

## Performance assessment of innovative constructed wetland-microbial fuel cell for electricity production and dye removal

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### ABSTRACT

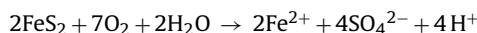
This research work deals with performance assessment of constructed wetlands-microbial fuel cell (CW-MFC) for electricity production and wastewater treatment. Microbial fuel cell consists of two chambers i.e. anaerobic and aerobic, where oxidation and reduction reactions take place. Constructed wetland also consists of aerobic and anaerobic zones where oxidation and reduction processes take place. These similarities in both technologies motivated us to design and develop a new type constructed wetland-microbial fuel cell. In this CW-MFC, the removal of dye and COD were investigated along with electricity generation. Experiments were performed in batch mode using different dye (methylene blue dye) concentration containing synthetic wastewater. Our results show that 76.2, 80.87, 69.29 and 93.15 percentage dye removal could be achieved after 96 h of treatment of wastewater containing 2000, 1500, 1000 and 500 mg l<sup>-1</sup> initial concentration respectively. Also, the CW-MFC is able to remove 75% of COD from wastewater with 1500 mg l<sup>-1</sup> initial concentration of dye. The maximum power density of 15.73 mW m<sup>-2</sup> and maximum current density of 69.75 mA m<sup>-2</sup> could be achieved during treatment of 1000 mg l<sup>-1</sup> initial dye concentration containing wastewater.

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### 1. Introduction

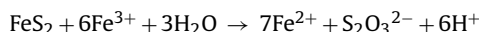
A microbial fuel cell (MFC) consists of an anode and cathode electrode similar to any battery. The voltage difference between the anode and cathode, together with the electron flow in the outer circuit, generate electrical power. Conventional two-chamber MFC consists of aerobic (cathode) and anaerobic (anode) compartments separated by a proton exchange membrane or salt bridge. In an MFC, electrochemically active bacteria oxidize the biodegradable organic matter present in the anodic chamber which generates electrons (e<sup>-</sup>) and protons (H<sup>+</sup>). Electron transfer occurs through the electrode (anode) integrated with an external circuit to the cathode. Protons diffuse through the proton exchange membrane (PEM) into the cathode chamber, where they combine with electrons and O<sub>2</sub> to form water. The organic substrates utilized at the anode may vary from the ordinary carbohydrates such as glucose and acetate, to more complex compounds such as starch, complex wastewater, sediments and various other organic and inorganic

constituents. The bacteria play a major role in the MFC. The electron transfer and the electrochemical reactions put together comprise the MFC. A schematic of the concept at the anode is illustrated in Fig. 1 with respect to oxidation of iron in the presence of *Acidithiobacillus ferrooxidans*. The main reaction in a typical leaching process involving pyrite is given as follow:

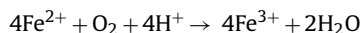


The above reaction shows how iron goes into solution in the presence of a sulphide reducing bacteria. The intermediate reactions are given below.

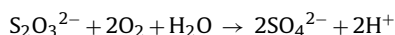
Step 1 (spontaneous):



Step 2 (iron oxidation in presence of bacteria):



Step 3 (sulphur oxidation in presence of bacteria):



The oxidation process releases electron that are transferred to the outer surface of the cell. This process of electron transfer is probably of the greatest interest at present because of its

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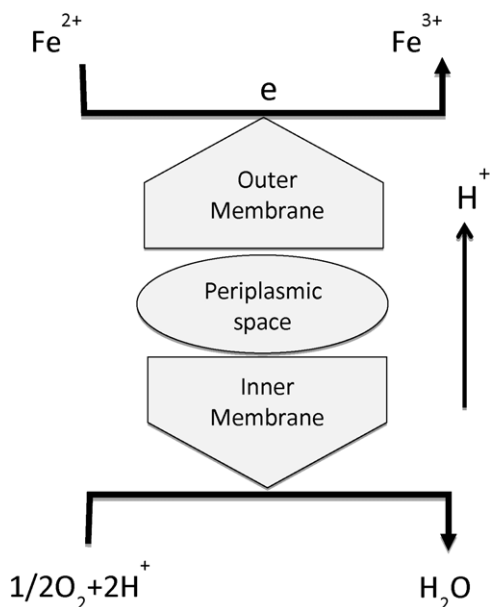


Fig. 1. Schematic of electron transfer in microorganism.

practical significance. A variety of organic matter can be used to substitute the anodic reaction. The metabolic variation along with the redox potential data, provides an insight into the microbial-electrodynamics which we have been pursuing in a different light (Misra et al., in press).

