Therefore, with the mutation induced by gamma irradiation, we obtained a mutant that increased electricity production by more than two times and also, two mutant strains that removed a comparable rate of COD removal compared to that of the mixed culture.

7.5 Conclusion

The usage of low-dose gamma radiation has increased the potential to produce bacterial electricity and to remove organic matter. Since chemical mediator was not used to increasing the electricity generation, this method is considered to be eco-friendlier on the sludge and environment.

Acknowledgements The author would like to acknowledge the International Islamic University, Malaysia for awarding fund via Publication RIGS Grant (Grant No. P-RIGS18-065-0065).

References

Aelterman, P., Rabaey, K., Pham, H. T., Boon, N., & Verstraete, W. (2006). Continuous electricity generation at high voltages and currents using stacked microbial fuel cells. *Environment Science and Technology*, 40(10), 3388–3394.

- Bolsunovsky, A., Frolova, T., Dementyev, D., & Sinityna, O. (2016). Low doses of gamma-radiation induce SOS response and increase mutation frequency in *Escherichia coli* and *Salmonella typhimurium* cells. *Ecotoxicology and Environmental Safety*, 134, 233–238.
- Cutler, T. D., & Zimmerman, J. J. (2011). Ultraviolet irradiation and the mechanisms underlying its inactivation of infectious agents. *Animal Health Research Reviews/Conference of Research Workers in Animal Diseases*, 12(1), 15–23.
- Fakhiruddin, F., Amid, A., Wan Salim, W. W. A., & Azmi, A. S. (2018). Electricity generation in Microbial Fuel Cell (MFC) by bacterium isolated from rice paddy field soil. *E3S Web of Conferences*, 34, 1–9. <https://doi.org/10.1051/e3sconf/20183402036>.
- Fatemi, S., Ghoreyshi, A. A., Najafpour, G., & Rahimnejad, M. (2012). Bioelectricity generation in mediator—Less microbial fuel cell: Application of pure and mixed cultures. *Iranica J Energy & Environment*, 3(2), 104–108.
- Fishbein, L. (1970). *Alkylating agent in chemical mutagens environmental effects on biological systems* (1st ed.). Academic Press.
- Habibi, M. B., & Pezeshki, N. P. (2013). Bacterial mutation; Types, mechanisms and mutant detection methods: A review. *European Sc. J*, 4(December), 1857–7881.
- Jena, N. R. (2012). DNA damage by reactive species: Mechanisms mutation and repair. *J Biosciences*, 37(3), 503–507.
- Lee, H., Song, H., & Kim, J. (2006). Effect of reverse voltage on proton exchange membrane fuel cell performance, pg. 205–208 in 2006 International Forum on Strategic Technology, IFOST. IEEE Xplore.
- Logan, B. E. (2009). Exoelectrogenic bacteria that power microbial fuel cells. *Nature Reviews Microbiology*, 7, 375–381.
- Mahdih, N., & Rabbani, B. (2013). An overview of mutation detection methods in genetic disorders. *Iranian J Pediatrics*, 23(4), 375–388.
- Min, J., Lee, C. W., & Gu, M. B. (2003). Gamma-radiation dose-rate effects on DNA damage and toxicity in bacterial cells. *Radiation and Environmental Biophysics*, 42(3), 189–192.
- Nimje, V. R., Chen, C. Y., Chen, H. R., Chen, C. C., Huang, Y. M., Tseng, M. J., Cheng, K. C., & Chang, Y. F. (2012). Comparative bioelectricity production from various wastewaters in microbial fuel cells using mixed cultures and a pure strain of *Shewanella oneidensis*. *Bioresource Technology*, 104, 315–323. <https://doi.org/10.1016/j.biortech.2011.09.129>.
- Oh, S., & Logan, B. E. (2007). Voltage reversal during microbial fuel cell stack operation. *Journal of Power Sources*, 167(1), 11–17.
- Parks, J. E. (2015). *The Compton effect-compton scattering and gamma ray spectroscopy*. Knoxville: The University of Tennessee.
- Quillardet, P., Frelat, G., Nguyen, V. D., & Hofnung, M. (1989). Detection of ionizing radiations with the SOS chromotest, a bacterial short-term test for genotoxic agents. *Mutation Research/Environmental Mutagenesis and Related Subjects*, 216(5), 251–257.
- Sun, D., Chen, J., Huang, H., Liu, W., Ye, Y., & Cheng, S. (2016). The effect of biofilm thickness on electrochemical activity of *Geobacter Sulfurreducens*. *International Journal of Hydrogen Energy*, 41(37), 16523–16528.
- Watford, S., & Warrington, S. J. (2021). *Bacterial DNA mutations*. Treasure Island (FL): StatPearls Publishing.
- Watson, V. J., & Logan, B. E. (2010). Power production in MFCs inoculated with *Shewanella oneidensis* MR-1 or mixed cultures. *Biotechnology and Bioengineering*, 105(3), 489–498. <https://doi.org/10.1002/bit.22556>.
- Wei, L., Han, H., & Shen, J. (2012). Effects of cathodic electron acceptors and potassium ferricyanide concentrations on the performance of microbial fuel cell. *International Journal of Hydrogen Energy*, 37(17), 12980–12986.
- Wen, Q., Wu, Y., Zhao, L., & Sun, Q. (2010). Production of electricity from the treatment of continuous Brewery wastewater using a microbial fuel cell. *Fuel*, 89(7), 1381–1385.
- Zhao, F., Harnisch, F., Schroder, U., Scholz, F., Bogdanoff, P., & Herrmann, I. (2006). Challenges and constraints of using oxygen cathodes in microbial fuel cells. *Environment Science and Technology*, 40(17), 5193–5199.