Decolorization of Congo red in Microbial Fuel Cell

K Priyanka

Anna University Chennai

A.merline sheela

Anna University Chennai

ILAMATHI R (✉ mathidmagnate@gmail.com)

CBIT: Chaitanya Bharathi Institute of Technology <https://orcid.org/0000-0003-0665-5561>

Research Article

Keywords: Congo Red, Dye decolourization, MFC, P.aeruginosa, Glucose

Posted Date: July 30th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-742079/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

In this research, three individual conditions (static, shaking and MFC) were tested for Congo Red decolorization. *Paeruginosa* MTCC 2582 has showed 96.1% decolourization under MFC condition with 85% COD reduction for the dye (100 μM). Microbial fuel cell of *Paeruginosa* can discharge the dual duty of degrading the recalcitrant dye with power generation. To understand the influence the growth curve, different substrate concentration of glucose (0-20 g/L) were selected to improve the performance of MFC. Results show that a larger open circuit potential of 0.691 V and a maximum power density of 1.9 mW m^{-2} was possessed for the degradation of 100 μM of dye at 10 g/L of glucose concentration. Further, the selection of optimum concentration of dye (200 μM) increased the open circuit potential to 0.844 V. The degraded metabolites were confirmed using UV-Vis and FTIR analysis. Biofilm formation on anode at optimal glucose concentration was studied by using SEM analysis.

1. Introduction

Textile industry is considered as the one of the major polluting industries by generating huge volume of wastewater with complex toxic compounds (Sen., 2003). Further, it is estimated that 10–15% of the dyes are lost in effluent during the dyeing process in textile industry. Wastewater from textile industry is one of the major sources of aromatic amines getting released into the environment leading to cumulative adverse effects. Another important sector to be considered is energy. Energy plays a critical role in the economic growth, progress and development, as well as poverty eradication and security of any country. In recent years, the industrial development and population growth have led to a surge in the global demand for energy. Present energy resource structure is unsustainable and the pollution control methods are high-energy-consuming and cost intensive.

Thus, it is wise to develop a technology that meets these two severe challenges. Microbial fuel cells (MFCs) are being explored extensively for sustainable energy production, waste disposal and reducing CO_2 emissions^[2]. Electricity production from biotechnological application employing the conversion of organic compounds with microbial fuel cells is a concept known for almost 100 years^[3]. However, it is only recently that the thoughts of utilizing MFC for practical applications have been entertained with an increase in power output. This was due to the paradigm shift in the exciting discoveries about microbial physiology related to electron transport, and the advancement of fuel cell technologies was established in the past few years^[4].

Microorganisms in MFC can discharge the dual duty of degrading the pollutants and generating power. Moreover, it yields 50–90% less solids to be^[5]. Microbial fuel cell (MFC) is one type of fuel cell, where the conversion of the chemical energy from the fuel to electrical energy is achieved by the catalytic action of microorganisms^[6]. It consists of anodic and cathodic compartments separated by a proton exchange membrane (PEM). Bacteria in the anodic chamber oxidize organic matter and transfer electrons to the cathode through an external circuit producing current. For each electron released, an equivalent proton must then diffuse across the proton exchange membrane to the cathode and therefore combine with the

electron and oxygen in the cathode to form water^[7]. Electric current generation is attributed by separating the microbes from oxygen or any other end terminal acceptor apart from anode^[8] for the MFC operation. Some areas where MFCs offer immediate prospect of application are powering gadgets^[9], biosensors^[10] and especially in wastewater treatment^[11]. It is known that wastewater from textile industry is one of the major sources of aromatic amines in the environment.

Various physical, chemical, biological treatment techniques were employed to remove colour from dye containing wastewaters. The physio-chemical methods which include Fenton's process, oxidation and ion exchange process are not used in developing countries due to their expenses, limited versatility and generation of waste products. Biodegradation has been limited to those compounds that are biodegradable^[12]. Not all dye compounds are susceptible to rapid and complete degradation. There are some concerns that the products of biodegradation may be more persistent or toxic than the parent compound. Biodegradation often takes longer time than other treatment methods. Evaluating performance of biodegradation finds it difficult because of no acceptable endpoints for biodegradation treatments.

