

**SIMULTANEOUS ELECTRICITY GENERATION AND
DECOLORIZATION OF TEXTILE DYES IN SINGLE-CELL AIR-
CATHODE MICROBIAL FUEL CELLS**

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**Programme : Molecular Biology-Genetics and
Biotechnology**

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**TEK-ODALI HAVA-KATOT MİKROBİYAL YAKIT HÜCRELERİNDE
EŞZAMANLI ELEKTRİK ÜRETİMİ VE TEKSTİL BOYALARININ
DEKOLORİZASYONU**

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FOREWORD

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ABBREVIATIONS

BEAMR	: Bioelectrochemically Assisted Microbial Reactor
BOD	: Biological Oxygen Demand
CEM	: Cation Exchange Membrane
HMFC	: Biohydrogen Microbial Fuel Cells
LME	: Lignin-Modifying Enzyme
MFC	: Microbial Fuel Cells
NASA	: National Aeronautics and Space Administration
PEM	: Proton Exchange Membrane
PTFE	: Polytetrafluoroethylene
TEA	: Terminal Electron Acceptor
WIN	: Water Infrastructure Network
WRF	: White Rot Fungi

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SIMULTANEOUS ELECTRICITY GENERATION AND DECOLORIZATION OF TEXTILE DYES IN SINGLE-CELL AIR- CATHODE MICROBIAL FUEL CELLS

SUMMARY

In textile industry, 10-15 % of the dyes are released as effluents of dyeing processes. Dye-contaminated wastewaters cause aesthetic problems, reduce light penetration and can harm organisms since they might contain metals, chlorides etc. Conventional waste treatment methods, such as absorption and coagulation, are expensive and they cause secondary pollution. Thus, biological approaches are more favorable for waste treatment.

Previous studies proved that Microbial Fuel Cells (MFC) can be used for biologic treatment of wastewaters. However, performance of MFCs in degradation of various dyes was not studied in detail. In this study, five air-cathode single chamber MFCs were constructed to determine the decolorization capability and extent of eight textile dyes (Remazol Black RL, Remazol Brilliant Blue BB Gran 133, Remazol Turquoise Blue G 133, Reactive Red 195, Reactive Yellow 145, Reactive Black 5, Remazol Blue RR, Reactive Blue 222) in the presence of a readily-oxidizable carbon source, while monitoring simultaneous electricity generation.

This study revealed that different textile dyes could be decolorized to various extents in the presence of a readily oxidizable carbon source, in MFCs. Decolorization rates appeared to be different for each dye. With the addition of high concentrations of each dye, a decrease in electricity production was observed, while smaller concentrations of dyes could be stimulatory for electricity production. It was also shown that the dye decolorization was a continuous process, and repetitive dye addition did not affect the performance. These results demonstrated that MFCs could be promising candidates of bioremediation of textile wastes with simultaneous power generation.

TEK-ODALI HAVA-KATOT MİKROBİYAL YAKIT HÜCRELERİNDE EŞZAMANLI ELEKTRİK ÜRETİMİ VE TEKSTİL BOYALARININ RENK GİDERİMİ

ÖZET

Tekstil sanayinde kullanılan boyaların % 10-15'i boyama aşamalarında tekstil atık suyuna karışmaktadır. Boya içeren atık sular estetik problemlere yol açmakta, ışık emilini azaltmakta ve metal, klorlu bileşikler vs içerirlerse sudaki canlılara zarar verebilmektedirler. En çok kullanılan geleneksel arıtma yöntemlerinden emilme ve çöktürme gibi yöntemler pahalı olmakta ve ikincil atıklar üretmektedir. Bu nedenle atık işlemede biyolojik yaklaşımlar çok daha elverişlidir.

Mikrobiyal Yakıt Hücrelerinin biyolojik atık işlemede kullanabileceği daha önceki çalışmalarla ispatlanmıştır. Fakat, Mikrobiyal Yakıt Hücrelerinin çeşitli boyaları giderme performansları detaylı olarak incelenmemiştir. Bu çalışmada; beş adet benzer Tek-Odalı hava-katot Mikrobiyal Yakıt Hücresinin, okside olabilen bir karbon kaynağı varlığında, sekiz tekstil boyasını (Remazol Black RL, Remazol Brilliant Blue BB Gran 133 %, Remazol Turquoise Blue G 133, Reactive Red 195, Reactive Yellow 145, Reactive Black 5, Remazol Blue RR, Reactive Blue 222) giderme ve eş zamanlı elektrik üretim kapasitesinin gözlenmesi amacıyla kurulmuştur.

Bu çalışma; çeşitli boyaların, okside edilebilir bir karbon kaynağı varlığında Mikrobiyal Yakıt Hücrelerinde giderilebileceğini göstermiştir. Renk giderim oranları her boya için değişiklik göstermektedir. Elektrik üretiminde, özellikle yüksek konsantrasyonlarda boya eklenmesi ile düşüş gözlenirken, daha düşük konsantrasyonların uyarıcı etki yapabileceği gözlenmiştir. Ayrıca boya gideriminin sürekli bir proses olduğu ve tekrarlı boya eklenmesi deneyinin performansı etkilemediği gösterilmiştir. Bu sonuçlar; Mikrobiyal Yakıt Hücrelerinin, tekstil atıklarının biyolojik olarak iyileştirilmesinde ümit vadeden bir yöntem olabileceğini göstermektedir.

1. INTRODUCTION

1.1 Textile Industry

1.1.1 Textile dyes

History of synthetic dyes began with the invention of first commercially successful synthetic dye in 1856 by Henry Perkin. It was reported that there are more than 100,000 different type and over 7×10^5 metric tons of dyes are produced to be used in textile, paper, food, cosmetics and pharmaceutical industries [Meyer 1981, Zollinger 1987, Carliell *et al.* 1995]. Today, the exact numbers on the quantity is unknown. By the year 2000, approximately 600 thousand tons of dyes were consumed by India, the former USSR, Eastern Europe, China, South Korea and Taiwan [Ishikawa *et al.*, 2000]. As of 1999, dyestuff market valued 6.6 billion US\$, of which, North America accounting for 1.2 billion US\$, Central and South America for 0.7 billion US\$, Western Europe for 1.2 billion US\$ and Asia for 2.7 billion US\$ [Will *et al.*, 2000].

Notably, 60% of the total dyes produced is consumed by the textile industry [Mohan, 2001]. In textile industry, dye effluents release in wastewaters after bleaching, neutralizing, dyeing, rinsing, washing and softening processes are, on average, 10-15% of the total dye utilized [Zollinger, 1987]. Amount of the effluent can vary according to the type of the dyes, dying procedure and dye-applied material (Table 1.1). Common textile dyes that are currently in use are Basic Dyes (2-3%), Direct dyes (5-20%), Disperse dyes (1-20%), Metal-complex dyes (2-5%), Sulfur dyes (30-40%), and Reactive dyes (20-50%) [Laing, 1991].

There are more than 9000 textile dyes registered in the color index according to chemical and application classification [Stolz, 2001]. The textile dyes are expected to be resistant against UV-fading and oxidizing agents [McKay *et al.*, 1979]. They are also supposed to be stable against biodegradation and they have very low affinity for many adsorbents [Reife *et al.*, 1993].

Table 1.1: Effluents of different dyes (Modified from O'Neill, 1999)

Dye application class	Loss to effluent (%)
Acid	5-20
Basic	0-5
Direct	5-30
Disperse	0-10
Metal Complex	2-10
Reactive	10-50
Sulfur	10-40
Vat	5-20

1.1.2 Azo dyes

The most common type of textile dyes are azo dyes. All azo dyes contain azo bonds ($-N=N-$). Azo groups are responsible for the color formation. Reactive dyes constitute 70% of all the azo dyes [Easton J. R., 1995]. These dyes have complex structure associated with the aromatic hydrocarbons, making them very stable [Carliell *et al.*, 1995]. The azo groups of reactive dyes also have a strong electron-withdrawal capability that protects the color against oxygenase attacks [Nigam *et al.*, 1996].

Main problem of the dye-contaminated wastewater is aesthetic concerns. Many dyes are visible at concentrations as low as 1 mg/L [Nigam *et al.*, 2000]. Dye concentrations in textile effluents can range between 10-200 mg/L. [Gähr F. *et al.* 1994, Glover B. *et al.* 1992, Shelley T. R. 1994, Wilmott *et al.* 1998, Pierce J. 1994]. Reactive dyes are released into the effluents in great quantities since they are hydrolyzed during dye processing without complete fixation [Stolz, 2001]. These high concentrations of dyes in effluents can reduce light penetration into the waters they pollute, which causes depletion in oxygen levels by inhibiting photosynthesis and increases in organic load. Some studies showed that dyes with azo linkages can also form carcinogenic breakdown products [Chung and Cerniglia, 1992; Shenai, 1995].

Some textile dyes contain metals, and other elements such as chlorine in their structures that harm aquatic organisms [Clarke E.A. and Anliker R., 1980; O'Neill *et al.* 1999, Hao *et al.*, 2000]. Therefore, for instance in UK, it was forbidden to release

synthetic chemicals into marine environment since September, 1997 [Robinson T. *et al.*, 2001].

1.1.3 Decolorization of dyes

As mentioned above, treatment of textile wastewaters has been a major problem for the past two centuries. There are various physiochemical methods for dye effluent treatment including flocculation, coagulation, adsorption, membrane filtration, precipitation, irradiation, ozonation, oxidation and biodegradation [Banat *et al.*, 1996; Slokar and Le Marechal, 1998; Churchley, 1994; Vandeviere *et al.*, 1998; Delee *et al.*, 1998; O'Neill *et al.*, 1999; McMullan *et al.*, 2001; Rai *et al.*, 2005; Van der Zee and Villaverde, 2005; Swaminathan *et al.*, 2003; Behnajady *et al.*, 2004; Wang *et al.*, 2004; Golab *et al.*, 2005; Lopez-Grimau and Gutierrez, 2005], (Table 1.2).

