Results & Discussions

Chapter-6

CHAPTER 6

6.1 Biodegradation of Reactive Orange 16 dye in a microbial fuel cell using mixed culture and its kinetics study

In this study, a double-chambered MFC inoculated with a mixed bacterial consortium was used for bioremediation of reactive orange 16 (RO16) dyes at a very high and variable concentration range of 100 to 1000 ppm.

6.1.1 Experiment design

MFC-2 (as described in section 3.2.2) with a working volume of 400 ml was operated for 3 months with different concentrations (100-1000 ppm) of Reactive Orange 16 dye using mixed culture. Samples were collected from the bottom of the MFC on daily basis and centrifuged for further analysis.

6.1.2 Result & Discussions

- **MFC operation**

Three parallel experiments including MFC with inoculums, batch systems with and without inoculums were carried out to study the performance of MFC. All the data were recorded in triplicate and the average was taken. At the start of the experiment, MFC was operated (without inoculums) with 100 ppm of dye in media to check the error produced by the system. The voltage produced during this period varies from 0.001 to 0.01 V. After this, 100 ppm dye solution along with 5% inoculums and other required media is added to the cell. On daily basis, 350 ml of the samples were taken back from MFC leaving only 50 ml of solution which acts as inoculums for the further experiment, and 350 ml fresh media along with 100 ppm dye were added in the setup. All other MFC parameters such as bacterial optical density, CFU, COD, and % color removal and elimination capacity in terms of COD and color were examined on the next day after the addition of fresh media which gives abundant time for the growth of microorganisms for CFU determination. Voltage and current were recorded on the computer continuously at 10 kΩ.