Due to such drawbacks of the various conventional methods the microbial fuel cells are now used for dye decolourization. Species like *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Proteus hauseri*, *Geobacter sulfurreducens*, *Beta Proteobacteria*, *Trametes versicolor*, *Shewanella oneidensis* and others have been used for dye decolourization and electricity generation in microbial fuel cell ([13];[14];[15];[16]). For the improved dye degradation efficiency with power production, secondary metabolites of *Pseudomonas sp.* can work as redox mediator in MFCs^[17]. Considering all these points, this study was designed to investigate the efficiency of *Pseudomonas aeruginosa* for dye decolorization with electricity generation in an MFC system. The objectives of the study were to study the efficiency of decolourization of Congo red dye by *Pseudomonas aeruginosa* in MFC. MFCs decolourization conditions and electricity production by *Pseudomonas aeruginosa* were studied in detail. Substrate was regarded as one of the mainly essential factors that affects the power generation in MFCs. MFCs are able to utilize different sources of substrates for production of bioelectricity ([18]; [19]). The present study also focused on evaluation of MFC performance for different concentrations of substrates at anode chamber.

2. Materials And Methods

2.1. FEED SOLUTION

In anode compartment, Minimal salt medium (MSM) was used as the feed Solution with the following composition per litre: KH_2PO_4 – 13.60 g; Glucose – 10.00 g; Na_2SO_4 – 9.47 g; NaOH – 2.33 g; NH_4Cl – 0.45 g; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ – 0.17 g; $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ – 1.00 mg; $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ – 23.0 mg; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ – 15.0 mg. Glucose was added as the energy source to the anode chamber. The salts were of analytical grade and purchased from CDH Chemicals. About 50mM Phosphate buffer (pH 7.0) was used in the cathode compartment as catholyte.

2.2. DYE STOCK SOLUTION

The sulfonated azo dye – Congo Red dye ($C_{32}H_{22}N_6O_6S_2Na_2$) of molecular weight 696.66 of commercial quality from Sisco Research Laboratories Pvt. Ltd., Maharashtra was used and its non-hydrolysed molecular structure is given in Figure.1. 10M dye stock solution was prepared for the reactive dye and was used for further studies.

2.3 MICROORGANISM AND ITS CULTURE CONDITION

Pseudomonas aeruginosa MTCC 1274 was obtained from Microbial Type Culture Collection, Institute of Microbial Technology, Chandigarh, India and maintained on Nutrient agar medium. The slants of *Pseudomonas aeruginosa* in Nutrient agar medium was stored at 4°C in the refrigerator and sub cultured every month. All the culture media, unless otherwise stated, were sterilized at 15 lbs/inch² pressure (121°C) for 15 minutes. *Pseudomonas aeruginosa* MTCC 1274 was taken from slants and suspended in 0.85% sterile sodium chloride was inoculated in the synthetic feed solution of anode compartment, pH 7.0. Flasks were incubated (32°C, 150rpm) in a rotary shaker for overnight. Streak plating was done in the minimal salt medium with 1% agar. Samples were withdrawn aseptically at regular time intervals from the freshly inoculated culture and were analysed for cell density at OD 600nm. Growth rate of *Pseudomonas aeruginosa* on feed solution was prepared as given in 3.2.1. and was investigated. Thus, the maximum decolourization of the sulfonated azo dye mentioned in 3.2.2. during the growth of *Pseudomonas aeruginosa* in feed solution was studied.

2.4. MFC CONSTRUCTION AND ITS OPERATION

The Double Chambered Microbial Fuel cell (DCMFC) consists of two (Anode and Cathode) bottles of 500 mL (18*8 cm) filled with effective volume up to 400mL with graphite electrodes (3.1*1.3 cm). The bottles were joined by a glass bridge containing a proton exchange membrane (Nafion TM 117, Dupont Co.) held by a clamp between the flattened ends of the two glass tubes (inner diameter 1.8 cm) fitted with rubber gaskets. Both Anode and Cathode are made of graphite without any coating. Electrodes was made to be positioned at a distance of 8 cm on either side of PEM (Fig. 2).

