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Modification of bacterial cell membrane to accelerate decolorization of textile wastewater effluent using microbial fuel cells: role of gamma radiation

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ABSTRACT

The aim of the present work was to increase bacterial adhesion on anode via inducing membrane modifications to enhance textile wastewater treatment in Microbial Fuel Cell (MFC). Real textile wastewater was used in mediator-less MFCs for bacterial enrichment. The enriched bacteria were pre-treated by exposure to 1 KGy gamma radiation and were tested in MFC setup. Bacterial cell membrane permeability and cell membrane charges were measured using noninvasive dielectric spectroscopy measurements. The results show that pre-treatment using gamma radiation resulted in biofilm formation and increased cell permeability and exopolysaccharide production; this was reflected in both MFC performance (average voltage 554.67 mV) and decolorization (96.42%) as compared to 392.77 mV and 60.76% decolorization for non-treated cells. At the end of MFC operation, cytotoxicity test was performed for treated wastewater using a dermal cell line, the results obtained show a decrease in toxicity from 24.8 to 0 (v/v%) when cells were exposed to gamma radiation. Fourier-transform infrared (FTIR) spectroscopy showed an increase in exopolysaccharides in bacterial consortium exposed to increasing doses of gamma radiation suggesting that gamma radiation increased exopolysaccharide production, providing transient media for electron transfer and contributing to accelerating MFC performance. Modification of bacterial membrane prior to MFC operation can be considered highly effective as a pre-treatment tool that accelerates MFC performance.

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Modification of cell membrane; textile wastewater; microbial fuel cell; gamma radiation; exopolysaccharides; *Bacillus* sp.

1. Introduction

Microbial Fuel cells (MFCs) are constructed to convert chemical energy to electrical energy using microbial cells as an alternative catalyst to chemicals. It has many applications in energy production, wastewater treatment, and other bioremediation processes (Jiang, Zhao, Zhang, Zhang, & Lee, 2009). The use of MFCs in the treatment of textile wastewater has been reported as an alternative approach to classical chemical and physical textile wastewater treatment due to its efficiency and low cost. The decolorization process can increase upon optimizing the operation conditions (Eslami et al., 2019), by adding redox mediators in the anodic chamber (Gomaa, Fapetu, Kyazze, & Keshavarz, 2017) or through incorporating a novel MFC design. During an MFC process, electrons are transferred from the microorganism to the anode, and then they flow through a circuit to the cathode. The electron transfer is considered one of the main complex but important steps in MFC operation (Schroeder, 2007). The mechanism by which electrons transfer is categorized into direct and mediated electron transfer pathway. In direct electron transfer, the electroactive cells are in contact with the electrode usually growing as a biofilm. Electroactive biofilm formation was reported to be imperative for the efficient operation of MFCs (Winaikij, Sreearunothai, & Sombatmankhong, 2018). Both gram-negative and gram-positive bacteria are known to form highly structured, multilayered biofilms, the former was reported to reach about 50 µm, while the latter was reported to form 38 µm (Kumar, Singh, & Zularisam, 2016).

This key process is governed by the biofilm growth on the anode; therefore, many attempts have been made to enhance the electron transfer by increasing the biofilm formation in order to enhance MFC performance (Angelaalincy et al., 2018). Two approaches are usually followed, one is to induce changes in the material of the anode such as using conductive polymer (Liu, Wu, & Gu, 2015), modified electrode surface (Eaktasang, Kim, Lee, Park, & Kim, 2012) or carbonized plants that give three-dimensional electrode surface to provide higher surface area for enhanced bacterial biofilm (Karthikeya et al., 2015). Another approach is to induce changes to the bacteria itself by perforating the cell membrane (Liu, Qiao, Lu, Song, & Li, 2012) or adding a chemical compound to increase cell attachment (Gomaa, Selim, Fathy, & Hamed, 2019). The latter

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approach depends on inducing changes to the cell membrane to increase hydrophobicity or exopolysaccharides in order to increase biofilm formation on the anode. Biosurfactants have been reported to enhance cell permeability, reduce cell membrane resistance, and increase mass transport through the bacterial cell membrane (Gao et al., 2017), therefore increasing both biodegradation and MFC performance (Sotirova, Spasova, Galabova, Karpenko, & Shulga, 2008; Zhang et al., 2017). In analogy, gamma radiation can be used as a physical approach to induce changes to bacterial cells. Gamma radiation is a form of ionizing radiation, it can induce a myriad of responses to cells, and the responses range from interacting with different cellular molecules, altering gene expression, damaging nucleic acids, or causing cell death (Shuryak, 2019). But at low doses, it can induce the production of exopolysaccharides in bacterial cells (Guesmi et al., 2019) and therefore, increase biofilm formation. From this standpoint, modifying bacterial cell membrane using gamma radiation may be considered the key step that contributes to both direct and mediated electron transfer and therefore, enhancing MFC performance. The aim of the present work was to use gamma radiation as a pre-treatment process and study its effect on bacterial cell surface charge, permeability, and biofilm formation on the anode. The target is to enhance textile wastewater treatment using MFC.