Recent studies indicate that MFCs may provide opportunities for a sustainable production of energy from biodegradable compounds (Rabaey and Verstraete, 2005). MFCs have been widely tested for different pollutants treatment such as oxidation of carbohydrates (Chaudhuri and Lovley, 2003), COD removal from the landfill leachate (Frew and Christy, 2006) and oxidation of ferrous iron ( $\text{Fe}^{2+}$ ) in acid mine drainage (Cheng et al., 2007). In recent years, single chamber microbial fuel cells have also been developed, which have anode at the bottom and cathode at the top of the reactor. Single chamber microbial fuel cell has previously been tested for azo dye removal (Sun et al., 2009). Despite its utility and potential benefits to treat wastewater, the overall MFC technology is still in its infancy. Any attempt towards the development of this technology for field level application is surely worthwhile.

Textile industry produces wastewater that contains several types of dyes. These dyes are designed to resist degradation with time, exposure to sunlight, water, soap and oxidizing agent, and cannot be easily removed by conventional wastewater treatment processes due to their complex structure and synthetic origin (Wang et al., 2008). Thus, dye compounds existed in wastewater is not easily degradable. The dye induced colour in dye polluted water blocks sunlight that is so essential for many photo-initiated chemical reactions necessary for aquatic life of water bodies. Azo dyes are the most prominent group of synthetic colourants for various purposes. Azo dyes molecule has one or more azo ( $\text{N}=\text{N}$ ) bridges linking substituted aromatic structures. This double bonded azo linkage is responsible for colour production either by absorption or scattering of visible light. It is reported that annually a significant amount of azo dyes are lost to domestic and industrial waste waters during textile processing (Seshadri et al., 1994). Methylene blue is a redox-dye. It can cause some harmful effects, such as heartbeat increase, vomiting, shock, cyanosis, jaundice, quadriplegia, and tissue necrosis in humans (Yi and Zhang, 2008). Therefore,

it is necessary to develop effective and cost efficient processes for dye removal. More recently, dye removal in different type of MFC has been attempted by various researchers (Sun et al., 2009; Chen et al., 2010; Hou et al., 2012) and in constructed wetlands (CW) (Davies et al., 2009; Freire et al., 2009) as a treatment technology for their removal from wastewater. Azo dyes removal in CW has been investigated by Noonpui and Thiravetyyan (2012). They demonstrated that maximum 97% of dye removal in CW; however, the removal efficiency found to be a function of structure and size of the dye molecule. Moreover, they emphasized that the plant, soil, and microorganisms might all influence the efficiency of dye removal in a CW. Application of CW for treatment of dye-rich textile wastewater has also demonstrated 84% COD removal, in addition to the dye removal (Bulc and Ojstrek, 2008).

Our main motivation to treat wastewater by combining CW and MFC is as follows. We realize that there are several processes in nature where redox conditions are prevailing like flooded systems, natural wetlands, etc. These conditions can be utilized for the development of in situ, nondestructive microbial fuel cell, which may directly apply to a large scale field level activity. CWs are the replica of natural wetlands, which are already in field level use for wastewater treatment and CWs have different aerobic and anaerobic zones throughout their bed depth and water column. This similarity forms the basis of our investigation to develop a constructed wetland-microbial fuel cell. The rationale behind this work is to use the stratified redox conditions of constructed wetlands as cathode and anode compartments for developing a constructed wetlands-microbial fuel cell. Furthermore, to the best of our knowledge, there are no research results available which reports on integration of wetland and microbial fuel cell for dye containing wastewater treatment. This work is the first of its kind towards integration of MFC and CW. The process may apply to the field level wetlands for dye containing or other type of wastewater treatment and simultaneous production of electricity. The outcome of the new integrated process may develop a cost-effective clean and green technology for simultaneous producing electricity and wastewater treatment for field level application.