The electrodes were made to get attached using copper wire to give external circuit connection. Prior to use electrodes were soaked in 100% ethanol for 1 hour followed by 1M HCL for 1 hour and then in deionized water for a period of 24 h. The anode compartment was filled (400 mL) with MSM (Minimal Salt Medium) containing reactive azo dye as mentioned in 3.2.2. and the cathode compartment was filled with phosphate buffer pH 7.0 as catholyte. The system was operated at room temperature (32°C). Each chamber was provided with sample port, wire point inputs (top), inlet and outlet ports and anode chamber were sealed to ensure anaerobic microenvironment.

The anode was purged with Nitrogen gas for 20mins at an interval of 24 h to maintain the anaerobic condition in the anode chamber while the cathode was sparged with air (110mL/min) using an aquarium pump. The anodic chamber prior to start-up was inoculated with selectively enriched and adopted

Pseudomonas aeruginosa through designed synthetic feed suspension of 1mL (approximately 3×10^6 cells). Constant COD removal and stable voltage generation were considered as indicators for stable operating conditions.

2.5. ANALYTICAL PROCEDURE

2.5.1 PHYSICOCHEMICAL ANALYSIS

UV-Visible absorbance scans of clarified (centrifuged) sample of Congo Red dye was performed in a spectrophotometer. The Congo Red dye concentrations were measured at λ_{max} 499 nm. Dye concentration was calculated based on the absorption measured at the dye peak absorption wavelength in the visible range. Decolorization percent was evaluated using following equation

$$\% \text{ Decolorization} = [(\text{Initial absorbance} - \text{Observed absorbance}) / \text{Initial Absorbance}] \times 100.$$

The samples were centrifuged at 3500 rpm for 20 min by using a centrifuge (REMI R-8C). This was done for all samples for separation of biomass prior to measurement of absorbance. The chemical oxygen demand (COD) was determined in accordance with the Standard Methods for the Examination of Wastewater treatment. The initial COD of glucose medium was investigated by theoretical chemical oxygen demand on the basis of its stoichiometric reaction with oxygen. The final COD was measured and COD removal was calculated as; $[(\text{COD}_{\text{in}} - \text{COD}_{\text{out}}) / \text{COD}_{\text{in}}] \times 100\%$. Where COD_{in} is the influent COD and COD_{out} is the effluent COD.

FTIR analysis of CR before and after treatment was monitored on a Thermo Scientific IR 200 FT-IR spectrophotometer. The FTIR spectra were then recorded between 4000 and 500 cm^{-1} , at a rate of 16 nm/s .

2.6 MICROSCOPIC ANALYSIS

SEM analysis of the biofilm on anode was investigated at high resolution to confirm the bacterial infestation [20]. The anode material was removed at the end of the experiment, was rinsed with the sterile medium (distilled water), and then were immersed in 5% formaldehyde overnight to fix the samples. Chips of $1 \text{ cm} \times 1 \text{ cm}$ were cut for SEM analysis. Before observation the anodic materials were collected and immersed in the 5% formaldehyde overnight for fixing samples, followed by washing with detergent. Samples were then dried and prepared by gold sputtering before imaging.

3. Results And Discussion

3.1 Decolourization performance under Static, Shaking and MFC condition and removal of COD

Pseudomonas aeruginosa exhibited higher dye-decolourizing activity when operated under DCMFC condition (80.56%), whereas agitated culture showed less decolourization efficiency (49.25%). At static