Coagulation and adsorption methods are most preferred by the textile industry. However, these methods are inefficient when working with high effluent volumes. They also generate excessive amount of sludge which requires additional treatment and high chemical usage, creating additional pollution problems. As a result, high operational costs are unavoidable for these techniques [Hao *et al.*, 2000; Sokolowska-Gajda *et al.*, 1996]. Therefore, novel biological treatments that could offer ecologically friendlier and cheaper solutions must be employed.

Table 1.2: Advantages and disadvantages of conventional treatment technologies

Physical and/or chemical methods	Advantages	Disadvantages
Oxidation	Rapid process	High energy costs and formation of by-products
Adsorption	Good removal of a wide range of dyes	Absorbent requires regeneration or disposal
Membrane technologies	Removes all dye types	Concentrated sludge production
Coagulation/flocculation	Economically feasible	High sludge production

Azo dyes are degraded in two steps: First the azo bond must be cleaved [Wuhrmann *et al.*, 1980] and then intermediates must be mineralized. The latter is a particularly important step for public health because these intermediates such as benzidine, 2-naphtylamine and other aromatic amines are known to be toxic [Anliker, 1979].

Dyes are designed to be recalcitrant. However, many microorganisms (filamentous fungi, bacteria, yeast and algae) can successfully degrade dyes and mineralize intermediates if the environmental conditions are suitable. This process is relatively inexpensive, has low operational costs and the end products are not toxic.

WRF are the best-known microorganisms in biodegradation. *Basidiomycetous* fungi are known to have high capabilities of lignin mineralization. These abilities are mainly due to the wide variety of LMEs they secrete. These extracellular LMEs possess a wide substrate range, therefore, they can degrade a wide variety of xenobiotics [Barr and Aust, 1994; Pointing, 2001; Scheibner *et al.*, 1997] as well as various recalcitrant dyes [Glenn and Gold, 1983; Pasti-Grigsby *et al.*, 1992; Paszczynski *et al.*, 1992; Spadaro *et al.*, 1992]. Many dyes and simulated textile effluents were decolorized by WRF and it was shown that fungi such as *Aspergillus niger* also remove dyes by adsorption. Textile dye effluents are harder to treat in comparison to pure dye solutions. In very few studies azo-chromophore containing textile effluents were shown to be decolorized by WRF [Knapp and Newby, 1999; Wesenberg, 2002].

Researchers found that some peroxidase-producing bacterial strains (e.g. some *Streptomyces* species, gram-negative bacteria such as *Sphingomonas chlorophenolicus*) can degrade textile dyes [Cao *et al.* 1993; Paszczynski *et al.* 1992]. Bacteria can break azo bonds under aerobic, anaerobic (methanogenic) and anoxic conditions. For aerobic degradation, bacteria should be exposed to simple azo dye compounds for a longer time for adaptation. During the period of adaptation, bacteria produce dye-specific reductases. However, anaerobic bacterial reduction is not specific to the type of dye. It was shown that under anaerobic conditions many textile dyes can be degraded [Delee *et al.*, 1998]. Reactive water soluble dyes are also degradable under anaerobic conditions [Carliell *et al.*, 1996]. The only drawback of this method is that carcinogenic aromatic amines might be formed as by-products of the reaction. These amines can be degraded if a secondary aerobic condition is added to the anaerobic process. Panswad and Luangdilok (2000) reported that with this integrated technique, bisazo vinylsulphonyl, anthraquinone vinylsulphonyl and anthraquinone monochlootriazine reactive dyes could be degraded effectively.

It was shown that *P. chrysosporium* can decompose Orange II, Tropaeolin 0, Congo Red, Azure B, Amaranth, New Coccine, Orange G, Tartrazine and Poly R-478 [Cripps *et al.*, 1990; Chagas and Durrant, 2001; Couto *et al.*, 2000a; Couto *et al.*, 2000b]. Algae cultures (*Chlorella pyrenoidosa*, *C. vulgaris* and *Oscillatoria tenuis*) also have azo reductase activity which degrades azo dyes into aromatic amines [Liu and Liu, 1992].

Most azo dyes are stable against microbial attacks; consequently, mixed bacterial cultures may be more advantageous than pure cultures in textile wastewater treatment. Different strains may attack the dye molecule differently, or one strain can help the degradation of a semi-decomposed molecule by another strain. Utilizing the wide diversity of microorganisms also increases the chances of decolorization of different dyes in the same effluent. However, microbial consortia can change during the processes which might be considered either as advantageous in terms of possibly increased degradation efficiency, or disadvantageous in terms of treatment stability.

Finally, as another option, usage of pure enzymes can be efficient and risk-free for the environment, nevertheless, extraction and separation processes increase operational costs which is detrimental for industrial scale applications. Furthermore, degradation rate highly depends on pH [Bhunia *et al.*, 2001] which makes the process even harder to handle.

All the problems mentioned above call for new technologies in waste treatment for dye effluents, and MFC technology may be considered as one of these in order for environmentally benign waste treatment and energy production.

Table 1.3: Some of the dye-degrader organisms (Modified from Forgacs *et al.*, 2004)

Organism	Dye	Reference
Prokaryota		
<i>Gram Negative Bacteria</i>		
<i>Pseudomonas luteola</i>	Crystal Violet, Red Pigment 2B, Red Pigment V2 Reactive Red 22	Hu (1998, 2001) Chang et al. (2001) Chen (2002)
<i>Gram Positive Bacteria</i>		
<i>Streptomyces viridosporus</i>	Poly R-478	Ball and Colton (1996)
<i>Geotrichum candidum</i>	Reactive Blue 5	Lee et al. (2000)
	Reactive Black 5, Reactive Red 158, Reactive Yellow 27	Maximo et al. (2003)
Eukaryota		
<i>Yeasts—Ascomycota</i>		
<i>Aspergillus niger</i>	Congo Red	Fu and Viraraghavan (2002)
<i>Filamentous fungi—Basidiomycota</i>		
<i>Bjerkandera adusta</i>	Amaranth, Remazol Black B, Remazol Orange, Tropaeolin O Reactive Blue 15, Reactive Blue 38	Swamy and Ramsay (1999a,b) Heinfling-Weidtmann et al. (2001)
<i>Phanerochaete chrysosporium</i>	Amaranth Disperse Orange K-GL, Everzol Yellow 4GL, Everzol Red RBN, Everdirect Supra yellow PG, Everzol Turquoise Blue G Indigo Carmine	Swamy and Ramsay (1999a,b) Kapdan et al. (2000) Podgornik et al. (2001), Gemeay et al. (2003)
	Poly R-478	Couto et al. (2000a), Mielgo et al. (2002)
	Red HE 8B	Sani et al. (1998)
	Remazol Black B	Swamy and Ramsay (1999a,b)
	Remazol Turquoise Blue	Conneely et al. (1999)
<i>Pleurotus ostreatus</i>	Eosin Yellowish, Evans Blue Phenol, Red Poly B 411	Eichlerova et al. (2002)
<i>Pycnoporus cinnabarinus</i>	Reactive Blue 19	Balan and Monteiro (2001)

1.2 Microbial Fuel Cells

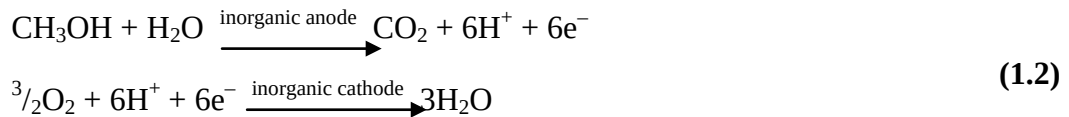
1.2.1 Definition and principles of MFC

MFCs can convert chemical energy into electricity by oxidizing inorganic or organic substrates.

Inorganically fueled devices (Usually metals are used as catalysts):



Organically fueled devices (Complex chemicals are used with the help of inorganic catalysts):



[Bullen *et al.*, 2006]

The bacterial biofilm that preferentially forms on the anode oxidizes substrates anaerobically. Consequently, this reaction generates electrons and protons as by-products. The electrons, directly or with the help of mediators, transfer externally from cell's respiratory system to the TEA in the cathode where reduction occurs while protons transfer internally. This flow of electrons from anode to cathode creates a redox potential (Volt) that results in electricity generation (Figure 1.1).

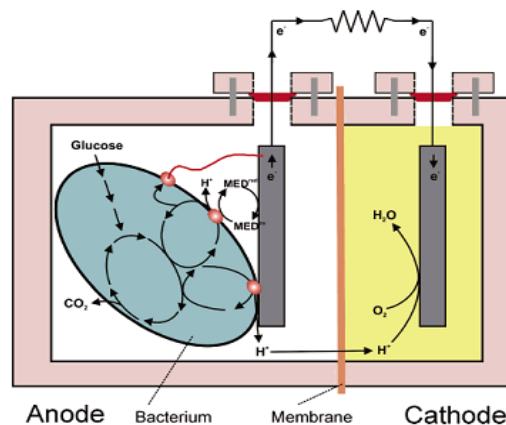


Figure 1.1 : Working principle of a dual-chamber MFC (Logan *et al.*, 2006)

1.2.2 Brief history

The relationship between biology and electricity was first demonstrated in the late eighteenth century by Luigi Galvani. He observed the first bioelectrical response in a frog's isolated leg where an electrical stimulus was transmitted through the tissue [Galvani, 1791].

In 1910, first MFC was built by placing platinum electrode into liquid media of yeast or *Escherichia coli* and it was proved that microorganisms could generate and deliver electricity [Potter, 1910].

Inspired by Potter's idea, Barnett Cohen built a potentiostat-poised half cell and he managed to generate 35 V with a batch of fuel cells [Cohen, 1931].

In late 1950s and 1960s, the need for treatment of organic waste made MFC studies popular again. In these years, NASA was searching for an alternative energy source for long-haul space travels and this called for new research on MFCs due to its potential of both generating electricity and waste disposal [Shukla, 2004; Bullen *et al.*, 2006]. However, researchers failed to generate electricity in consistent rates [Lewis, 1966; Davis and Yarbrough, 1962; Shukla *et al.*, 2004].