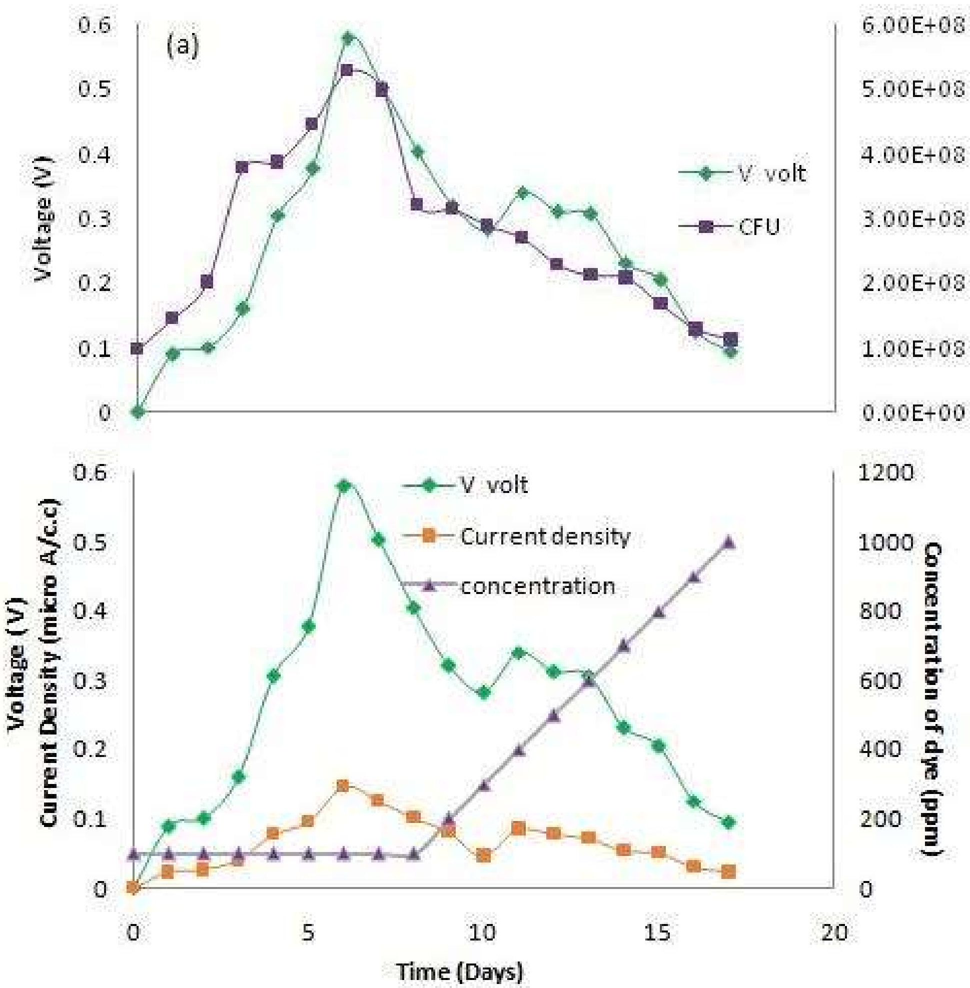

Figure 6.1(a) Variation of voltage and current over time and (b) Variation of voltage and Colony-forming unit with time.

It was observed from **figure 6.1(a)** that the voltage and microbial population increased rapidly with time in the MFC and achieved maximum value on the 6th day (0.57 V) of operation. During this period the substrate (dye+media) replenished continuously keeping dye concentration constant at 100 ppm. **Figure 6.1 (b)** indicates that voltage was maintained in the range of 0.56 to 0.57 V during the 6th to 8th day of operation at 100 ppm dye concentration. To study the effect of dye loading on electricity production, the dye concentration in the MFC was increased from the 9th day onwards. A sharp decrease in voltage output was observed in the MFC on the 9th day and then voltage output stabilized above 0.30 V up to the 12th day corresponding to 500 ppm of dye. Above 500 ppm and corresponding to the 13th day onwards the voltage output again started decreasing continuously and this behavior of MFC is similar to the behavior observed by another researcher (Ghoreyshi et al., 2011) who found that most of the dyes were remained unconsumed at high concentration in the MFC. The maximum power density of 0.08 W/m^3 was obtained at 100 ppm of dye **(Figure 6.2)**.