2. Materials and methods

2.1. Textile wastewater effluent

Textile wastewater effluent was obtained from a textile factory located in 6th October district at the end of reactive dye operation day. Chemical oxygen demand (COD) was measured according to ASTM (1995), while total suspended solids, pH, biological oxygen demand (BOD), conductivity, and color were measured according to the APHA manual (2012). The tests were conducted at the Microanalytical center at the Faculty of Science, Cairo University.

2.2. Enrichment and characterization of anodic bacteria

MFC systems were H-type two-chambered systems with two identical Duran bottles held together with external metal clamps. The electrodes were made of carbon fiber and were cut to 4 × 4 cm. The anode and the cathode compartments were separated with a cation-exchange membrane CMI-7000 (Membranes International USA). The anaerobic anode compartment containing 200 ml working volume was purged with nitrogen gas for 10 min through a 0.22 µm pore size diameter filter prior to inoculation; the media used were MSM prepared according to Gomaa et al. (2017), which contained the following (g/L): NH_4Cl 0.46, KCl 0.225, $MgSO_4.7H_2O$ 0.117, NaH_2PO_4 2.5, Na_2HPO_4 4.11, $(NH_4)_2SO_4$ 0.225; a vitamin mixture and trace mineral solution were added (1%), and 500 mg/L casein hydrolyzate and 2.2 g/L sodium pyruvate were also added. Cathode chamber contained 200 ml working volume of 100 mM potassium ferricyanide in 50 mM sodium phosphate buffer (pH 7). MFC systems were incubated at 37° C in an incubator. A 24 hr inoculum (20%) was added to the anodic chamber. The external resistance used was 1000 Ω. The biofilm formed on the anode was used for bacterial characterization of the consortium.

2.3. Next generation sequencing (NGS) of anodic consortia and identification of the predominant strain

The genomic DNA from the wastewater samples was extracted with Sigma Aldrich GenElute DNA isolation kit (Sigma Aldrich, UK). The extracted DNA was tested for purity with A260/A280 ratio and quantified using a NanoDrop (Nano-1000, Thermo Scientific, USA) spectrophotometer. The samples were sent to NovoGene Genome Sequencing Company, China, for 16 S rRNA amplicon sequencing.

The DNA concentration and purity were monitored on 1% agarose gel and the concentration of DNA was diluted to 1 ng µL^{-1} using sterile water. PCR amplification was carried out with Phusion High-Fidelity PCR Master Mix (New England Biolabs) for the 16 S V4 region using 515 F-806 R primers. The PCR products were then quantified on 2% agarose gel and the samples that produced bright bands in the 400–450 bp region were used for subsequent analysis. The PCR products were purified using the Qiagen Gel Extraction Kit (Qiagen, Germany). The libraries were generated on NEBNext Ultra DNA Library Prep Kit for Illumina and the quality assessed on Qubit 2.0 Fluorometer (Thermo Scientific) and Agilent Bioanalyser 2100 system. The samples were sequenced on the Hiseq 2500 platform to generate 250 bp paired-end reads. Following that, paired-end read data were exported in FASTQ format and were processed using Quantitative Insights Into Microbial Ecology (QIIME software ver. 1.8) pipeline (Caporaso et al., 2010). Read sequences were joined, quality checked, and clustered into operational taxonomic units (OTUs) with the Uclust method (Edgar, 2010). The taxonomic assignment of the major OTUs was checked using Classifier (Wang, Garrity, Tiedje, & Cole, 2007); the data were represented as the relative abundance of top genus. The sequencing data were deposited in Genbank SRA accession: PRJNA526831.

2.4. Pre-treatment of anodic bacteria

As pre-treatment, anodic bacteria were exposed to gamma radiation at the following doses: 0, 0.5, 1, 1.5,

2 2.5, and 3 kGy. Gamma radiation was performed at the Indian Cobalt Unit located at the National Center of Radiation Research and Technology (NCRRT) radiation facility at a dose rate of 0.9 KGy/h.

2.5. Dielectric property studies for bacterial cell membrane

The dielectric measurements were carried out using LCR meter HIOKI 3531 (manufactured in Japan), the frequency range was from 40 kHz to 1 MHz. The measured parameters were capacitance (F) and conductance (S) for bacterial cell membrane. The bacterial consortium obtained at the end of the first MFC operation was lightly washed with phosphate-buffered saline (pH7) and re-suspended in double-distilled water. Description of the plate conductivity cell and platinum electrodes was previously described (Gomaa, Selim, & Linz, 2013). In the present study, the values of conductance were chosen at 1 kHz for comparison of the different samples as the mid-point in the plateau of the dispersion curve. The measured parameters were conductivity, which reflects cell membrane permeability (μS/cm), and cell membrane charge, which represents relative permittivity. While permittivity is in μF/cm, the plotted data represent relative value to non-irradiated cells; therefore, it is relevant to 1.

2.6. Fourier-transform infrared (FTIR) spectroscopy

All gamma-irradiated samples were tested for changes in their functional groups using Fourier-transform infrared (FTIR) spectroscopy. The test was performed using the attenuated total reflection (ATR) mode. Upon gamma irradiation, the bacterial samples were placed directly on the crystal and scanning was performed from 400 to 4000 cm^{-1} using FTIR, BRUKER VERTEX 70 device at Central Lab. Unit, NCRRT, Cairo. The data were plotted as wave number vs. T (%).