In 1963, MFCs became commercially available in the markets as an energy source for radios, signal lights and other marital appliances [Shukla, 2004]. However, they were later replaced by the alternative new energy sources such as solar photovoltaics.

In 1980s, H. Peter Bennetto made a series of experiments and managed to produce electricity by operating pure cultures of bacteria with artificial mediators to aid electron transfer [Bennetto *et al.*, 1981; 1983; 1985; Roller *et al.*, 1984; Delaney, 1984]. Additional studies showed that these MFCs enhanced efficiency of electron transfer and reaction rate [Lithgow *et al.*, 1986; Emde *et al.*, 1989; Park and Zeikus, 2000]. On the other hand, recent studies also demonstrated that some bacteria, *Geobacter sulfurreducens* [Pham *et al.*, 2003; Bond and Lovley, 2003], *Geobacter metallireducens* [Bond *et al.*, 2002; Bond and Lovley, 2003], *Shewanella putrefaciens* [Kim *et al.*, 1999; Kim *et al.*, 2002], *Clostridium butyricum* [Park *et al.*, 2001], *Rhodospirillum rubrum* [Chaudhuri and Lovley, 2003] and *Aeromonas hydrophila* [Pham *et al.*, 2003], could transfer electrons directly to the electrodes without the help of mediators.

1.2.3 MFC designs

MFCs must be designed according to the needs of high power generation and coulombic efficiency. However, constructional costs must be decreased before commercialization.

The most basic and cheapest MFC design is the H shaped MFC with two-chambers that are usually made of two bottles (Figure 1.2 F). These two bottles are connected with a tube that contains a separator which is mostly a CEM. This membrane allows passage of protons, but not the substrates or bacteria in media. CEM or PEM can be made of mostly nafion, ultrex or a salt bridge (Figure 1.2A, 1.2F). However, the salt bridge MFC is inefficient in power generation because of the high internal resistance. H-shaped MFCs are proper for research experiments that neglect the power generation. In these systems power production may be improved by using additional electron acceptors such as ferricyanide. However, ferricyanide alone is not efficient for power generation, therefore, modification on other factors such as anode and cathode should be considered as well.

In air-cathode systems, cathode is exposed directly to oxygen, thus, requirement for an ion-exchange membrane may be unnecessary (Figure 1.2C, 1.2D, 1.2E). Air-cathode MFCs allow greater power production due to the use of oxygen as direct electron acceptor. This system was first developed in Penn State University [Liu and Logan, 2004]. A cubic reactor contained anode and cathode placed on the opposite ends. In such a setup, the cathode must be permeable for oxygen, but impermeable for liquids, in order to prevent leaks. This is solved by coating cathode surface with PTFE. In order to prevent oxygen diffusion to anode that could inhibit anodic reaction and reduce Coulombic efficiencies, ion-exchange membranes may still be used.

Although batch systems can produce high electricity, gradual reduction of this level is unavoidable due to the source limitations. To overcome the problem, continuous systems are developed. In this design, there is an outer cylinder (a cathode) and a concentric inner cylinder (an anode) that contains media. Another continuous design is an upflow fixed-bed reactor where the media flow through porous anode to the cathode chamber (Figure 1.2H).

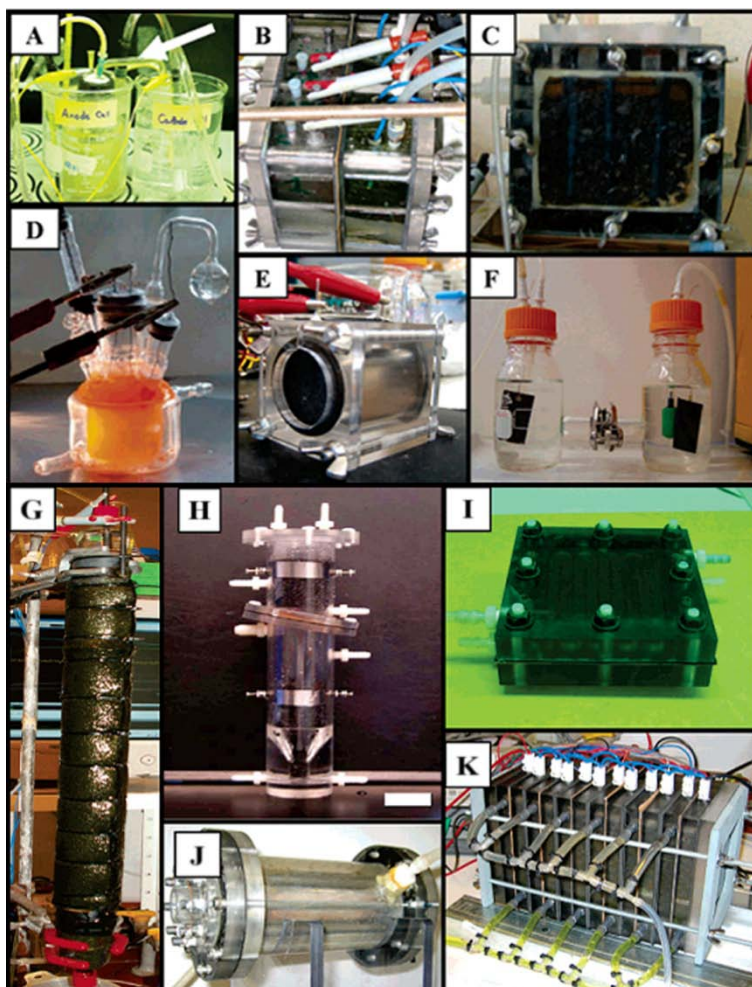


Figure 1.2 : MFC designs (Logan *et al.*, 2006)

Flat plate MFCs aim to reduce internal resistance by decreasing the space between electrodes (Figure 1.2I). Nevertheless, it was shown that electrodes spaced too close did not significantly improve power generation [Min and Logan, 2004].

In stacked systems, plates containing the electrodes are placed inside the cell to increase electricity generation (Figure 1.2K). However, experiments demonstrated that stacked cells exhibited reversal of electricity polarity from time to time [Aelterman *et al.*, 2006]. Further studies shown that depletion in carbon source could cause these voltage reversals [Oh and Logan, 2007].

HMFC employ the fermentation reaction to produce H_2 gas [Schröder *et al.*, 2003]. Normally, hydrogen reactors, that utilize either bacteria or mediators, aim to produce hydrogen, not electricity. However, in the design of Schröder and co-workers, hydrogen was produced in one container and electricity was generated in another container. In anode section, bacteria catalyses the reaction that convert substrate into

hydrogen and for the oxidation of H_2 to generate electricity, chemical catalysts should be added to the anode. The disadvantage of system is inadequate oxidation of substrate in the cell. This problem was surpassed with the invention of photobiological HMFCs [Rosenbaum *et al.*, 2005]. In this example, *Rhodobacter sphaeroides* was used. This bacterium could convert volatile organic acids (some end products of glucose fermentation e.g. acetic acid) to H_2 . It was reported that requirement of sunlight was a limiting factor even though the substrate was utilized more efficiently and the power generation was acceptable.

1.2.4 Applications of MFC

It is estimated that petroleum reserves will be depleted in approximately 200 years and alternative energy sources are needed urgently. MFC technology directly uses various carbon sources as fuel; as a result, not only that it produces less waste, but also it can bioremediate waste. By complete oxidation of carbohydrates to CO_2 and water; 16×10^6 J/kg (about 5 kWh), of electrical energy is obtained. That equals to nearly half of the energy obtained from the same amount of octane.

Up to now, there are only two commercial MFC applications. In one setup, MFCs has been used for starch plant wastewater treatment in a pilot application for over 5 years [Gil *et al.*, 2003]. In another application, a *Shewanella* sp. containing MFC was used as a BOD sensor to measure BOD in sewage [Kim *et al.*, 2003; Chang *et al.*, 2005; Moon *et al.*, 2004].

One of the most promising applications is hydrogen production by HMFCs. In HMFCs, electrons derived from bacterial reactions unite with the protons on the cathode to produce hydrogen gas instead of electricity. Since the actual intention here is to produce hydrogen, these systems are also called BEAMRs or biocatalyzed electrolysis systems rather than true MFCs [Heilmann, 2005; Liu *et al.*, 2005a; Logan and Grot, 2005; Rozendal and Buisman, 2005; Rozendal *et al.*, 2006]. The first hydrogen producing system was developed by Liu *et al.* [2005b]. It was a reactor made of two glass culture bottles separated by a CEM (Nafion). The anode was plain carbon cloth and the cathode was made of platinum coated carbon paper (0.5 mg Pt/cm^2). Up to now, four different BEAMRs were studied. Three of them were fueled with acetate and the other one was fueled with wastewater. These systems can provide an economical way of hydrogen production since the energy need of reaction is supplied by the substrate itself.

1.2.5 MFC and dye degradation

In USA, treatment of more than 126 billion liters of domestic wastewater costs over \$25 billion annually [WIN, 2001]. Studies demonstrated that MFC systems can be fueled with wastewaters, which gives an opportunity for wastewater treatment [Liu *et al.*, 2004; Feng *et al.*, 2008; Min and Logan, 2004; He *et al.*, 2005]. Microorganisms in MFC can achieve both degrading effluents and producing electricity. Furthermore, it was proved that MFC can accomplish the bioremediation of some contaminants [Gregory and Lovley, 2005; Lovley, 2006]. With these discoveries, it is very rational to assume that MFC can remediate textile wastewaters. It is known that many organisms that are found naturally in MFC biota can degrade some of the textile dyes [Table 1.4]. Recent discoveries presented the dye-degradation ability of MFCs [Sun, J. *et al.*, 2009, Cao *et al.*, 2009; Liu *et al.*, 2009].