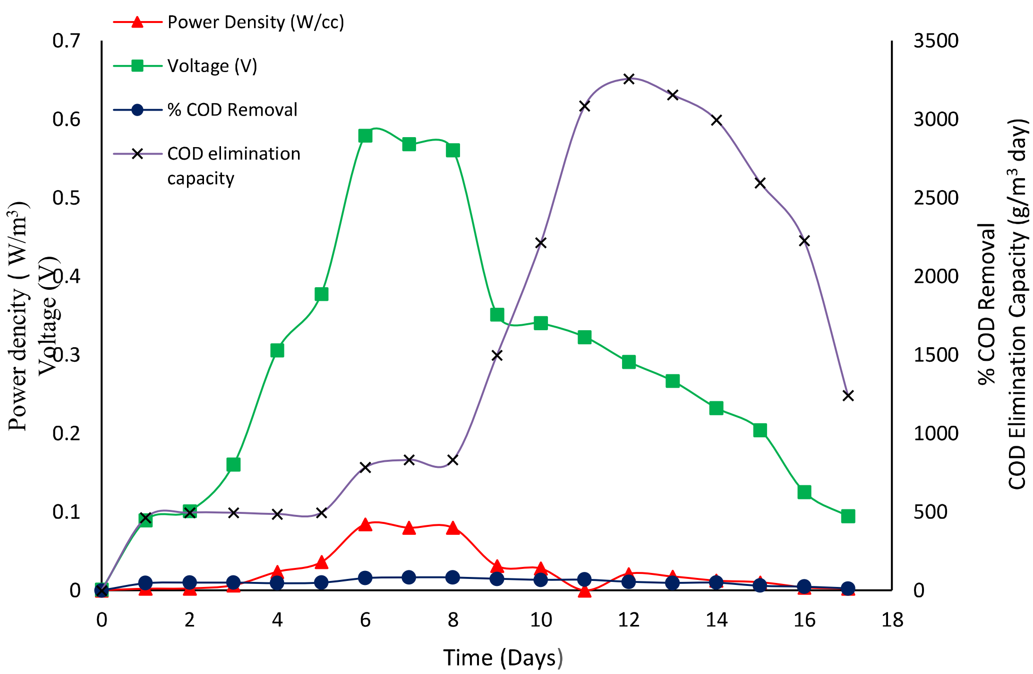

Figure 6.2 Relation between power density and COD removal over time.

Since the CFU value in the MFC represents the number of bacteria (**Figure 6.1(a)**) which is directly correlated with the production of electrons by bacterial metabolism and consequently generation of voltage, current, and power density(Arbianti et al., 2017). Even though there was more biodegradation in the MFC at a higher loading rate (200 to 500 ppm) of the substrate and this phenomenon was supported by color removal **(Figure 6.3),** COD removal **(Figure 6.4),** and CO_2 production **(Figure 6.5)** but the voltage obtained was not as high as produced with 100 ppm of dye.

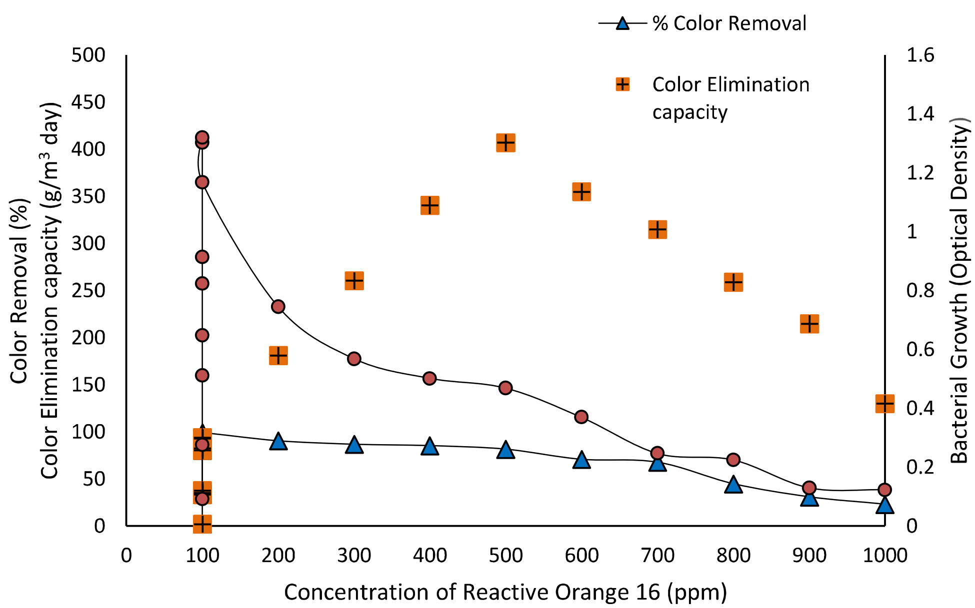

Figure 6.3 Variation of % color removal and bacterial growth with dye concentration.

Higher color removal (till 500 ppm) showed there is no inhibitory effect because of the substrate. It was expected that the voltage will also increase accordingly with an increase in biodegradation but voltage did not increase with the increase in biodegradation. This phenomenon may be attributed to the higher affinity of Nafion (PEM) for most of the cationic species other than proton produced at the anode side during the biodegradation process. Due to the high production of cation species other than proton at a high loading of the substrate (100 to 500 ppm), the movement of the proton through Nafion significantly reduced and thus reducing the voltage output of the MFC. Similar behavior of MFC was observed by other researchers particularly in the degradation of complex compounds like dyes that follow multiple metabolic pathways and produce more metabolites (Rajamanickam et al., 2017; H. Wang et al., 2019). Beyond 600 ppm (14^{th} day onwards) the sharp decline in the voltage may be due to substrate inhibition. The current density also followed a similar pattern as shown in **figure 6.1 (b).** The maximum current density was obtained for 100 ppm of dye and was found to be 0.14 A/m^3.