## 2. Materials and methods

### 2.1. Dye containing synthetic wastewater preparation

A synthetic wastewater containing sucrose as a carbon source was used throughout the study. The composition of the synthetic wastewater are as follows:  $8 \text{ g l}^{-1}$  sucrose,  $0.5 \text{ g l}^{-1}$   $\text{NH}_4\text{Cl}$ ,  $0.2 \text{ g l}^{-1}$   $\text{KH}_2\text{PO}_4$ ,  $0.2 \text{ g l}^{-1}$   $\text{K}_2\text{HPO}_4$ ,  $0.25 \text{ g l}^{-1}$   $\text{MgCl}_2$ ,  $20 \text{ mg l}^{-1}$   $\text{CoCl}_2$ ,  $10 \text{ mg l}^{-1}$   $\text{ZnCl}_2$ ,  $10 \text{ mg l}^{-1}$   $\text{CuCl}_2$ ,  $4 \text{ mg l}^{-1}$   $\text{CaCl}_2$ ,  $10 \text{ mg l}^{-1}$   $\text{MnCl}_2$ . Methylene blue dye was purchased from M/s S.D. Fine-Chem Ltd., India. In the experiments, the required amount of methylene blue dye was mixed with synthetic wastewater for making the dye containing wastewater of 500, 1000, 1500 and  $2000 \text{ mg l}^{-1}$  concentration. Fig. 2 illustrates the chemical structure of methylene blue.

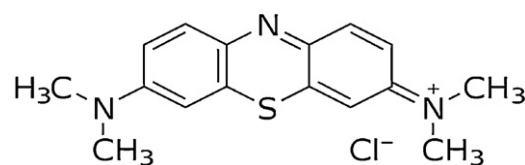
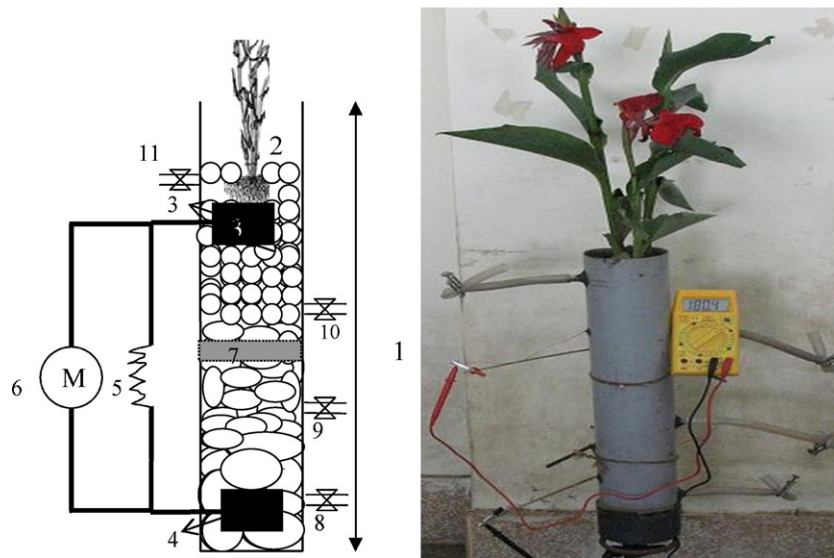


Fig. 2. Chemical structure of methylene blue.



**Fig. 3.** Schematic of novel constructed wetlands cum microbial fuel cell (not scaled) [(1) gravel filled constructed wetlands cum microbial fuel cell microcosm planted with *Canna indica*, (2) rhizospheric zone, (3) cathode, (4) anode, (5) resistance, (6) multimeter, (7) layer of glass wool, (8–11) sampling ports].