For the first time, Sun *et al.* (2009) proved the dye decolorization of Active Brilliant Red X-3B by using a single-chamber MFC with a microfiltration membrane air-cathode. This study aimed to resolve the effects of different co-substrates and dye concentrations on degradation of Active Brilliant Red X-3B and electricity production. It was concluded that decolorization of Active Brilliant Red X-3B was higher than conventional anaerobic dye treatment when MFC technology was used. Furthermore, dye decolorization was not strongly inhibited with the increased concentration of dyes (Figure 1.3), while electricity generation decreased (Figure 1.4).

Table 1.4: Some of the dye-degrader organisms found in MFCs (Modified from Forgacs *et al.*, 2004)

Organism	Dye	Reference
Prokaryota		
<i>Gram Negative Bacteria</i>		
<i>Aeromonas hydrophila</i>	Acid Orange 7, Acid Red 106, Direct Orange 39, Direct Yellow 4, Direct Yellow 12, Reactive Black NR, Reactive Blue 160, Reactive Blue 222, Reactive Red 198	Chen et al. (2003)
<i>Desulfovibrio desulfuricans</i>	Reactive Orange96	Yooetal. (2001)
<i>Escherichia coli</i>	Ethyl Red, Methyl Red Reactive Red 22 Reactive Red 22	Nakanishi et al. (2001) Chang and Lin (2001) Chang and Kuo (2000)
<i>Geobacter sulfurreducens</i>	Anthraquinone-2, 6-disulfonate	Cervantes et al. (2003)
<i>Klebsiella pneumoniae</i>	Methyl Red	Wong and Yuen (1998)
<i>Gram Positive Bacteria</i>		
<i>Clostridium perfringens</i>	Amaranth Bromophenol Blue, Crystal Violet, Methyl Orange	Semde et al. (1998) Kim et al. (2002a,b)
Eukaryota		
<i>Yeasts—Ascomycota</i>		
<i>Saccharomyces cerevisiae</i>	Reactive Blue 19	Aksu and Donmez (2003)

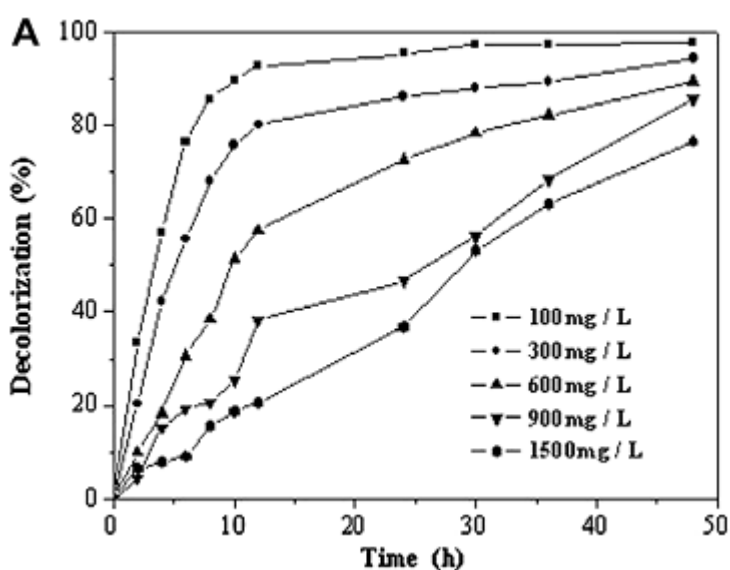


Figure 1.3 : Decolorization rate of Active brilliant Red X-3B in MFCs (Sun *et al.*, 2009)

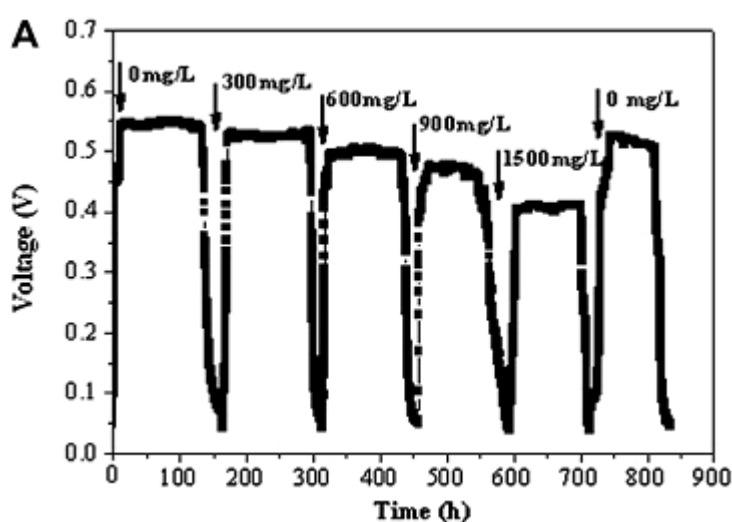


Figure 1.4 : Electricity generation during increasing dye concentration (Sun *et al.*, 2009)

Liu *et al.*, (2009) demonstrated that the electrons that were captured directly from the respiration of *Klebsiella pneumoniae* strain L17 could be transferred to the dyes, degrading methyl orange, Orange I and Orange II via this reduction pathway.

The study of Cao *et al.* (2009) focused on the electricity generation from various substrates during degradation of Congo red and the further clarification of the degradation pathway of this dye by using air-cathode single chamber MFC.

Even though, the degradation of azo dyes with MFC technology was demonstrated in these studies, some important points were yet to be elucidated. Two of the previous

work demonstrated the degradation of a single dye while the other study was focused on the redox potentials of a few azo dyes in different conditions with pure culture inoculated MFCs. There is no comprehensive study that demonstrates the comparative degradation of several azo dyes and discusses the microbial communities responsible, in using air-cathode single chamber MFCs inoculated with activated sludge, while observing the electricity generation, simultaneously. Activated sludge has a dynamic structure because of having a wide range of microbial diversity. Better understanding this dynamic structure could be very beneficial in efficient decolorization of different dyes.

1.2.6 Aim of the study

The main purpose of this study is to determine the biodegradation capability of air-cathode single-chamber MFCs for eight different textile dyes by using sludge inoculated air-cathode MFCs at different concentrations and study the effect of dyes on electricity production.

2. MATERIAL AND METHODS

2.1 Construction of Air Cathode MFCs and Monitoring Electricity Production

Five air-cathode MFCs were constructed from plexiglas, as shown below (Figure 2.1). Anode and cathode were placed at the opposite edges of the cylindrical structure with a total working volume of 12 mL (Figure 2.2).

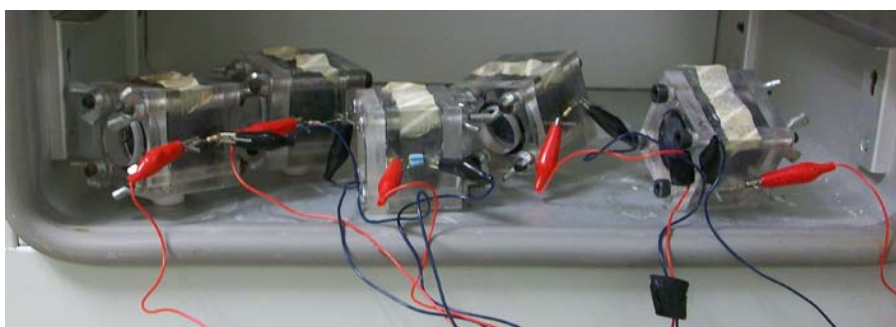


Figure 2.1 : Five air-cathode MFCs

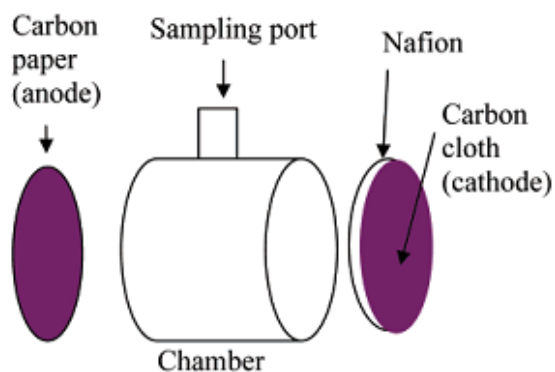


Figure 2.2 : Structure of MFC

Anode and cathode were connected via a 100Ω resistor to the multimeter data acquisition system (Keithley 2700 multimeter, USA) to monitor voltage output every 45 seconds. Data was stored in the ExceLINX program (Keithley, USA), (Figure 2.3).



Figure 2.3 : Operation of MFCs

2.2 Anode and cathode preparation

Carbon (graphite) cloth was used as anode material. Cloth fiber layers of anode were coated with PTFE layers. Air-exposed surfaces of the cathode carbon fibers were coated with PTFE and carbon powder while inner surfaces of cathode carbon cloth were coated with platinum as catalyst and nafion as the binder (According to Cheng *et al.*, 2006b).

2.3 Inoculum and cultivation

Activated sludge that was kindly provided from an industrial nitrification pool of a local yeast production company (PAKMAYA Izmit Plant, Turkey). MFCs (excluding MFC 1) were initially inoculated with 6 mL of the activated sludge and filled with the company waste that was rich in molasses and their derivatives. In contrast with the other MFCs, MFC 1 had been in use for more than 2 years by now, and had been inoculated with an activated sludge that was received from an aerobic reactor setup of the environmental engineering department of Istanbul Technical University. When a stable electricity production was observed, this complex medium was replaced with a phosphate-buffered medium containing acetate as carbon source for acclimatization of cells. Acetate containing medium was prepared as follows (per liter): Sodium acetate (2 g), NH_4Cl (0.31 g), $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ (5.84 g), $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ (15.47 g), KCl (0.13 g), a mineral solution (12.5mL) and a vitamin solution (12.5mL) [Lovley and Phillips, 1988]. (Appendix C)

After the 24-hour dye decolorization studies, MFC media were replaced by fresh ones that did not contain the dyes to standardize the MFCs because of the variation in electricity generation.