Further, substrate inhibition was confirmed by fitting experimental values to the Monod model. But for the higher dye loading, the Monod model does not fit the experimental value, therefore, the Haldane inhibition model was used (Chakraborty, 2016; Li et al., 2016). The voltage obtained in the present study was higher than reported values in the literature. Since exact data on voltage produced by Reactive Orange 16 dye in double-chambered MFCs is not available, therefore data were compared with other dyes and shown in **Table 6.1.**

Table 6.1 Electricity production using dyes

Dyes used	**Design**	**Electrical parameters**	**Decolorization**	**Reference**
Congo red (200 mg/L)	Single-chamber air cathode MFC	23.50 mW/m^2	88 % in 36 h	(Dai et al., 2020)
azo dye X-3B(200 mg/L)	biofilm electrode reactor microbial fuel cell coupled	1.05W/ m^3	92.2 % in 24 h	(X. Cao et al., 2019)
reactive blue 19 (50 mg /L)	Double chambered	19.22 mW/m^2	89 % in 48 h	(H. Wang et al., 2019)

Azo dye X-3B (200 mg/L) with glucose (150 mg//L)	Stacked MFC	0.37 W/m^3	98 % in 24 h	(X. Cao et al., 2019)
Reactive orange 16 (500 ppm)	Single chambered	2887 ± 13 $\mu W/m^2$	65 % in 48 h	(Ilamathi et al., 2019)
Congo red (300 mg/L)	two-chamber microbial fuel cells	215 mW/m^2	97% in 30 hr	(Hou et al., 2019)
Reactive red 198 (500 mg/L)	calcium alginate bead encapsulated bacterial cells	-	96.20% after 72 h	(Unnikrishnan et al., 2018)
Acid Orange 7(50 mg/L)	Single chambered up-flow membrane-less	167.4 ± 11.6 mV	80.6% in 24 h	(Thung et al., 2018)
Methyl orange (25 mg/L)	Photoelectron cat- alytic double chambered	0.11 W/m^2	84.5% a in 36 h	(Han et al., 2017)
Reactive orange 16 (100-1000)	Double chambered	0.57 V 0.08 W/m^3	98 % in 24 h for 100 ppm	**Present study**

- **COD removal efficiency**

For the characterization of organic matter present in the textile industry wastewater, a very common parameter chemical oxygen demand (COD) is used instead of biological oxygen demand (BOD) because of its simplicity and quick results than BOD (Babu et al., 2015; Chouler and Di Lorenzo, 2015). **Figure 6.4** showed the relation between COD removal, COD elimination capacity per day Vs Dye Concentration. The graph showed an increasing trend of COD removal concerning the time at 100 ppm of the dye concentration. 80% COD removal was obtained at maximum growth (**Figure 6.1(a))** and voltage. Afterward, with an increased concentration of dye, COD removal showed a declining trend.