## 2.2. Analysis and measurements

The analysis of chemical oxygen demand (COD) was carried out according to the open reflux method of APHA standard (APHA, 1998). This was done both at the start and at the end of the experiments. The pH measurements were made before and after the experiments using a pH meter (M/s Hanna Instruments). The extent of colour removal was determined by the UV visible spectrophotometry using a Perkin Elmer make spectrophotometer ( $\lambda$ -35 double beam). At first, the  $\lambda_{\max}$  of methylene blue was determined by scanning the absorption of different dye concentrations between 200 and 800 nm wavelength. The  $\lambda_{\max}$  value of methylene blue was found to be 660 nm. Then further experiments were carried out at this wavelength. All the samples were analysed immediately after collection. The decolourization efficiency (DE) was calculated by applying the equation given below:

$$DE(\%) = \left( \frac{A_0 - A}{A_0} \right) \times 100$$

where  $A_0$  the initial absorbance and  $A$  is the observed absorbance after treatment. The voltage ( $V$ ) and current ( $I$ ) were measured using a digital multimeter (Mastech, Mas8301) and converted to power ( $P=IV$ ). The power density ( $\text{mW m}^{-2}$ ) and the current density ( $\text{mA m}^{-2}$ ) were calculated by dividing the power and current with the surface area ( $\text{m}^2$ ) of the anode ( $A$ ).

## 2.3. Constructed wetland cum microbial fuel cell microcosm

A vertical flow constructed wetland microcosm was fabricated in the laboratory with a PVC pipe having an internal diameter of 10.5 cm and length of 62 cm. It was filled with gravel (2–4 mm) and planted with *Canna indica* plant species. *C. indica* is robust plant species and used in several constructed wetlands studies (Yadav et al., 2010; Cui et al., 2010). The upper end of microcosm was left open, while the lower end was sealed with epoxy materials. In this microcosm, four sampling ports were provided for collecting samples from different depths of bed according to requirement. Stoppers were used to close the open ends of the sampling ports. For measuring electricity, an arrangement similar to a traditional MFC was made inside the constructed wetland microcosm. A cathode

electrode was placed near to the rhizospheric zone. It was introduced with an idea that this zone will be more aerobic as compared to the deeper zones due to the air diffusion from the immediate outer atmosphere and air leakage from the rhizosphere. The anode was placed near to the bottom of microcosm with an idea that this zone will be comparatively anaerobic and suitable for anodic reaction of MFC. Insulated copper wires were used to connect both the electrodes. The surface area of both the anode and the cathode was  $40.93 \text{ cm}^2$  and the distance between the anode and the cathode was 32.5 cm. Fig. 3 demonstrates a schematic of this constructed wetland-microbial fuel cell and other relevant specifications of the microcosm is given in Table 1.

## 2.4. Experiment start up and sampling

Marine sludge collected from the Bay of Bengal, India was used as inoculum for inoculating the CW-MFC. About 200 g (wet) of marine sludge was mixed in the synthetic wastewater and then poured into the CW-MFC. Earlier, the same sludge was used in another microbial fuel cell work in our laboratory, which showed the good results in terms of COD reduction and current generation (Yadav et al., in press). We believe that this sludge contains mixed culture of active microbial community which has considerable potential of electricity generation and other pollutants removal. In the beginning of the experiments, only synthetic wastewater with inoculum was fed to the microcosm and kept for two weeks in

**Table 1**

Detail Specification of constructed wetland-microbial fuel cell.

Thickness of the PVC pipe	2 mm
Thickness of the electrode	5 mm
External diameter of the column	10.9 cm
Internal diameter of the column	10.5 cm
Void volume of the column	2330 ml
Volume of the column	5000 ml
Distance between the sampling port 1 to 2	20 cm
Distance between the sampling port 2 to 3	23 cm
Distance from top to the column sampling port 1	7 cm
Distance from top to the column sampling port 4	50 cm
Total surface area of the anode	$40.93 \text{ cm}^2$
Total surface area of the cathode	$40.93 \text{ cm}^2$