2.7. MFC performance

Two MFC systems were set up, each containing textile wastewater, and were inoculated separately with (1) non-pre-treated bacteria, (2) 1 kGy gamma-irradiated bacterial consortium; this dose was chosen based on the highest cell conductivity obtained using dielectric spectroscopy. MFC design and media used are described above.

2.8. Post-MFC biofilm assay

To confirm biofilm formation, 0.5 × 0.5 cm pieces of carbon cloth anode from each of the MFC setups were used for the following assays: exopolysaccharides (EPS) were measured by placing the carbon cloth pieces in sterile falcon tubes containing 95% ethanol, the tubes were incubated at 4°C overnight to release surface-bound exopolysaccharides (Nehad & El-Shamy, 2010). Ethanol was removed and exopolysaccharides were assayed quantitatively using the phenol-sulfuric method (Chaplin & Kennedy, 1986), absorbance was measured at 490 nm, and glucose was used as standard. Biofilm surface-bound proteins were extracted according to a modified method of Castellanos, Ascencio, and Bashan (1997). The harvested cells were re-suspended in 10 ml 6 M urea for 90 min at 22°C. The cell suspension was centrifuged at 1600 g for 10 min at 10 °C, the supernatant was used to detect the protein content using Lowry method (1951) using bovine serum albumin (BSA) as a standard. Biofilm assay was performed using a crystal violet microtiter plate reader at 560 nm. For scanning Electron Microscopy (SEM), the cut pieces were dried at 50°C then glued separately onto brass stubs using a double-sided adhesive tape and were coated with a thin layer of gold under reduced pressure using sputtering unit model JFC-1100E (Japan). The images were captured at magnifications of 200 and 3500 X using an electron beam high voltage of 30 kV, JOEL JSM 5400 Scanning Electron Microscope (Japan). Micrographs were captured using Orion digitizer software. Carbon cloth anode pieces for pre-treated bacterial samples were compared to those of control (unused) carbon cloth electrodes.

2.9. Post-MFC analysis of treated textile wastewater

BOD, COD, conductivity, and TSS were all assayed as described earlier in the 'textile wastewater analysis' section. Decolorization was calculated as the percentage of removal using the following equation:

$$\text{Decol (\%)} = \frac{IA - IF}{IA} \times 100$$

where I_A is the initial absorbance and I_F is the final absorbance. Cytotoxicity was assayed as 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide tetrazolium (MTT) using dermal cell culture according to the method described by Tolosa, Donato, and Gomez-Lechon (2015).

2.10. Statistical analysis

All experimental data indicated on the graphs and tables are the mean value of triplicate experiments, the error bars in the graphs represent the standard deviation of the mean (SD). Statistical analysis of data was conducted by one-way analysis of variance (ANOVA) using Microsoft Excel statistics package.

3. Results

3.1. Taxonomic assignment

The obtained results in Figure 1 show that the genus *Bacillus* had a predominance of 80.6% and 87.5%