2.4 Dye decolorization studies

Eight different textile dyes (Remazol Black RL, Remazol Brilliant Blue BB Gran 133 %, Remazol Turquoise Blue G 133, Reactive Red 195, Reactive Yellow 145, Reactive Black 5, Remazol Blue RR, Reactive Blue 222) used for the decolorization assays were kindly provided by Setaş Kimya company (Istanbul, Turkey). Dyes were dissolved in distilled water that contains 10 µl of 10 mM NaOH. Dye solutions were stirred overnight on a magnetic stirrer. Dye concentrations of 40, 80, 160 and 320 mg/L (Dye/acetate buffer) were studied for decolorization experiments.

From the beginning of dyes addition, 300 µl of media samples were drawn from each MFC (MFC 1, MFC 2, MFC 3, MFC 4 and MFC 5) every 2 hours throughout an incubation period of 24 hours. The samples were then centrifuged at 9000 rpm for 5 minutes to remove the particulate matter (Sigma 1-14 microfuge, Germany). From each cell, 200 µl of supernatants were transferred to the 96-well microplates. Decolorization of textile dyes was monitored using a microplate spectrophotometer (Benchmark Plus Microplate Reader, Bio-Rad, USA). The incubation medium without dyes was used as blank for spectrophotometric readings. Electricity production was monitored in parallel, using multimeter.

For repetitive dye application studies, each different dye was added to MFC 1-4 in the concentration of 40 mg/L every 24 hours for five days. Every 12 hours, 300 µl of media samples were drawn from each MFC (MFC 1, MFC 2, MFC 3 and MFC 4). The samples were then centrifuged at 9000 rpm for 5 minutes and 200 µl of supernatant samples were transferred to the microplates. Decolorization of textile dyes was then monitored as described.

3. RESULTS AND DISCUSSION

3.1 Decolorization

In the current study, spectrophotometric data demonstrated that MFCs could efficiently decolorize the entire textile dyes in question (Table 3.1). However, while all the MFCs were identical and received the same inocula (except for MFC 1), decolorization rates of the same dyes were different in each MFC. The highest decolorization results were obtained in MFC 1 for almost all of the dyes tested. MFC 3 was the next best in dye decolorization. The lowest performance was obtained from MFC 5.

The UV–visible absorption spectra revealed that MFCs decolorized 80%, 50%, 85%, 60%, 75% and 70% of Remazol Black RL, Remazol Turquoise Blue G 133, Reactive Yellow 145, Reactive Red 195, Reactive Black 5, Reactive Blue 222 and Remazol Blue RR, respectively (Table 3.1). Remazol Brilliant Blue BB G 133 was decolorized to the highest extent, with approximately 90%.

Decolorization rates were usually inversely proportional with the increase in applied dye concentrations in the MFCs. However, with Remazol Blue RR and Remazol Black 5, increases in dye concentration did not significantly affect decolorization rates.

To the best of our knowledge, dye biodegradation is best accomplished by filamentous fungi. These dyes are recalcitrant due to the azo, nitro, and sulfo groups they contain [Pagga and Brown, 1986; Kulla *et al.*, 1983; Michaels and Lewis, 1985; Shaul *et al.*, 1991]. It was shown that a white rot-fungus, *Phanerochaete chrysosporium*, could efficiently degrade various azo dyes, the triphenylmethane dyes and some aromatic pollutants [Bumpus and Aust, 1987; Bumpus and Brock, 1988; Bumpus *et al.*, 1985; Cripps *et al.*, 1990; Hammel, 1989; Paszczynski and Crawford, 1991; Paszczynski *et al.* 1991].

Even though textile dyes are resistant against bacterial degradation, under anaerobic conditions many bacteria can mineralize these dyes [Carliell *et al.*, 1996;

Chinkewitvanich *et al.*, 2000; Razo-Flores *et al.*, 1997; Bell *et al.*, 2000; Plumb *et al.*, 2001; Weber and Adams, 1995; Willetts *et al.*, 2000; Talarposhti *et al.*, 2001; Yoo *et al.*, 2001; Isik and Sponza, 2005; Van der Zee and Villaverde, 2005]. A few studies showed that especially sulfonated azo dyes may be mineralized under aerobic conditions by several bacteria, albeit, low efficiency. Therefore, bacterial degradation did not attract much attention [Zimmermann *et al.* 1982, 1984; Sarnaik and Kanekar, 1999; Blhmel *et al.*, 1998]. Although the cell design used in the current study was an air-cathode model, formation of both aerobic and anaerobic regions in the cells is possible. Therefore, both aerobic and anaerobic bacterial dye degradation could have taken place.

Removal of dyes may be accomplished mainly via chemical degradation, adsorption onto surfaces and sedimenting in the reactor. Here, no significant sedimentation or surface adsorption was detected. This suggests that all the dyes were chemically processed by the bacteria. The nature of the degradation was not the focus of the current work, and therefore, have not been studied in detail. As one of the most important indications of dye degradation is color removal, spectrophotometric studies were conducted here. The results showed effective color removal in the presence of a carbon source.

Table 3.1: Decolorization ratio following the treatment in MFCs

DYES	40 mg/l					80 mg/l				
	MFC 1	MFC 2	MFC 3	MFC 4	MFC 5	MFC 1	MFC 2	MFC 3	MFC 4	MFC 5
Remazol Black RL	88	64	84	69	93	97	77	87	65	87
Remazol Brilliant Blue BB G 133	98	96	95	96	89	98	91	96	95	97
Remazol Turquoise Blue G 133	77	67	49	55	61	63	39	45	47	57
Reactive Yellow 145	96	93	96	95	89	96	90	94	94	89
Reactive Red 195	83	78	77	68	79	82	70	77	75	67
Reactive Blue 222	72	56	73	73	58	77	67	70	71	64
Remazol Black 5	77	72	75	81	68	80	76	76	74	78
Remazol Blue RR	78	73	58	69	56	80	75	80	76	75

DYES	160 mg/l					320 mg/l				
	MFC 1	MFC 2	MFC 3	MFC 4	MFC 5	MFC 1	MFC 2	MFC 3	MFC 4	MFC 5
Remazol Black RL	84	76	83	79	75	82	74	86	82	59
Remazol Brilliant Blue BB G 133	89	91	95	93	87	88	86	97	93	60
Remazol Turquoise Blue G 133	56	47	55	53	59	38	32	43	32	37
Reactive Yellow 145	93	78	88	86	72	90	69	75	73	65
Reactive Red 195	65	31	51	44	46	85	42	56	53	42
Reactive Blue 222	73	49	64	63	58	75	51	60	62	54
Remazol Black 5	79	69	75	74	67	78	73	75	76	60
Remazol Blue RR	85	75	81	79	68	84	60	76	72	59

3.2 Electricity Production

The electricity production from acetate in five MFCs (MFC 1-5) was monitored for 24 hours as one batch. As stated before, electricity production varied among each individual MFC. This variation in electricity production could possibly be partly explained by the complexity of the mixed bacterial culture and the rapidly changing dynamics among the bacterial species until an equilibrium is reached.

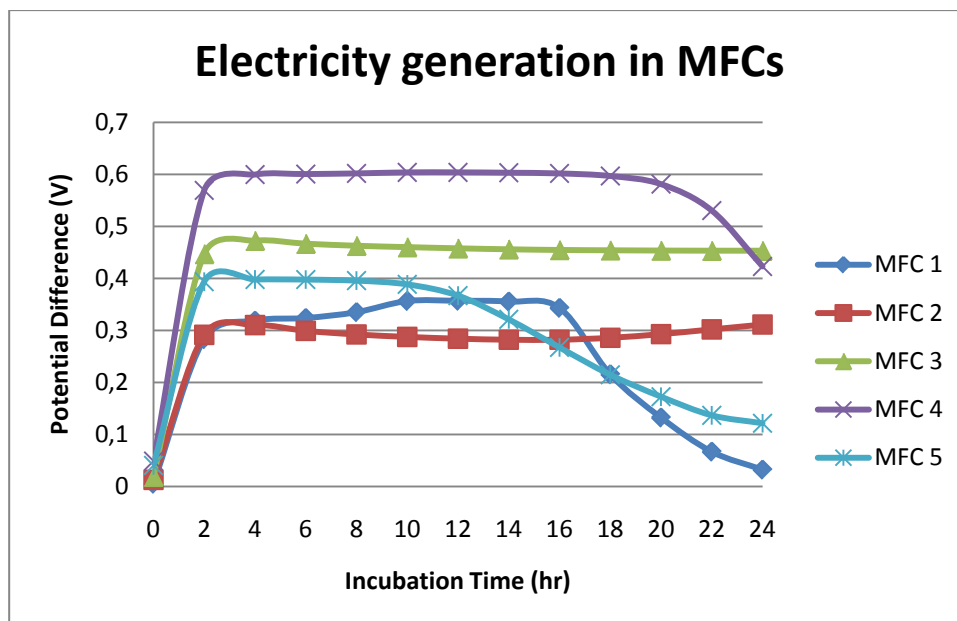


Figure 3.1 : Electricity production in the five MFCs

In terms of electricity production from a simple carbon source, MFC 4 was the most successful that could generate about 0.6 V. This voltage is one of the highest that was gained in similar type of single cell MFCs reported so far. MFC 2 generated the least electricity among all of the MFCs, with 0.3 V at maximum (Figure 3.1).

Power generation curves indicated that there was a decrease in power with respect to the depletion of carbon source. It appeared that the microbial consortia in the MFC 3 provided a more stable and efficient consumption of the carbon source, as the power generation continued without drops for 20 hours. Other cells demonstrated slower depletion of the carbon source, while providing less power.

The most successful decolorization results were obtained in MFC 1 in degradation of all the dyes tested. The performance of this cell was the best, and this can possibly be explained with the fact that this was the cell that had been working for longer time than others did, and therefore it was more stable. It was found that for some of the dyes examined, degradation efficiency and power generation was related. The level of power generation was inversely proportional to the applied dye concentration of various dyes (Figure 3.2, Figure 3.3 and Figure 3.4).