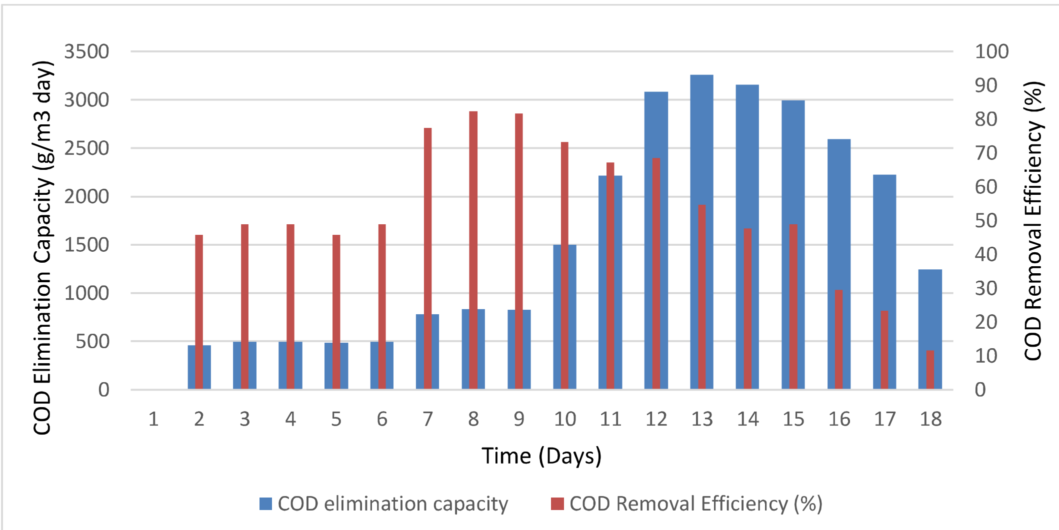

Fig 6.4 Relation between COD Removal Vs Dye Concentration

For 100-400 ppm of dye, COD removal was obtained in the range of 80-40 %. But the COD elimination capacity increased continuously from 100 to 500 ppm **(Figure 6.4)** showed effective biodegradation in the MFC at the anode side but after 500 ppm elimination capacity also decreased along with a reduction in voltage and power production. After 500 ppm, COD removal decreased and reached 10 % at a high loading of dye (1000 ppm). A very low COD value is because most of the dye remained as such in the solution at the anode side at high concentrations and resulted in substrate inhibition (Pazarlioglu et al., 2012).

Figure (6.2 and 6.3) depicts the behavior of MFCs at different loading of dye. Power density continuously increased and reached a maximum value of 0.08 W/m^3 at 100 ppm of dye. Afterward, it decreased continuously even if the concentration of dye was increased. The variation in power density and voltage followed the trend similar to the variation of COD removal. 88 % COD removal was achieved by Thung et al. (Thung et al. 2018) for acid orange 7 at 50 ppm and 79% COD removal was achieved for reactive orange 16 at a concentration of 100 ppm by others (Spagni et al., 2010). Present data were found to be either comparable or better as reported by other researchers (Srinivasan et al., 2011).

- **Color removal**

Almost 100 % color removal was achieved for 100 ppm of dye within 24 h of operation of MFC. From **figure 6.3**, it was clear that with the increase of bacterial density, color removal increases for 100 ppm of dye. As the concentration of dye increased from 100 to 500 ppm bacterial growth decreased up to a certain level and consequently,, % color removal decreased but overall color elimination capacity increased till 500 ppm showed effective biodegradation in the system. After 500 ppm color elimination and % color removal both decreased. This phenomenon may occur due to the increase of toxicity for microorganisms at higher concentrations of dye(Raj et al., 2012; Sponza and Mustafa, 2018). Till 400 ppm, 85 % color removal was achieved within 24 h. But after increasing concentration from 500 to 1000 ppm although decolorization takes place in MFC but not in 24 h. Decolorization rate decreased and time taken for 600 ppm dye for 50 % removal increased to 3 days, this is because of decreased bacterial density and increased toxicity due to dye. Ilamathi et al. (Ilamathi et al., 2019)have reported the decolorization of Reactive orange 16 (500 ppm) in single-chambered with 65 % removal efficiency in 48 h. Present data obtained were high as reported by others (Ilamathi et al., 2019; Spagni et al., 2010) and comparable to other reported data in batch biodegradation (Harry-asobara and Kamei, 2019; Wang et al., 2013).