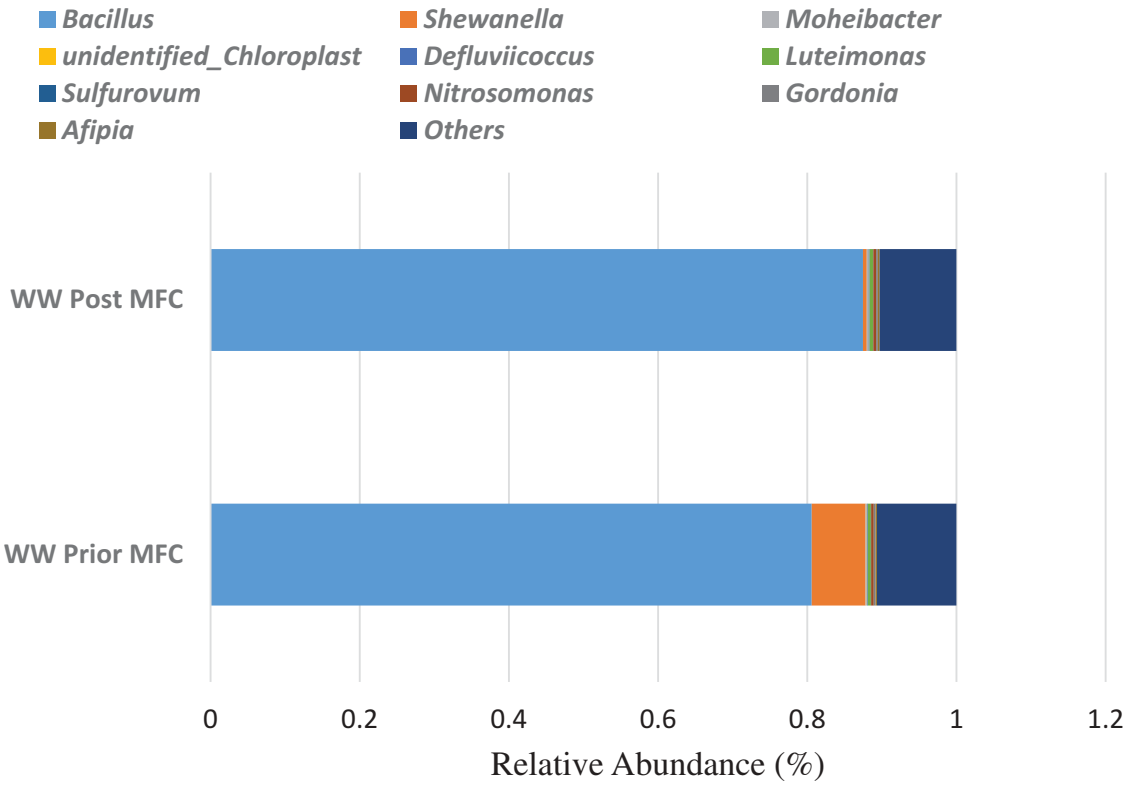

Figure 1. Representation of relative abundance of operational taxonomic unit (OTU) for textile wastewater prior to, and post-MFC operation. The results are based on 16 S rRNA gene sequences.

before and after MFC operation, respectively. *Shewanella* was detected as well but with a less presence that reached 7.2% before MFC operation and decreased further to 0.5% after MFC operation. Other reported genera were *Moheibacter, Defluvicoccus, Luteimonas, Sulfurovum, Nitrosomonas, Gordonia, Afipia*, but were all reported to be of less significant values. A 10% was reported as other unidentifiable genera in both MFCs before and after the operation.

3.2. The effect of gamma irradiation on anodic bacteria

In order to study the effect of gamma radiation on anodic biofilm bacteria, bacterial cell permittivity, and cell conductivity were studied using dielectric spectroscopy. The results in Figure 2 show that exposing the bacteria to increasing doses of gamma radiation resulted in an increase in both cell permittivity and cell conductivity, the maximum values reached 4- and 3.54-fold, respectively, at 1 kGy; however, the values dropped gradually with increasing doses.

The FTIR spectrum for textile wastewater enriched bacteria exposed to different gamma irradiation doses is shown in Figure 3. The figure shows peaks at 3394 and 2931 cm^{-1} which are assigned for –OH stretching frequency of –NH and –CH stretching groups, respectively. The peak at 1629.7 cm^{-1} represents the carboxylate group; it was also reported to represent ring stretching of galactose. The peaks from 1457 to 1381 cm^{-1} represent COO^- groups and >C = O stretch groups and C–O bond from COO^- groups. The peak at 1070.4 cm^{-1} represents symmetric –CH bending. The peaks from 1200 to 915 cm^{-1} suggest the presence of monosaccharides like glucose or galactose. The absorption peak at around 621 cm^{-1} could be attributed to stretching alkyl halides. All the peaks suggest that EPS is increasing upon exposure to gamma radiation in quantity but not in quality.

3.3. Study of MFC performance

The anodic bacteria were that used for cell exposure to gamma radiation had an effect on MFC operation. Figure 4(a) shows that cell exposure to gamma radiation as a pre-treatment increased the voltage/current, the maximum reaching 180 mV, as compared to 140 mV for non-treated cells. The same trend can be seen for polarization curves (Figure 4(b)), the maximal points were 0.38, 0.15 mW/m^2, respectively.

3.4. Scanning electron microscopy

Following MFC operation, the anodes were used to detect biofilm formation. Therefore, Scanning Electron Microscopy (SEM) was used to reveal deposition of cells on the carbon electrode for gamma-irradiated cells. However, SEM does not represent exact numbers but can only represent visual adhesion (Figure 5).