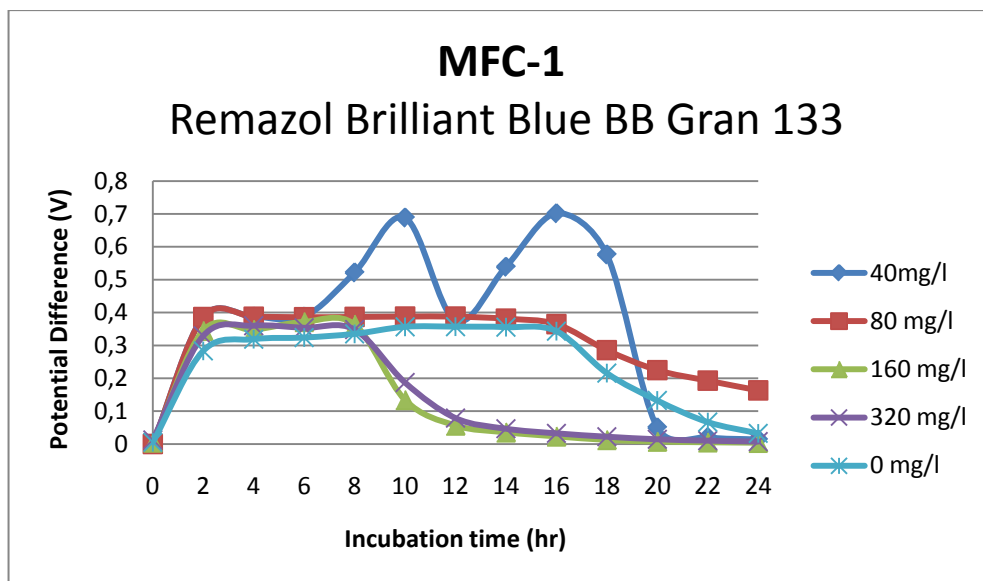


Figure 3.2 : Energy generation in MFC 1 and decolorization of Brilliant Blue

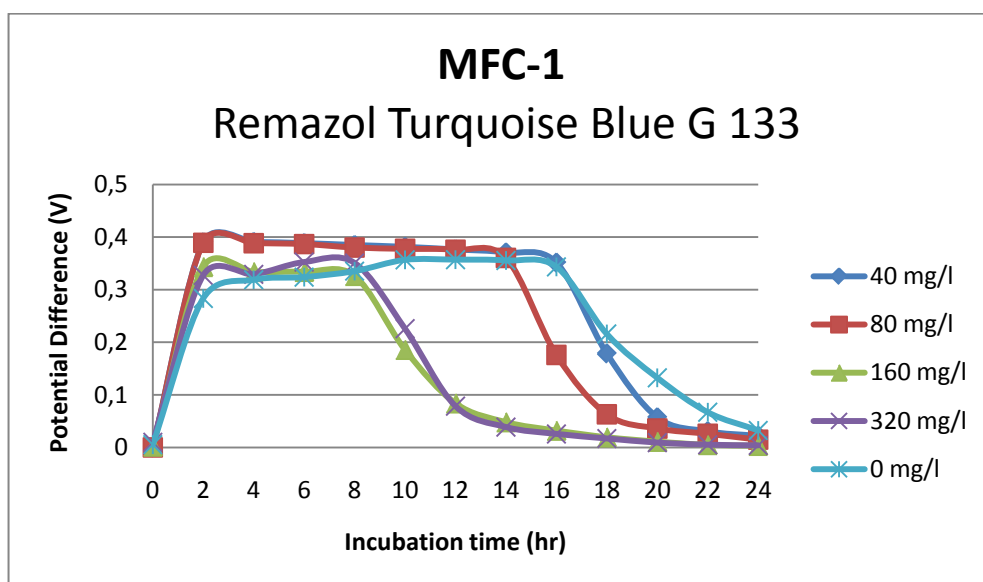


Figure 3.3 : Energy generation in MFC 1 and decolorization of Turquoise Blue

No significant decrease was detected in electricity production with the addition of higher concentrations of Reactive Yellow 145, Remazol Blue RR, Reactive Black 5 and Reactive Blue 222 (Figure 3.5, Figure 3.7, Figure 3.8 and Figure 3.9).

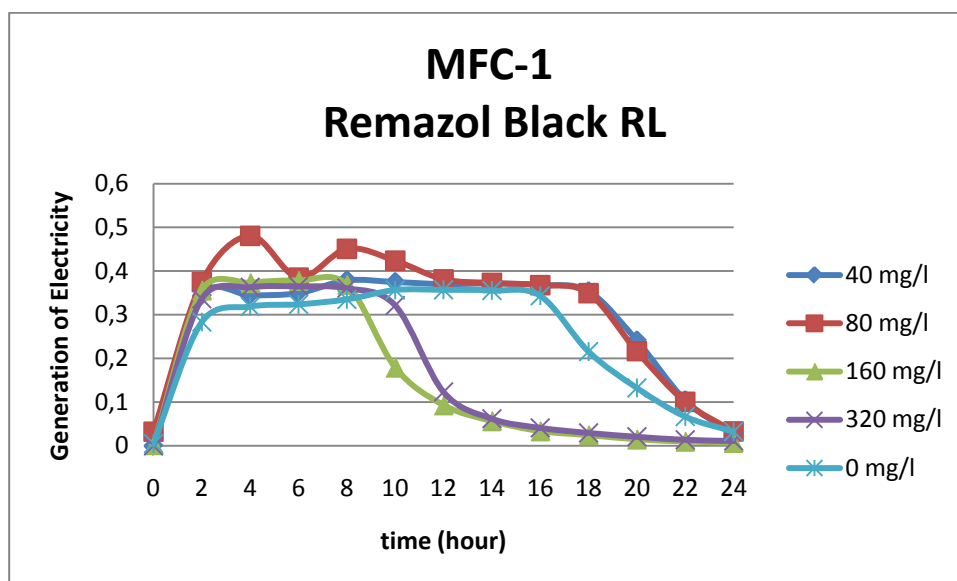


Figure 3.4 : Energy generation in MFC 1 and decolorization of Remazol Black RL

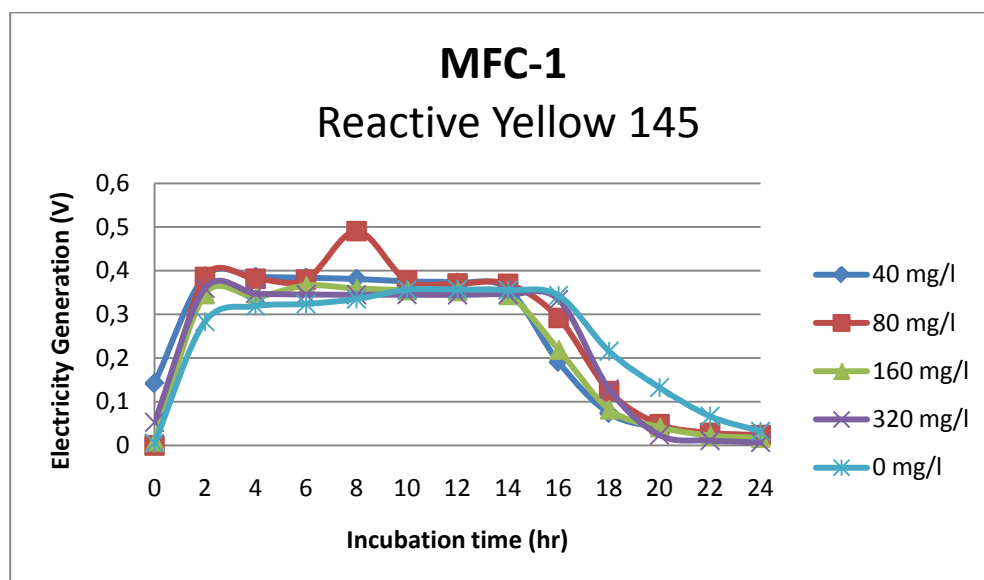


Figure 3.5 : Energy generation in MFC 1 and decolorization of Reactive Yellow 145

At the concentrations of 40 and 80 mg/L of Reactive Red 195, no power generation could be detected in MFC 1. This might probably have been caused by an experimental error (Figure 3.6).