- **CO_2 production**

In the anode chamber, CO_2 production was analyzed and depicted in **figure 6.5**. Initially, there was a rapid increase in CO_2 production as a concentration of dye increased to 400 ppm. After 400 ppm, although CO_2 was produced with a higher concentration than 100 ppm but the production rate was decreased. The maximum concentration of CO_2 was found to be 0.85 % at 500 ppm which is higher than the atmospheric concentration of 0.003% and this phenomenon confirms the biodegradation of reactive orange dye 16 in MFCs.

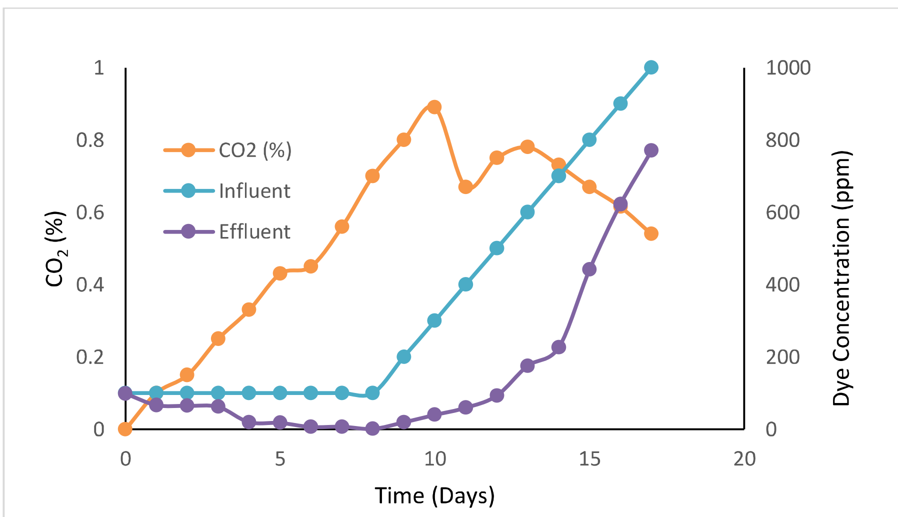

Figure 6.5 Relation between CO_2 production and Dye concentration

After 500 ppm, CO_2 production decreased and reached 0.54 %, and this value showed that after 500 ppm biodegradation rate in MFC decreased which can be attributed to substrate inhibition (Chaudhuri and Lovley, 2003; Inan Beydilli and Pavlostathis, 2005; Saba et al., 2017).

- **Growth Kinetic Study**

To evaluate growth kinetics, the biomass growth data from different initial reactive orange dye concentrations from experiments were plotted **(Figure 6.6)**. It was observed that with the increase of reactive orange concentration, specific growth rate (μ) increases till 500 ppm but after 500 ppm specific growth decreased with increased concentration of dye along with the decline of electricity production. Experiments cleared that after a certain concentration of dye, dye behaves as an inhibitory substrate. Monod kinetics were fitted with these data and found that for a higher concentration of dye, the Monod model does not fit with the experimental value. To validate experimental data, the Haldane model is used to find out the inhibitory concentration of dye and the value of the Haldane growth parameter was calculated as μ_{max} (maximum specific growth rate), K_s(half-saturation constant), K_i (Substrate inhibition constant) as 0.42 day^{-1}, 206.2 ppm and 447.12 ppm respectively. The value of substrate inhibition constant obtained from the kinetic study (447.12 ppm) and the experiment (500 ppm) is approximately the same which validates substrate inhibition in a microbial fuel cell with Reactive Orange 16 as a dye.