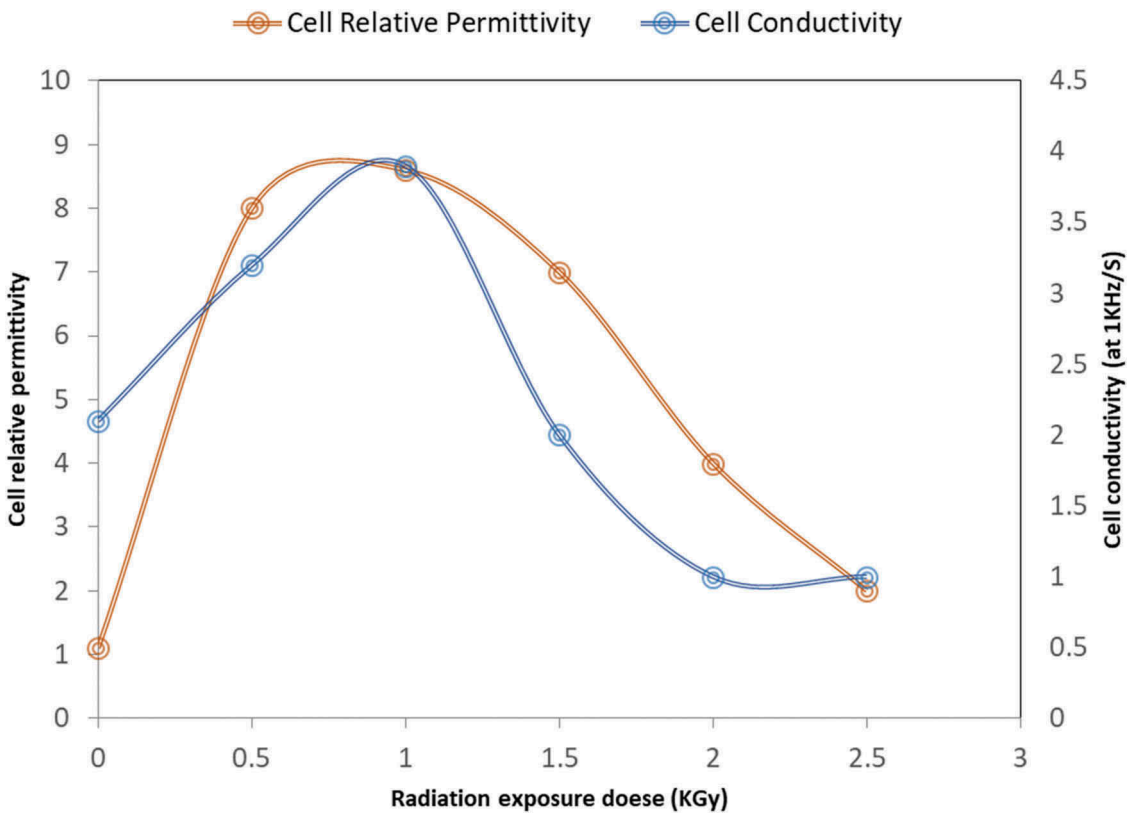

Figure 2. Dielectric measurements of anodic biofilm exposed to gamma radiation.

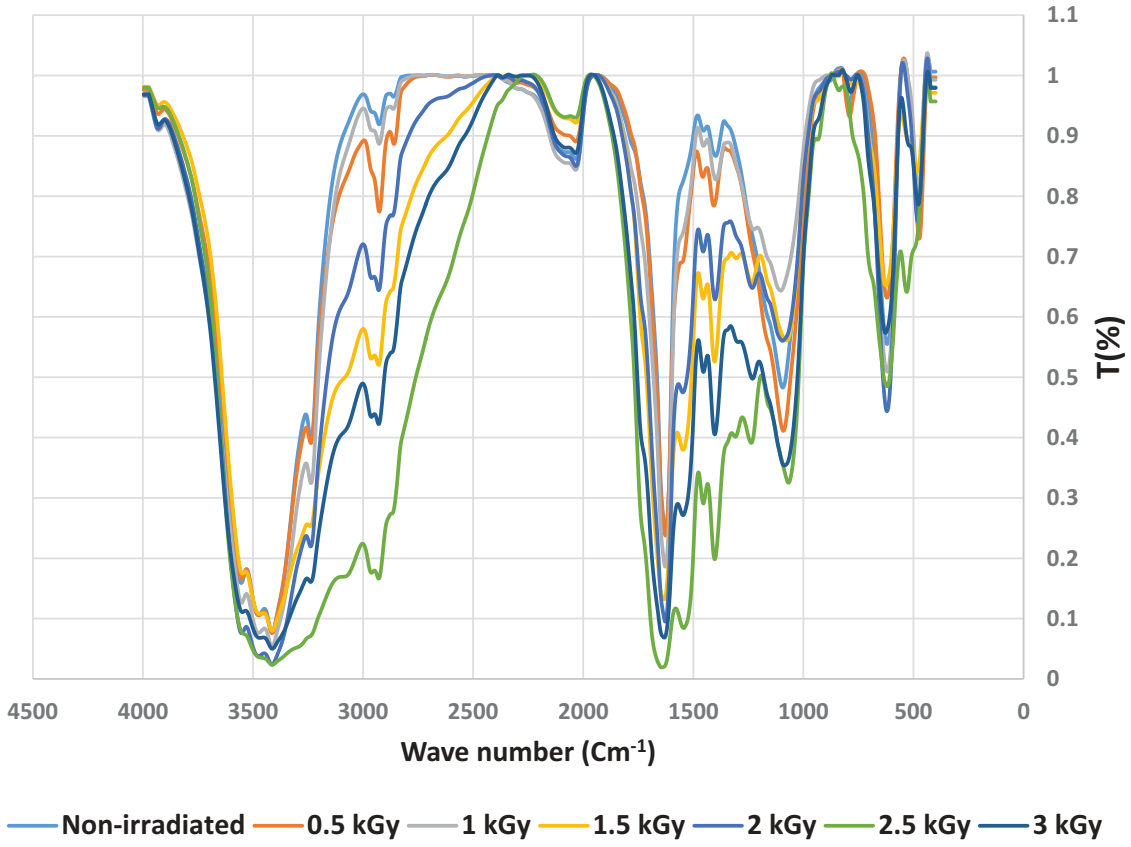

Figure 3. FTIR spectrum for anodic bacteria exposed to different doses of gamma radiation.