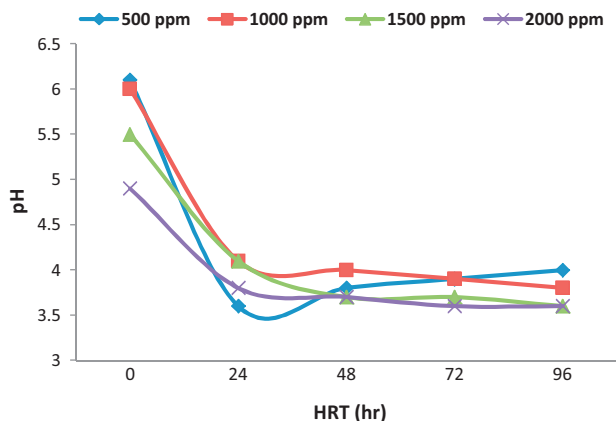


Fig. 4. Variation in pH of the samples at different concentration.

order to be stabilized. For sampling purpose at desirable time, desirable amount of wastewater was withdrawn from the two middle sampling ports and mixed well prior to any analysis.

### 3. Results and discussion

#### 3.1. pH Profile

The pH of the wastewater was measured at the beginning and at the end of every batch experiment. In case of  $500 \text{ mg l}^{-1}$  of initial dye concentration containing wastewater treatment, pH was found to be of 6.1 and 4.0 at the beginning and at the end of experiment respectively. When the dye concentration was changed to  $1000 \text{ mg l}^{-1}$ , the corresponding pH was 6.0 and 3.8. Similarly for  $1500 \text{ mg l}^{-1}$ , the pH was recorded as 5.5 and 3.5. The pH variation at different levels for different concentrations of dye is illustrated in Fig. 4. It is clear that in the lower part of the microcosm, anaerobic condition was prevailing which helped in breaking down the organic materials of wastewater into fatty acids. This shifted the pH towards the acidic side. Production of fatty acids by biodegradation of organic materials in the absence of oxygen tends to reduce the pH, and the process is well-defined according by Ong et al. (2009). In a previous study with similar reactor (Yadav, 2010), dissolved oxygen (DO) concentration was found to be  $3.4$  and  $0.7 \text{ mg l}^{-1}$  in the samples collected from upper most and lower most sampling ports respectively. Mohan et al. (2009) reported that DO concentration of catholyte in the range of  $3.5$  to  $4.5 \text{ mg l}^{-1}$  is sufficient for treating high organic wastewater ( $18.6 \text{ g COD l}^{-1}$ ;  $56.8 \text{ g TDS l}^{-1}$ ) in MFC. It is assumed that anaerobic condition of the lower part of CW-MFC might have provided the suitable environment where microbial oxidation of the substrates releases electron which may have consumed by oxidized form of methylene blue and gets converted into the reduced colourless form. Similar mechanism has been reported by Ong et al. (2005) for the treatment of methylene blue in an up-flow anaerobic sludge blanket reactor. Garg et al. (2004) has reported almost no effect in the range of 2–10 pH on methylene dye adsorption of saw dust generated from rosewood plant, activated carbon of the same sawdust, and commercially activated carbon. Ma et al. (2004) reported that pH has no obvious effect on the COD and methylene blue removal efficiency. We believe that at lower pH, presence of excess  $\text{H}^+$  ions may compete with dye cations for the adsorption sites on gravel which cause lower dye removal as observed in the present case. Similar results were reported by Kahraman et al. (2011) at lower pH.

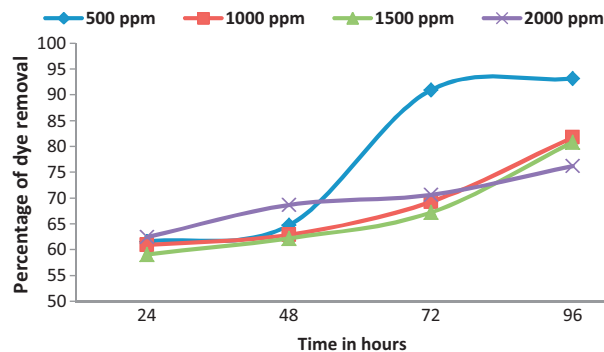


Fig. 5. Dye removal percentage at different concentration.