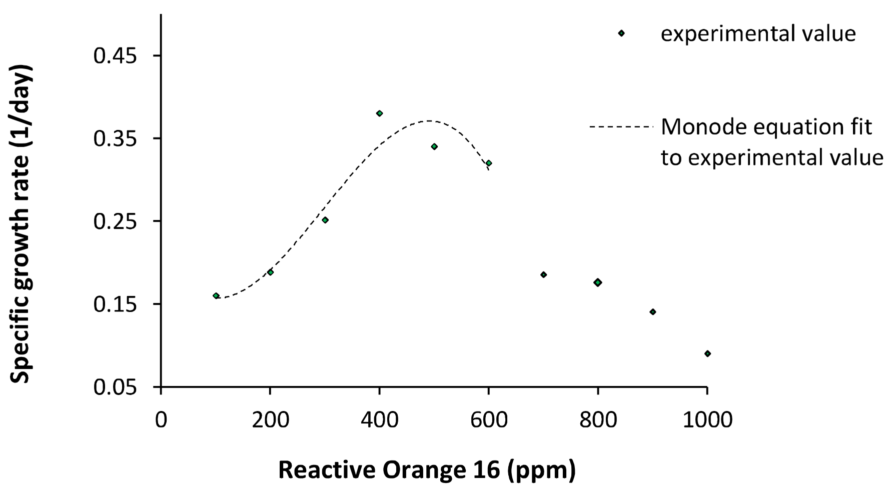

Figure 6.6 Monod Equation fitted till 400 ppm of dye (obtained experimental value)

Since none of the literature is available for the biodegradation kinetics of reactive orange 16 in microbial fuel cells. So direct comparison of the Haldane kinetic parameters of the present study with existing literature is not possible. But, **table 6.2** contains some comparable data based on batch biodegradation of dye in reported literature, which contained data of μ_{max} (specific growth rate) or V_{max} (maximum decolorization rate (mg/L h)) (Inan Beydilli and Pavlostathis, 2005; Srinivasan et al., 2011; Wang et al., 2013). Kinetics data of Reactive Orange 16 dye biodegradation is useful for the optimal design and operation of microbial fuel cells for treating textile waste.

Table 6.2 Haldane kinetic parameter for different dyes

Dyes used	μ_{max}/ V_{max}	K_s	K_i	Remarks	Reference
Reactive Red 2 (100 – 2000 mg/L)	109 mg/L h	8.6 mg /l	7285 mg/L	K_i> final dye concentration, there was no inhibition	(Inan Beydilli and Pavlostathis, 2005)[1]
Reactive Black 5 (100 – 1000 mg/L)	7.98 mg/L h	130.73	615.21	K_i < final dye concentration Inhibition was present	(Wang et al., 2013)
Yellow 2G (100–1000 mg/L))	0.49 mg/L h	263.20	1039	K_i> final dye concentration, there was no inhibition	(Srinivasan et al., 2011)[7]
Six reactive dyes (Red F3B, Black BL, Yellow HE4R, Red	0.037 - 0.15 h^{-1}	651.04–1372.88 mg/L	5681.81–18727.59 mg/L	K_i < final dye concentration Inhibition was present	(Gnanapragasam et al., 2011)

HE7B, Blue HERD, and Yellow F3R) (1000 – 6000 mg/L)					
Reactive orange 16 (100 -1000 mg/L)	0.42 day^{-1}	206.20 ppm	447.12 ppm	K_i < final dye concentration Inhibition was present	Present study

6.1.3 Concluding remarks

In the present study, decolorization and electricity production using Reactive Orange 16 dye in the MFC at high concentrations were successfully demonstrated. The maximum voltage of 0.57 V was observed at 100 ppm of dye. Almost 100 % color removal was achieved at 100 ppm of dye within 24 h. Haldane kinetics and the experimental results both show that the color removal and COD removal decreased rapidly after 500 ppm due to substrate inhibition. The inhibition constant (K_i) which indicates the concentration above which substrate inhibition will dominate using was found to be 447.12 ppm Haldane kinetic model. The results indicate a very good agreement between inhibition constant (K_i) and experimentally obtained value of concentration (500 ppm) above which substrate inhibition occurred. The result suggested that the MFC may be a good option of bioreactor for treatment of dye below 500 ppm along with the extra advantage of electricity generation.