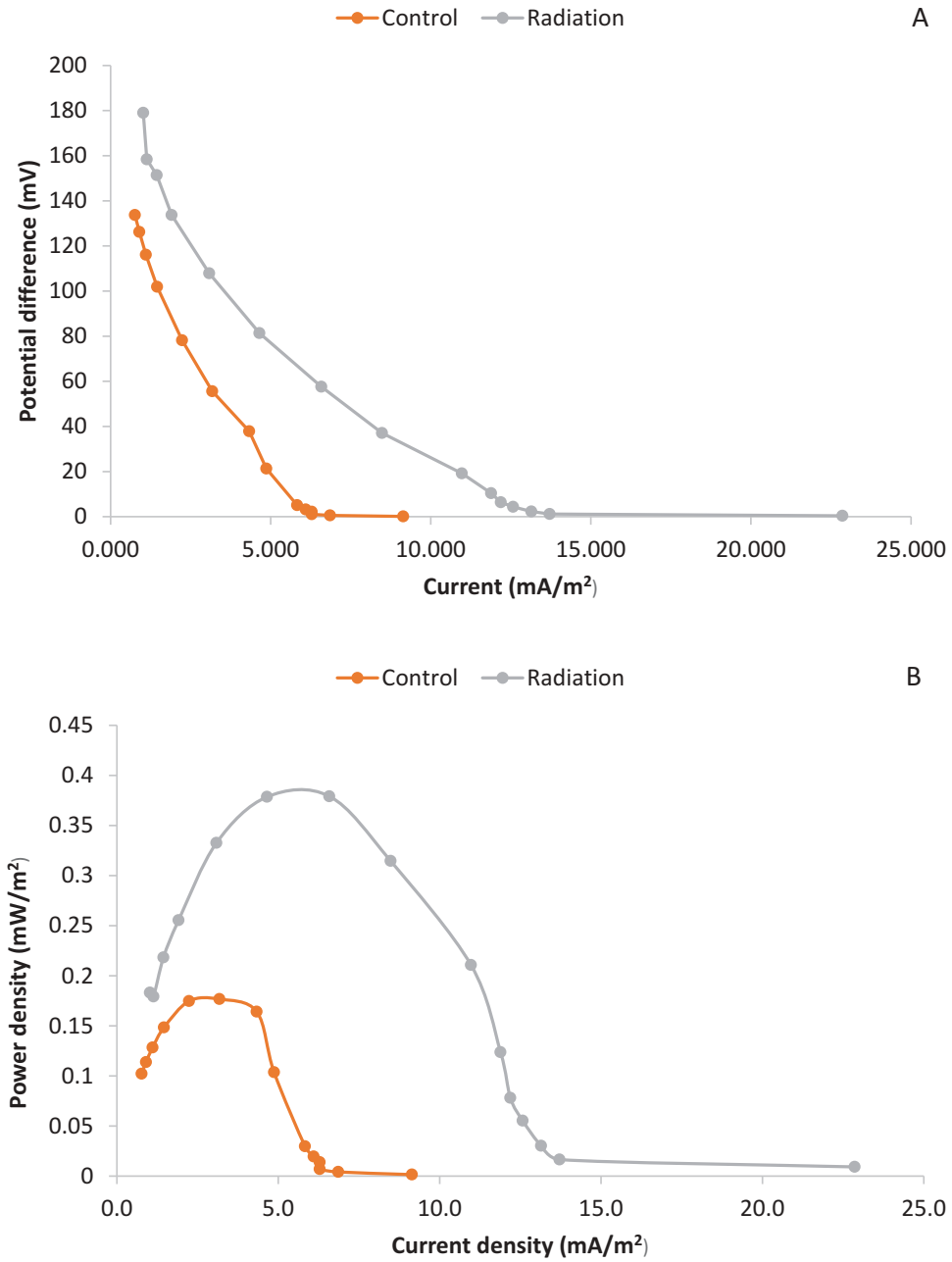

Figure 4. Voltage vs. current (a) and polarization curve (b) of MFCs examined in this study.

3.5. Study of some chemical and physical parameters and toxicity at the end of MFC performance

The results in Table 1 show that while conductivity of the textile wastewater increased for pre-treated cells at the end of MFC operation which was 4.98 mS/cm as compared to 0.9 mS/cm for cells with no prior treatment. Gamma-irradiated pre-treatment showed a decrease in both COD and BOD. Bacterial cell relative permittivity increased from 1.49 to 3.26 for non-treated and gamma-irradiated cells, respectively. Bacterial cell membrane conductivity also increased from 0.22 to 2.7 µS/ml, for non-treated and gamma-irradiated cells, respectively. Exopolysaccharides increased from 20.29 to 68.1 mg/ml for the samples mentioned above. Detecting biofilm using crystal violet showed absorbance that increased from 0.4 to 1.2, respectively. The decolorization increased from 60.76 to 94.42, respectively. Studying the toxicity of the textile wastewater after the MFC treatment, the results show IC_{50} value 24.79 for control MFC with no prior treatment to the cells and 0 value for gamma-irradiated cells.