condition only 16% decolourization of Congo red (CR) was observed after 120 h (Fig. 3). At static condition bacterial growth rate 0.074 h^{-1} , this evident that the sulfonated diazo dye Congo red is recalcitrant to bio decolourization under static condition ^[21]. At shaking condition bacterial growth rate improved to 0.304 h^{-1} which resulted in 50% of dye decolourization. The growth rate of bacteria was observed to be 0.688 h^{-1} for MFC condition resulted in higher decolourization efficiency of CR dye. The time required for decolourization was decreased with MFC condition. This result proved that the decolourization was not to the adsorption of cells but due to the metabolic activity of bacterial cells ^[22]. The average rate of decolourization was increased up to 12 h in MFC condition. On continuous acclimatization in DCMFC 100% decolourization was observed within 48 h. Removal of COD was 85% under MFC condition. Hou et al., 2011 ^[23] reported 68.9 % removal of COD at MFC condition whereas Isik and Sponza (2003) ^[24] reported only 57% removal of COD under anaerobic condition while 87% of CR decolourization at aerobic condition.

3.2 Effect of glucose concentration on dye decolourization and power density

CR decolourization efficiency and power production by *Pseudomonas aeruginosa* were investigated under different glucose concentration (0 g/L, 5 g/L, 10 g/L, 15 g/L and 20 g/L). Initially all MFCs were operated under open circuit condition to evaluate its performance when no load was applied. The OCV was recorded at a time interval of 3 h and the recorded data was averaged for every 24h. Similarly, samples were withdrawn aseptically at regular time intervals from the inoculated MFC and analysed for cell density at OD 600 nm. As shown in Fig. 4, the growth curves of *P. aeruginosa* cultured under anaerobic condition in MFC displayed a typical three-stage growth cycle: an adaptive phase (0–4h), an exponential growth phase (5–14h), and a stationary phase (after 14h) approximately for all five sets.

The growth of bacteria was maximum at 10 g/L (0.858 OD at 11th hr). When the glucose concentration was 10g/L the maximum growth rate was expressed. Hence, this concentration was used for further study. Moreover, Fig. 5 shows the performance of MFC under different glucose concentration at 100 μM of CR concentration. Initially, an open circuit voltage of 0.184V, and a maximum power density of 1.3 mW m^{-2} was observed after culturing time of 264 h. Later for 5 g/L of glucose concentration an OCV of 0.483 V, and a maximum power density of 1.7 mW m^{-2} was observed for the culturing time of 480 h. Then for 10 g/L of glucose concentration an OCV of 0.691 V, and a maximum power density of 1.9 mW m^{-2} was observed for the culturing time of 576 h. For 15 g/L of glucose concentration an OCV of 0.426V, and a maximum power density of 0.7 mW m^{-2} was observed for the culturing time of 336 h. Finally, for 20 g/L of glucose concentration an OCV of 0.458 V, and a maximum power density of 0.4 mW m^{-2} was observed for the culturing time of 216 h.

Concerning the working power densities at 220 ohm external resistance with the duration time of 72 h shows the variation in power densities at different culture time. Therefore, from the growth curve study a culture time of 3 h, corresponding to the middle of log phase would be suggested in this study. From

Fig. 4 shows that the culture time of 4 h, corresponding to the transition of log phase to stationary phase in the growth curve. Therefore, higher the growth influences the bacteria metabolism and growth in *Pseudomonas aeruginosa* in MFC [25]. Better power performance was observed in stationary phase of the growth curve and can further be suggested.

Comparing the power performance among different concentration of glucose, 10 g/L of glucose concentration would produce an optimal performance for the studied cases. Further, the MFC operated with 10 g/L of glucose concentration had prolonged stationary phase which improves the electrical activity in the MFC. Previous study by Jafary et al., 2013 [26] investigated that the substrate concentration enhancement resulted in improved MFC performance. But, in this study, with 10 g/L of glucose the DCMFC will produce 1.9 mW m⁻² under the optimal condition of glucose.

3.3 Effect of glucose concentration on different dye decolourization

The decolourization activity of *Pseudomonas aeruginosa* in MFC system was studied using CR at various initial concentrations with different glucose concentrations. DCMFC system inoculated with *Pseudomonas aeruginosa* can decolourize up to 200 µM of dye concentration, however higher concentration seemed to be toxic for the cell growth in MFC. The dye decolourization was strongly inhibited at 500 µM in the MSM medium. From Table 1, it was evident that a maximum OCV of 0.844 V was observed for the initial concentration 200 µM of dye and 10 g/L of glucose concentration. Further the dye decolourization was also reported to be higher (96.1%).