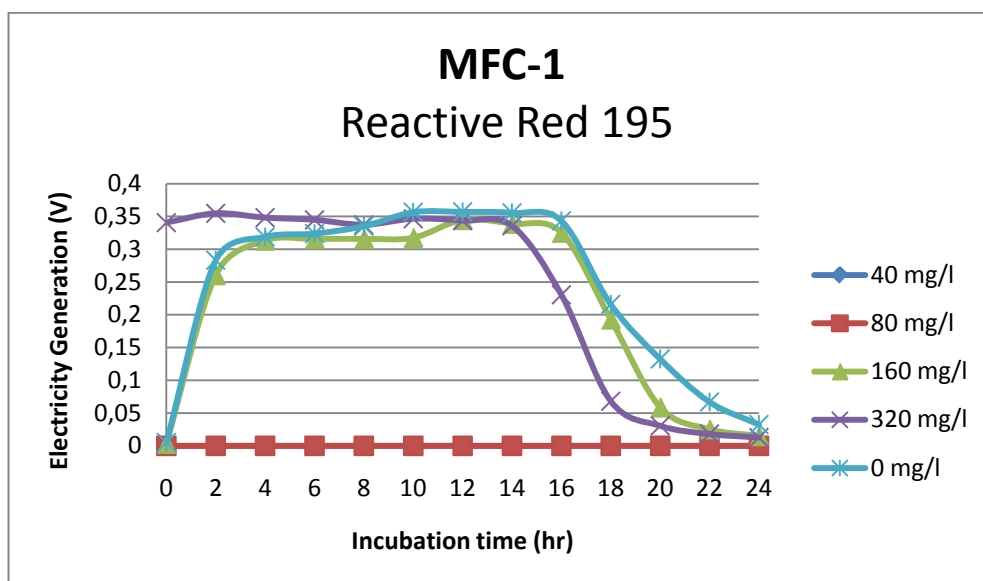


Figure 3.6 : Energy generation in MFC 1 and decolorization of Reactive Red 195

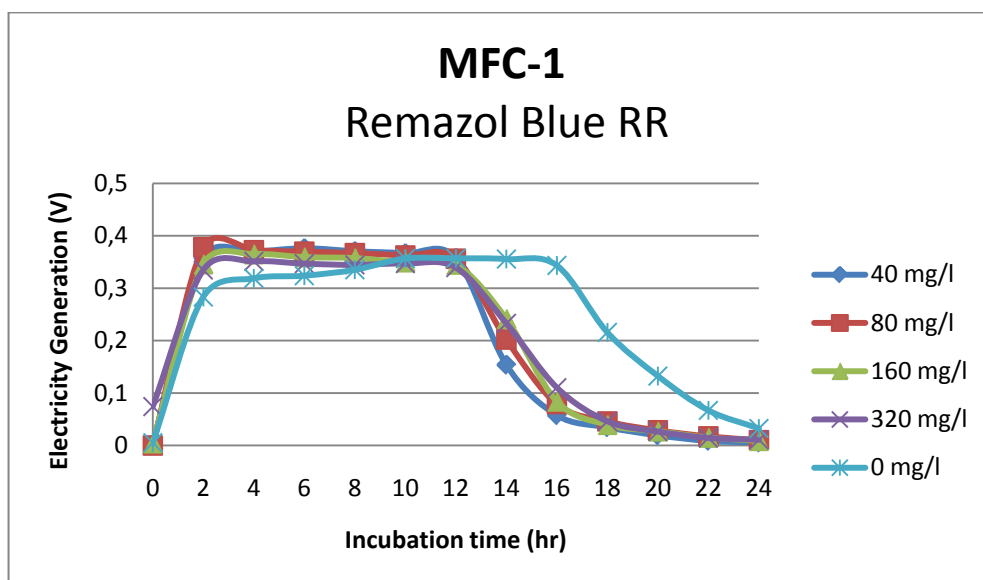


Figure 3.7 : Energy generation in MFC 1 and decolorization of Remazol Blue RR

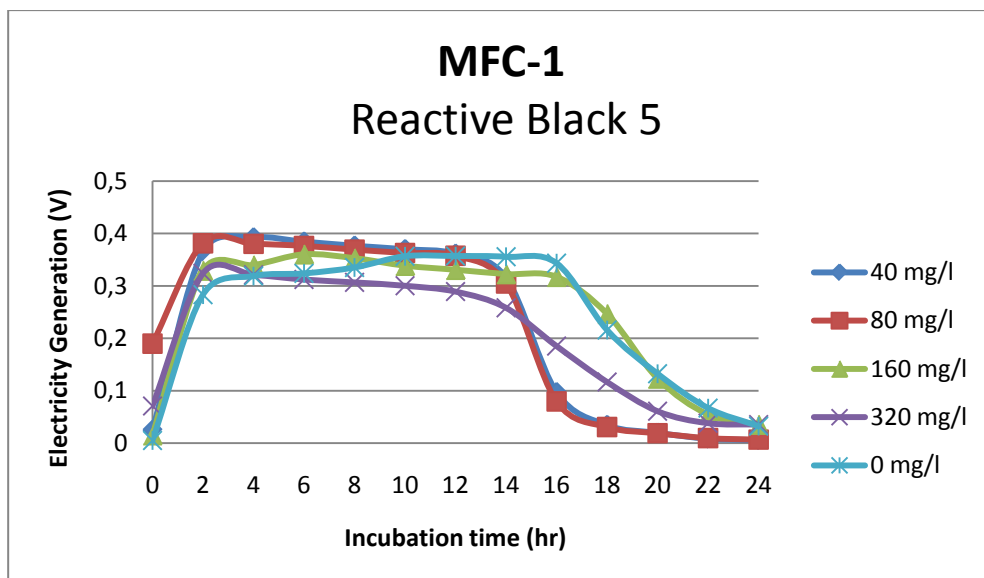


Figure 3.8 : Energy generation in MFC 1 and decolorization of Reactive Black 5

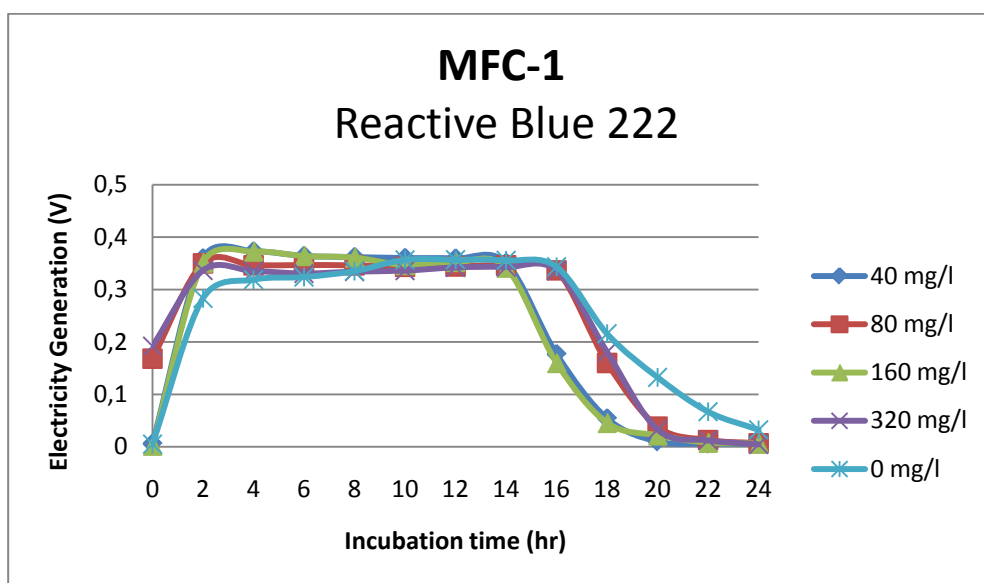


Figure 3.9 : Energy generation in MFC 1 and decolorization of Reactive Blue 222

Although MFC 2 could achieve decolorization of given textile dyes, there were no functional electricity production data that obtained throughout the experiments (Figure 3.10, Figure 3.11, Figure 3.12, Figure 3.13, Figure 3.14, Figure 3.15, Figure 3.16 and Figure 3.17).