4. Discussion

The presence of electroactive bacteria (EAB) is not restricted to a certain environment but it can be found in different ecosystems, including extreme environments (Chabret, Ali, & Achouak, 2015). Bacteria isolated from textile wastewater sludge has been reported to be efficiently used in MFCs for dye decolorization (Eslami et al., 2019). In the present study, MFC was used to treat textile

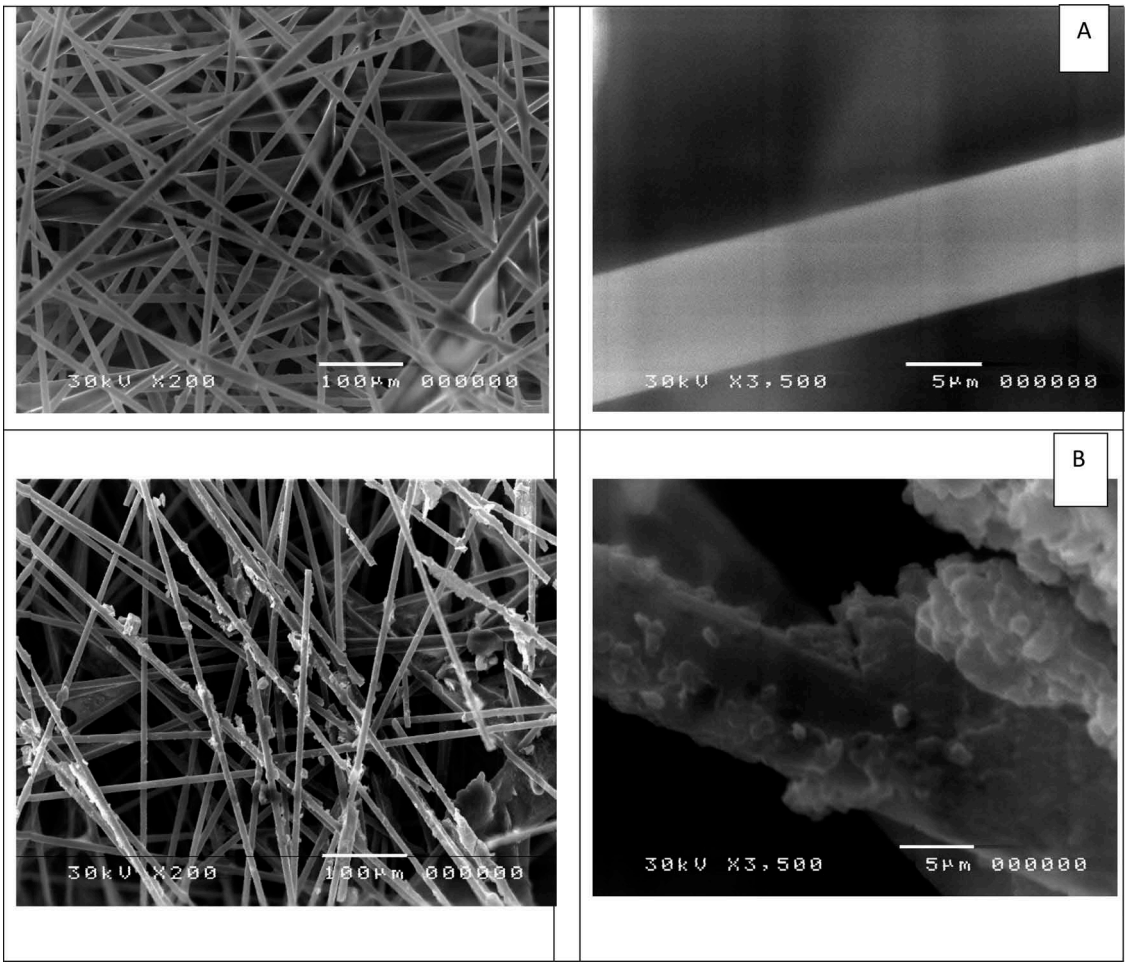

Figure 5. SEM images of the anode before and after MFC experiment at magnifications 200x and 3500x for anode (a) and bacterial anodes exposed to 1 kGy gamma radiation (b).

Table 1. Assay of different parameters at the end of MFC operation; anodic chamber was inoculated with non-treated bacteria and gamma-irradiated bacteria.

Assay	Non-treated bacteria	Gamma-irradiated bacteria
Textile wastewater conductivity (mS/cm)	0.90	4.98
COD (mg/l)	Above detecting limit	1479
BOD (mg/l)	Above detecting limit	7
Bacterial cell surface charge (relative permittivity) at 1 kHz	1.49	3.26
Bacterial cell permeability (conductivity) at 1 kHz (µS/ml)	0.22	2.70
Exopolysaccharides (mg/ml)	20.29 ± 0.11	68.10 ± 0.22
Biofilm (crystal violet protein in µg/ml)	0.4 ± 0.01	1.2 ± 0.13
Average voltage (mV)	218.884	554.67
Decolorization (%)	60.76	96.42
IC_{50} (v/v%)	24.79	0