Table 1
Effect of dye concentration under different glucose concentration

Concentration of glucose (g/L)	Maximum OCV (mV)			Maximum decolourization (%)		
	100 µM	200 µM	300 µM	100 µM	200 µM	300 µM
0	206	249	190	57.64	51.93	43.19
5	483	712	316	80.85	91.39	69.11
10	691	844	392	89.74	96.1	79.94
15	426	527	277	86.07	89.65	70.21
20	458	551	292	80.46	77.85	67.56

Table 2
Characteristics of dye wastewater
before and after MFC treatment

TESTS	SAMPLE	
	INITIAL	FINAL
COD (mg/L)	1036	148
TSS (mg/)	923	452
TDS (mg/L)	4211	2040
EC (mS/cm)	8.422	4.08

As for the performance of MFC under 100 μM of dye using 10 g/L of glucose concentration a maximum OCV of 0.691 V and a maximum decolourization of 89.74% was reported. Moreover, for 300 μM of dye concentration, a maximum OCV of 0.392 and a maximum decolorization of 79.94% was observed. These results would indicate that the glucose concentration of 10 g/L and 200 μM seems to be a better choice for better decolourization in MFC. Stable OCV production was achieved during CR decolourization with the presence of 10 g/L of glucose. Further, the dye intermediates of CR might have enhanced the MFC performance after degradation ^[27]. The test for TSS, TDS and EC were conducted for samples obtained from MFC at optimum condition (200 μM dye concentration and 10g/L glucose concentration) and the results are given in table. 2

3.4 Degradation analysis

CR colour removal in MFC was confirmed by UV-Visible analysis (200–800 nm) (Fig. 6). CR UV-Visible scanning depicted two peaks. One extended at 340 nm was due to the interaction between the aromatic hydrocarbon /polycyclic aromatic hydrocarbon groups and other chromophore group, and a peak at 495 nm, relative to the azo bond group and a large conjugated system of whole dye molecule. After 72 h treatment, a decrease in the major peak intensity at 495 nm was noted. This indicated that the dye structure especially the azo bond was broken and was degraded during MFC treatment. Surprisingly, the absorbance at 340 nm was also decreased which evident that interaction of the aromatic hydrocarbons and some chromophore group were partially destroyed. These results suggested that MFC treatment is able to degrade the CR dye with a complete breakdown of chromophore group. Unlikely Wang et al, 2017 ^[28] reported that complete breakdown of aromatic hydrocarbon seemed to be more difficult that the breakdown of azo double bond.

In order to elucidate the nature of the functional groups present in the untreated and treated sample, FTIR analysis was performed. The spectra of decolourized sample reveals shifting of some characteristic bands which depict changes in functional groups after decolourization compared to untreated dye sample (control) (Fig. 7). The FTIR spectrum of untreated sample and treated sample reveals several peaks and stretching as presented in Tables 3 and 4 respectively. FTIR analysis revealed the presence of aromatic compounds in untreated sample and alkenes in treated sample.