#### 3.2. COD and dye removal

The percentage of dye removal at different dye concentration containing wastewater was found to be in the range of 59–93 within 24–96 h of the treatment time. During the same period, the COD removal percentage varied between 10 and 75. The extent of dye and COD removal is represented in Figs. 5 and 6. It is observed that in about 96 h, at  $500 \text{ mg l}^{-1}$  dye concentration, 93.15% of dye was removed. Almost 80.0% of dye was removed within 96 h of treatment of 1000, 1500 and  $2000 \text{ mg l}^{-1}$  dye concentration containing waste waters. Methylene blue is a redox dye and used as mediator in microbial fuel cell at low concentration (Sund et al., 2007; Sevda and Sreekrishnan, 2012). In MFC, methylene blue is added as mediator to the growth media at specific concentrations. The oxidation of biodegradable substrates is carried out by microbes which generates the electrons. These electrons are consumed by the oxidized form of methylene blue. The methylene blue gets reduced to its colourless form. The reduced form of dye donates electron to the anode and returns to the oxidized form. It is assumed that in the present case too, above described mechanism is the probable biological removal mechanism of dye. Similar mechanism is also reported by Ong et al. (2005) for methylene blue removal in UASB reactor. Major removal mechanism of colour in the reactor may be attributed due to the adsorption on the surface of gravels, plants, and biomass which is gradually increased to a certain level depending upon the initial dye concentration. It decreases when the gravel is saturated with the dye. In present case, the average extent of dye removal is not very different from other studies reported in the literature which vary from 68 to 78%. We inferred that higher hydraulic retention time (HRT) can lead to better dye removal in constructed wetland system. Alternatively, use of a better adsorption capacity material as media can also lead to higher dye removal efficiency. As far as the COD removal is concerned, it

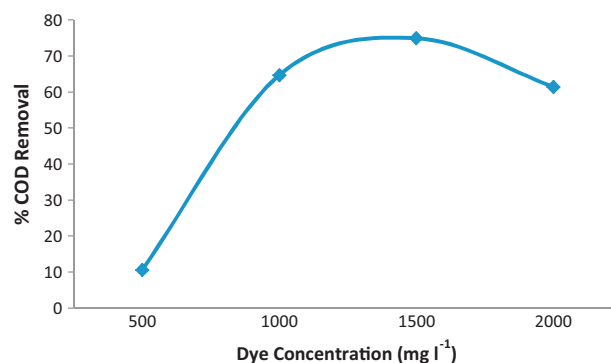


Fig. 6. COD removal percentage at different dye concentrations.



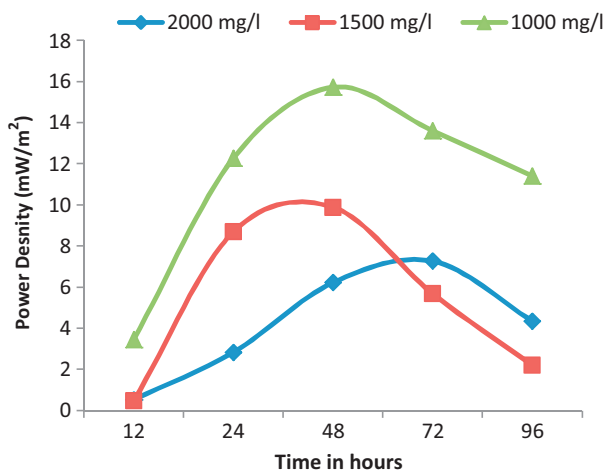


Fig. 7. Power density during treatment different dye concentrations containing wastewater.

was found surprisingly low for the lowest dye containing wastewater and it improved with the increase in the dye concentrations. It seems a higher dye concentration and a higher load of organic carbon source might have caused lower removal due to fast acidification and possible toxicity of microbes. This may be improved by maintaining the higher HRT.