wastewater, and therefore, it was tested for potential presence of EAB in the textile wastewater itself. Our results showed the predominance of *Bacillus* sp. over *Shewanella* sp. Although *Shewanella* sp. is widely known for its uses and application in MFCs, yet *Bacillus* sp. and other gram-positive bacteria were also reported to be electroactive; they respond to the addition of redox mediators and were reported to produce flavins and possess multiheme cytochromes (Costa, Carlson, Coates, Louro, & Paquete, 2015; Liu et al., 2010; Wu et al., 2014). A mixed gram-positive and gram-negative culture was reported to efficiently degrade dyes from real textile wastewater (Eslami et al., 2019). An MFC containing mixed bacterial consortium is considered more preferable than pure cultures because of the high durability and variation in substrate consumption (Winaikij et al., 2018). Gram-negative and gram-positive bacteria each follow different electron transfer mechanisms, while electrons pass across the outer cell membrane to cell surface in the former; electrons have to pass through a thick cell wall before being transferred to an electron acceptor (White et al., 2016). It is crucial to induce perforations in the thick cell wall of *Bacillus* sp., since it represents the majority of the bacterial population on the anode. Therefore, we used gamma radiation as a pre-treatment approach, the doses chosen were based on published work (Romanovskaya et al., 2002; Atique, Ahmed, Asaduzzaman, & Hasan, 2013). Spectroscopy was used to study gamma-irradiated cells. The dielectric properties' measurements are considered a noninvasive technique, it reflects cell physiology and properties of the cell membrane. The increase in permeability value is correlated

with an increase in exchange between the cytoplasm and the media (Patel & Markx, 2008). The use of different doses of gamma radiation on bacteria showed that there was an optimal dose of 1 KGy that was sufficient to increase bacterial cell permeability and surface charge, above which, those two parameters dropped. This result suggests that an optimal dose of gamma radiation is required to promote mediated electron transfer to increase bacterial cell permeability and bacterial cell surface charge. The addition of EDTA and oligo-electrolytes was also reported as chemical treatments that can induce membrane permeability resulting in up to four times increase in MFC performance, this increase was linked to changes in membrane fatty acid composition (Kumar et al., 2016). In the present study, we used ATR-FTIR to depict changes in bacterial consortium exposed to gamma radiation. Our results showed that both proteins and carbohydrates in the cell membrane changed upon exposure of bacteria to increased doses of gamma radiation, changes in peaks correlated to lipids were not obvious, and this is in agreement with EPS fingerprint as reported by Nambiara et al. (2018). ATR-FTIR spectroscopy is considered to be a comprehensive and sensitive method for detecting molecular changes in intact cells (Salman et al., 2010). Both cell surface proteins and EPS were previously found to act as key performers in bacterial adhesion to the anode when the cells were previously treated with palladium alpha-lipoic acid nanocomplex to form a biocomposite before adding it to the anodic chamber (Gomaa et al., 2019). EPS was mentioned as a key player in enhancing electron transfer (Angelaalincy et al., 2018). It was reported to be the transient media favorable for electron transfer as reported by Xiao et al. (2017). As a matter of fact, the pre-treatment of cells using gamma radiation has resulted in an increase in MFC performance, this confirms the effective result of gamma radiation.

SEM images often provide a general view of how the electroactive biofilm colonizing the electrode fibers. The results obtained show an increase in biofilm formation for gamma-irradiated cells when compared to non-treated cells. Similar results were obtained by Eaktasang et al. (2012) who induced changes to the electrode by acid treatment. Biofilm formation was confirmed quantitatively using crystal violet assay; a 3-fold increase was depicted and 3.35-fold increase in EPS when cells were exposed to gamma radiation. Microorganisms produce EPS, especially polysaccharides and proteins, as a means of protection against stress (Li et al., 2019). Gamma radiation was reported to induce EPS in *Bacillus* sp. it is thought to act as an antioxidant that protects bacterial cells from ionizing radiation and thus is considered a radio-protector (Guesmi et al., 2019). Besides EPS increase, total protein increased as well upon exposure to gamma radiation, this is attributed to an increase in protein synthesis, a response that is unanimous to all organisms exposed to environmental stress. One of the protein classes claimed to be released as stress response is the heat shock proteins (HSPs), these proteins act as molecular chaperones, and they play a role in protein folding, repair, and assembly in bacterial cells (Caillet, Millette, Dussault, Shareck, & Lacroix, 2008). MFCs inoculated with gamma-irradiated pre-treated cells did not only show the highest MFC performance, but it also exhibited high decolorization and low toxicity values for the treated wastewater. Enhancing decolorization of real textile wastewater was reported to decrease toxicity (Gomaa, Abdel El Kareem, & Fathey, 2011). Therefore, we can claim that gamma radiation affects bacterial cell permeability as well as induces EPS production as stress response, contributing to release of electrons and/or other metabolites, while EPS acted as the transient media for electron transfer, in addition to the overall increase in cell adhesion to the anodic surface. As far as we know this is the first report of using gamma radiation to trigger biofilm formation in MFCs.

5. Conclusion

In conclusion, membrane permeability and surface charge can be modified using gamma radiation and can be used as an effective approach to accelerate MFC performance. The use of gamma radiation enhanced EPS production which has been directly linked with improving extracellular electron transfer. In addition to that, the bacteria can be exposed to gamma irradiation, lyophilized, and stored in sterile sachets for individual use. Applying gamma radiation is worth further investigation as a safe and effective strategy to improve electron transfer in MFCs. This study is a proof of the possibility of modifying the cell membrane to increase biofilm formation and possibly release of intrinsic mediators. We are currently working on the later.

Disclosure statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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