Table 3
FTIR Peaks (cm^{-1}) of CR before MFC treatment

Sl. No.	Absorption (cm^{-1})	Appearance	Group	Compound class
1.	3452.30	weak, sharp	-	-
2.	3057.99	weak, broad	O-H stretching	Alcohol
3.	2292.49	weak, broad	$\text{C}\equiv\text{N}$ stretching	Nitrile
4.	2070.22	weak, broad	-	-
5.	1988.63	weak, sharp	C-H bending	aromatic compound
6.	1899.28	weak, broad	C-H bending	aromatic compound
7.	1581.53	medium, sharp	-	-
8.	1550.09	weak, sharp	-	-
9.	1500.85	weak, sharp	-	-
10.	1445.59	weak, sharp	-	-
11.	1399.10	weak, sharp	-	-
12.	1351.03	medium, sharp	O-H bending	Alcohol
13.	1322.18	weak, sharp	-	-
14.	1221.71	strong, sharp	C-O stretching	vinyl ether
15.	1175.35	strong, sharp	C-O stretching	Ester
16.	1121.71	medium, sharp	-	-
17.	1059.22	strong, sharp	C-O stretching	primary alcohol
18.	919.72	medium, sharp	-	-
19.	860.71	weak, sharp	-	-
20.	832.11	medium, sharp	C = C bending	Alkene
21.	783.62	weak, sharp	-	-
22.	750.72	medium, sharp	-	-
23.	724.55	weak, sharp	-	-
24.	695.18	medium, sharp	-	-
25.	638.02	strong, sharp	-	-
26.	591.72	medium, sharp	-	-

Sl. No.	Absorption (cm ⁻¹)	Appearance	Group	Compound class
27.	552.55	weak, sharp	-	-
28.	524.06	weak, sharp	C-Br stretching	halo compound

Table 4
FTIR Peaks (cm⁻¹) of CR after MFC treatment

Sl. No.	Absorption (cm ⁻¹)	Appearance	Group	Compound class
1.	3277.83	strong, broad	O-H stretching	carboxylic acid
2.	2112.40	weak, broad	C≡C stretching	Alkyne
3.	2012.27	weak, broad	-	-
4.	1636.52	medium, sharp	C = C stretching	conjugated alkene
5.	1080.26	weak, sharp	-	-
6.	988.06	weak, broad	-	-
7.	922.49	weak, broad	-	-
8.	862.41	weak, broad	-	-
9.	662.11	weak, broad	-	-
10.	606.27	weak, broad	-	-
11.	567.38	weak, sharp	-	-

3.5 Bacterial adhesion on anode

The surface of the anode electrode was visualized by scanning electron microscope SEM (FEI-Quanta FEG 200F) to determine microbial attachment and formation of a biofilm on the anode electrode surface. It was removed after 29 days incubation period. SEM Images of bacterial attachment on Anode from MFC Setup used for decolourization of dye concentration (200µM) along with glucose concentration (10g/L) is represented in Fig. 8. The average size of the microbe was obtained as 1µm.

The formation of a biofilm begun when bacteria sense certain environmental cues and then trigger the transition to life; these environmental signals vary among microbial communities. *P. aeruginosa* biofilms can be found on any substrate surface suitable for its growth [29]. Some studies [30] have shown that bacteria develop biofilms on surfaces when nutrients are provided at sufficient levels in the solution; the cells will then enter back into the solution when the nutrients are scarce. This starvation response is a key factor regulating biofilm formation [31]. Changes in environmental factors cause changes in microbial state. Xu found that *Desulfovibrio vulgaris* adhered to carbon steel surfaces under starvation conditions to acquire extra energy from steel [32]. Additionally, microorganisms are capable of complex behaviours

that best benefit their reproduction. Mutual interactions in biofilms result in biofilm formation, which enables sessile organisms to stay on surfaces and exhibit improved growth.

Ilamathi et al., 2019 ^[17] reported that the presence of *P. aeruginosa* biofilm on electrode surface strongly improved the redox behaviour when compared to uncolonized electrodes. From the SEM analysis (Fig. 8) and OCV generation (Fig. 5) it is understood that the presence of *P. aeruginosa* biofilm on electrode surface improved the redox behaviour.

4. Conclusion

Several concentrations of carbon substrates at the range of 0–20 g/L were studied at different dye concentration at the range of 100–300 μ M. The growth curve of bacteria revealed that the growth was maximum at 10 g/L glucose as carbon source. At the optimum growth condition (10 g/L glucose concentration) of *P. aeruginosa* and 100 μ M dye concentration, the decolourization was tested at static, shaking and MFC condition it was found that the maximum decolourization at less time was at MFC condition. The FTIR analysis revealed the presence of aromatic compounds in untreated sample and alkenes in treated sample. From the SEM analysis obtained at optimum condition (10 g/L glucose and 200 μ M dye concentration) it was understood that the presence of *P. aeruginosa* biofilm on electrode surface improved the redox behaviour.