### 3.3. Bioelectricity generation

The maximum power density and current density was found to be  $15.73 \text{ mW m}^{-2}$  and  $69.75 \text{ mA m}^{-2}$  respectively. The power density and the current density are represented in Figs. 7 and 8. It is observed that for any concentration of dye, the initial current and power density increases to a peak value and after that it decreases gradually. The probable reason of such behavior may be linked to the higher rate of breakdown of biodegradable material which eventually slowed down. The power density and the current density values were decreased with the increase in the dye concentration in wastewater. The average power density and current density was found to be  $11.30 \text{ mW m}^{-2}$  and  $58.38 \text{ mA m}^{-2}$  respectively for  $1000 \text{ mg l}^{-1}$  dye concentration which were further reduced to  $4.59 \text{ mW m}^{-2}$  and  $31.24 \text{ mA m}^{-2}$  respectively in case of  $1500 \text{ mg l}^{-1}$  of dye in wastewater. Decrease in power density with an increase in concentration of dye in wastewater is due to increase in the level of toxicity. Similar findings were also reported by other researcher where methylene blue was applied as mediator. Such systems showed toxic

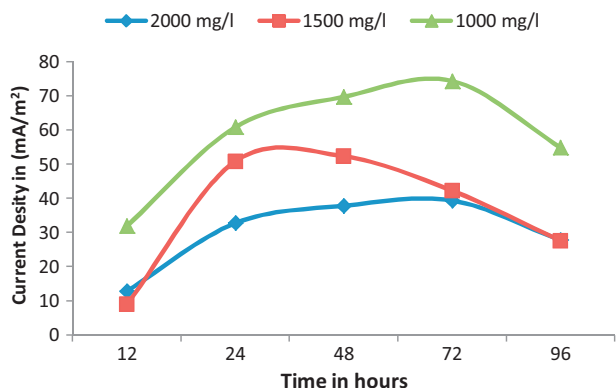


Fig. 8. Current density during treatment different dye concentration containing wastewater.

effects when the concentration was high (Sevda and Sreerishnan, 2012). During the treatment of various dye concentration, it was noticed that plant biomass was also reduced with the increase in dye concentration in wastewater. This also indicates that increased dye concentration adversely affects the plant growth due to the inherent build-up of toxicity. The plant biomass reduction was found to be 8.83, 20.84, 30.57 and 27.98% for 500, 1000, 1500 and  $2000 \text{ mg l}^{-1}$  of dye concentration respectively. The maximum power density was found to be  $15.73 \text{ mW m}^{-2}$  after 48 h of contact time during the treatment of wastewater containing  $1000 \text{ mg l}^{-1}$  methylene blue. As far as the dye removal was concerned, it was found to be fairly stable and did not reduce significantly with the increase of dye in wastewater. This indicates that the dye removal was achieved by other abiotic mechanism such as adsorption on gravel and other surfaces. The methylene blue is widely used as mediator in microbial fuel cell (Sevda and Sreerishnan, 2012; Rahimnejad et al., 2011) as it helps in trapping and drawing the electrons from microbial membrane bound reactions involving electron and supplying those electrons to the anode electrode. It qualifies as a substantial mediator as it does not decompose easily in the long term redox cycling by electrochemically active bacteria. This fact supports the findings of our study too where the extent of dye removal was not significantly reduced with an increase of dye concentration in the wastewater.

## 4. Conclusions

In this work, an innovative CW-MFC was designed, developed, and its feasibility was tested. Results clearly establish the feasibility of simultaneous bioelectricity generation and treatment of wastewater in CW-MFC. A maximum of 93.15% dye removal was achieved at 96 h of treatment from the wastewater containing  $500 \text{ mg l}^{-1}$  initial dye concentration. This CW-MFC was able to remove 75% of COD from wastewater containing  $1500 \text{ mg l}^{-1}$  initial concentration. During the treatment of  $1000 \text{ mg l}^{-1}$  initial dye concentration, the maximum power density and current density was recorded as  $15.73 \text{ mW m}^{-2}$  and  $69.75 \text{ mA m}^{-2}$  respectively. Overall, it is demonstrated that the CW-MFC is immensely beneficial for wastewater treatment in addition to renewable energy generation. While the field scale work is in progress, an attempt is also being made to study the microbial-electrodynamics from the electron transfer and kinetics standpoint.

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