Declarations

The authors declare that there is no conflict of interest.

References

1. Sen S, Demirer (2003) GN Water Res 37:1868–1878
2. Lovley DR (2006) Microbial fuel cells: novel microbial physiologies and engineering approaches. Curr Opin Biotechnol 17:327–332. doi:10.1016/j.copbio.2006.04.006
3. Shukla AK, Suresh P, Berchmans S, Rajendran A (2004) Biological fuel cells and their applications. Curr Sci 87(4):455–468
4. Ilamathi R, Jayapriya J (2018) Microbial fuel cells for dye decolorization. Environ Chem Lett 16(1):239–250
5. Holzman DC (2005) Microbe power. Environ Health Perspect 113(11):754–757
6. Oh SE, Logan BE (2005) Hydrogen and electricity production from a food processing wastewater using fermentation and microbial fuel cell technologies. Water Res 39(19):4673–4682
7. Logan BE, Murano C, Scott K, Gray ND, Head IM (2005) Electricity generation from cysteine in a microbial fuel cell. Water Res 39:942–952. doi:10.1016/j.watres.2004.11.019
8. Du Z, Li H, Gu T (2007) A state of the art review on microbial fuel cells: a promising technology for wastewater treatment and bioenergy. Biotechnol Adv 25:464–482. doi:10.1016/j.

9. Aelterman P, Rabaey K, Verstraete W (2006) Continuous electricity generation at high voltages and currents using stacked microbial fuel cells. *Environ Sci Technol* 40(10):3388–3394. doi:10.1021/es0525511
10. He, Z, Angenent, LT Application of bacterial biocathodes in microbial fuel cells *Electroanal*, 18 (2006), pp. 2009–2015
11. Rabaey K, Verstraete W (2005) Microbial fuel cells: novel biotechnology for energy generation. *Trends Biotechnol* 23:291–298. doi:10.1016/j.tibtech.2005.04.008
12. Min B, Cheng S, Logan BE (2005a) Electricity generation using membrane and salt bridge microbial fuel cells. *Water Res* 39:1675–1686
13. Kim HJ, Park HS, Hyun MS, Chang IS, Kim M, Kim BH (2002) A mediator-less microbial fuel cell using a metal reducing bacterium, *Shewanella putrefaciens*. *Enzyme Microb Technol* 30(2):145–152
14. Bond DR, Lovely DR (2003) Electricity production by *Geobacter sulfurreducens* attached to electrodes. *Appl Environ Sci Microbial* 69(3):1548–1555
15. Chaudhuri SK, Lovely DR (2003) Electricity generation by direct oxidation of glucose in mediatorless microbial fuel cells. *Nat Biotechnol* 21:1229–1232
16. Rabaey K, Boon N, Siciliano SD, Verhaege M, Verstraete W (2004) Biofuel cells select for microbial consortia that self-mediate electron transfer. *Applied Science Technology* 39:3401–3408
17. Ilamathi R, Sheela AM, Gandhi NN (2019) Comparative evaluation of *Pseudomonas* species in single chamber microbial fuel cell with manganese coated cathode for reactive azo dye removal. *International Biodeterioration Biodegradation* 144:104744
18. Rezaei, F., Richard, TL., Brennan, RA., Logan, BE. Substrate-enhanced microbial fuel cells for improved remote power generation from sediment-based systems, *Environ Sci Technol*, 41 (2007), pp. 4053–4058
19. Pant, D., Bogaert, GV., Diels, L., Vanbroekhoven, K. A review of the substrates used in microbial fuel cells (MFCs) for sustainable energy production, *Bioresour Technol*, 101 (2010), pp. 1533–1543
20. Kramer J, Soukiazian S, Mahoney S, Hicks-Garner J (2012) Microbial fuel cell biofilm characterization with thermogravimetric analysis on bare and polyethyleneimine surface modified carbon foam anodes. *J Power Sources* 210:122–128
21. Telke AA, Joshi SM, Jadhav SU, Tamboli DP, Govindwar SP (2010) Decolorization and detoxification of Congo red and textile industry effluent by an isolated bacterium *Pseudomonas* sp. SU-EBT *Biodegradation* 21(2):283–296
22. Jang MS, Jung BG, Sung NC, Lee YC (2007) Decolorization of textile plant effluent by *Citrobacter* sp. strain KCTC 18061P. *J Gen Appl Microbiol* 53(6):339–343
23. Hou B, Sun J, Hu Y (2011) Simultaneous Congo red decolorization and electricity generation in air-cathode single-chamber microbial fuel cell with different microfiltration, ultrafiltration and proton

- exchange membranes. *Bioresour Technol* 102:4433–4438.
<https://doi.org/10.1016/j.biortech.2010.12.092>
24. Işık M, Sponza DT (2003) Effect of oxygen on decolorization of azo dyes by *Escherichia coli* and *Pseudomonas* sp. and fate of aromatic amines. *Process Biochem* 38(8):1183–1192
 25. Ilamathi R, Sheela AM (2020) Performance analysis of microbial fuel cell operational parameters on reactive azo dye decolorization. *Desalination Water Treatment* 190:312–321
 26. Jafary T, Rahimnejad M, Ghoreyshi AA, Najafpour G, Hghparast F, Daud WRW (2013) Assessment of bioelectricity production in microbial fuel cells through series and parallel connections. *Energy Conv Manag* 75:256–262
 27. Xu B, Chen BY, Hsueh CC, Qin LJ, Chang CT (2014) Deciphering characteristics of bicyclic aromatics–mediators for reductive decolorization and bioelectricity generation. *Bioresour Technol* 163:280–286
 28. Wang N, Chu Y, Zhao Z, Xu X (2017) Decolorization and degradation of Congo red by a newly isolated white rot fungus, *Ceriporia lacerata*, from decayed mulberry branches. *International Biodeterioration Biodegradation* 117:236–244
 29. O'Toole G, Kaplan H/B, Kolter R (2000) Biofilm formation as microbial development. *Annu. Rev. Microbial.* 54 (1), 49 Communities
 30. Life F *Bacteria Environment Surface* 54, 49–79
 31. Kolter R, Siegele DA, Tormo A (1993) The stationary phase of the bacterial life cycle. *Annu Rev Micobial* 47:855–874
 32. Sherar BWA, Power IM, Keech PG, Mitlin S, Southam G, Shoesmith DW (2011) Characterizing the effect of carbon steel exposure in sulfide containing solutions to microbially induced corrosion. *Corrosion Sci* 53:955–960
 33. Xu DT (2014) Carbon source starvation triggered more aggressive corrosion against carbon steel by the *Desulfovibrio vulgaris* biofilm. *Int Biodeterior Biodegrad* 91:74–81

Figures

Figure 1

Percentage of CR decolourization at static, shaking and MFC condition

Figure 2

Growth curve of bacteria at different glucose concentration in MSM medium.

Figure 3

OCV (mV) observed at different glucose concentration and 100 μ M dye concentration in MSM medium in Double Chambered Microbial Fuel Cell.

Figure 4

Absorbance at different wavelength for samples taken from MFC setup containing 10g/L glucose concentration and 200 μ M dye concentration at different time intervals.

E:\USERS DATA\EXTERNAL\EXT 2020\FEBRUARY\PRIYANKA\CR.0	CR	Instrument type and / or accessory	17-02-2020
--	----	------------------------------------	------------

E:\USERS DATA\EXTERNAL\EXT 2020\FEBRUARY\PRIYANKA\A.0	A	Instrument type and / or accessory	17-02-2020
---	---	------------------------------------	------------

Figure 5

FTIR graph of Congo Red Dye a) Control b) Treated sample

Figure 6

SEM Images of bacteria attached on anode of MFC Setup with dye concentration (200 μ M) and glucose concentration (10g/L). a) Plain Graphite electrode b) Graphite electrode with *Paeruginosa* adhesion.