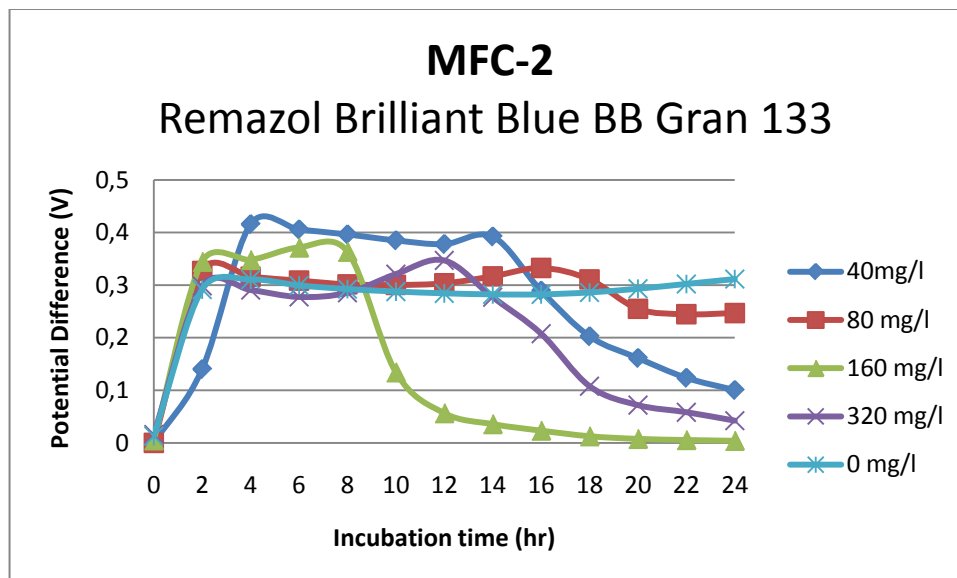


Figure 3.10 : Energy generation in MFC 2 and decolorization of Brilliant Blue

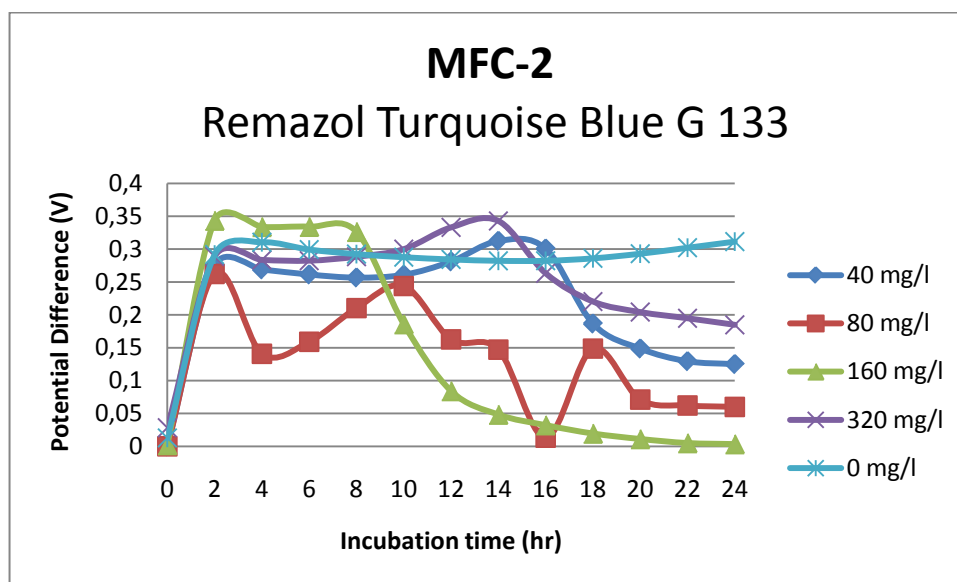


Figure 3.11 : Energy generation in MFC 2 and decolorization of Turquoise Blue

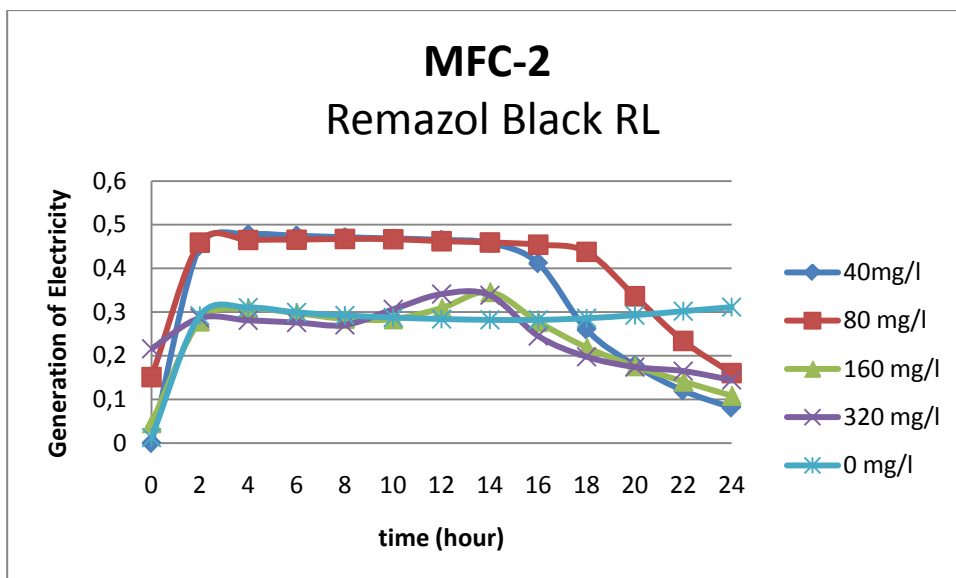


Figure 3.12 : Energy generation in MFC 2 and decolorization of Remazol Black RL

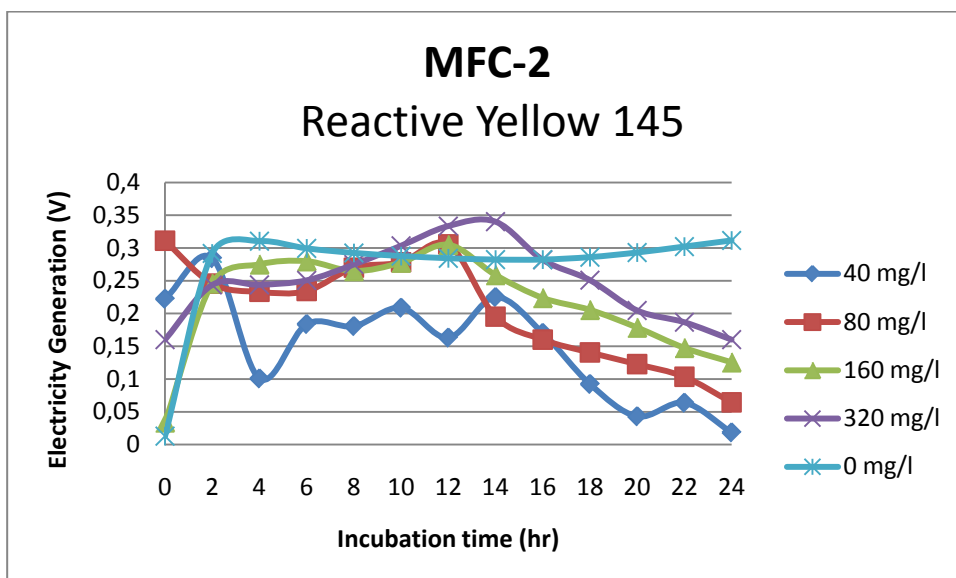


Figure 3.13 : Energy generation in MFC 2 and decolorization of Reactive Yellow 145

MFC 3 produced a high and stable power. The power generation lasted longer, and continued even after 24-hour incubation. However, with the addition of dyes, duration of power generation decreased (Figure. 3.18, Figure 3.19, Figure 3.20, Figure 3.21, Figure 3.22, Figure 3.23, Figure 3.24 and Figure 3. 25).

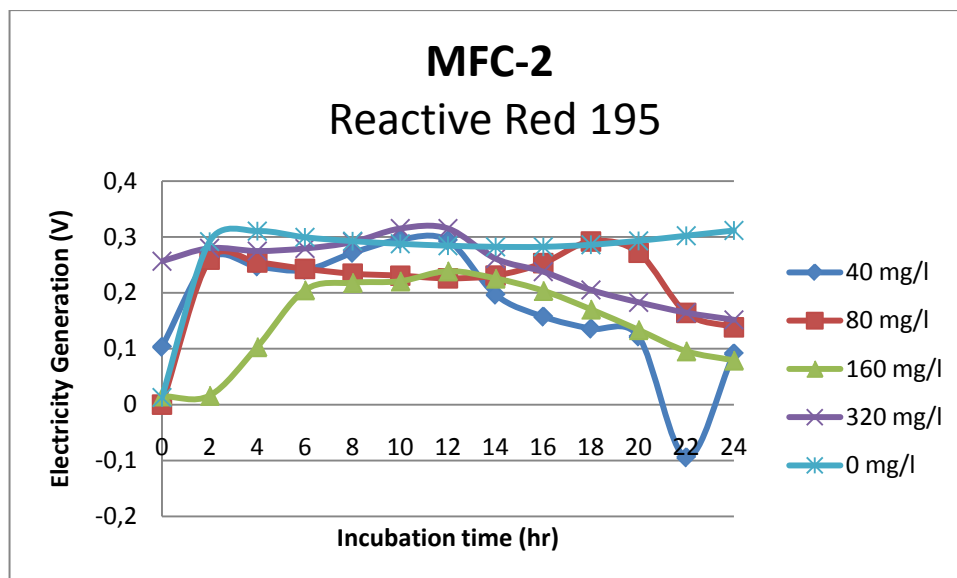


Figure 3.14 : Energy generation in MFC 2 and decolorization of Reactive Red 195

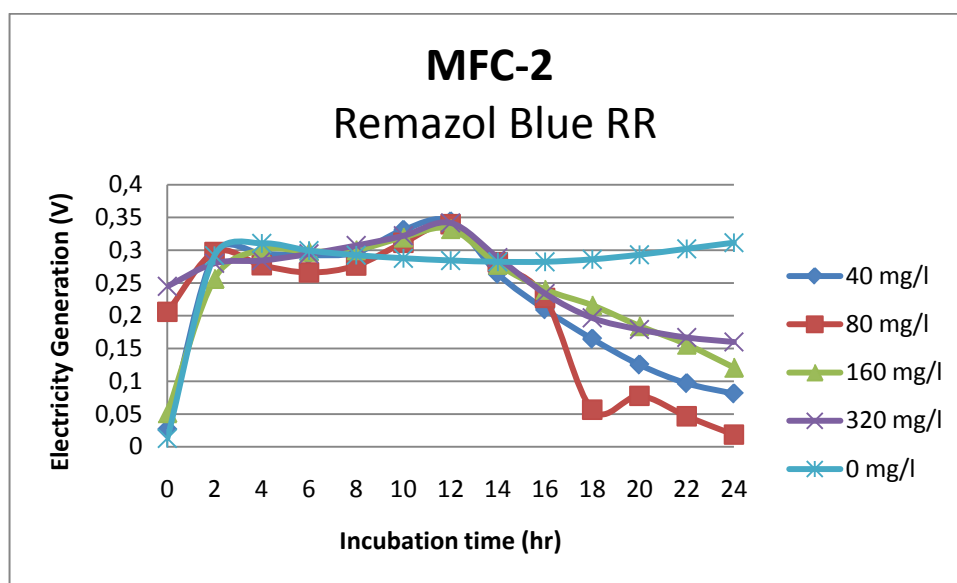


Figure 3.15 : Energy generation in MFC 2 and decolorization of Remazol Blue RR

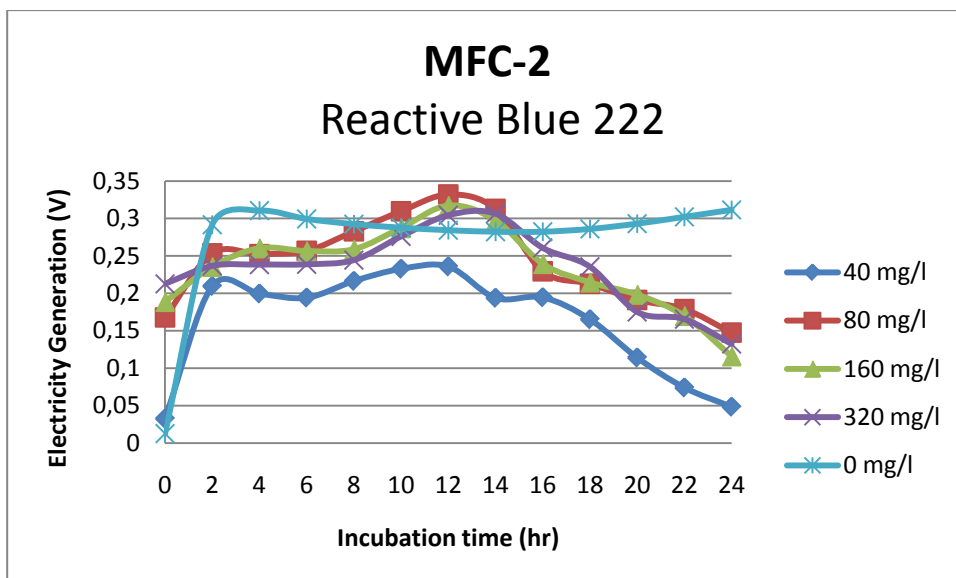


Figure 3.16 : Energy generation in MFC 2 and decolorization of Reactive Blue 222

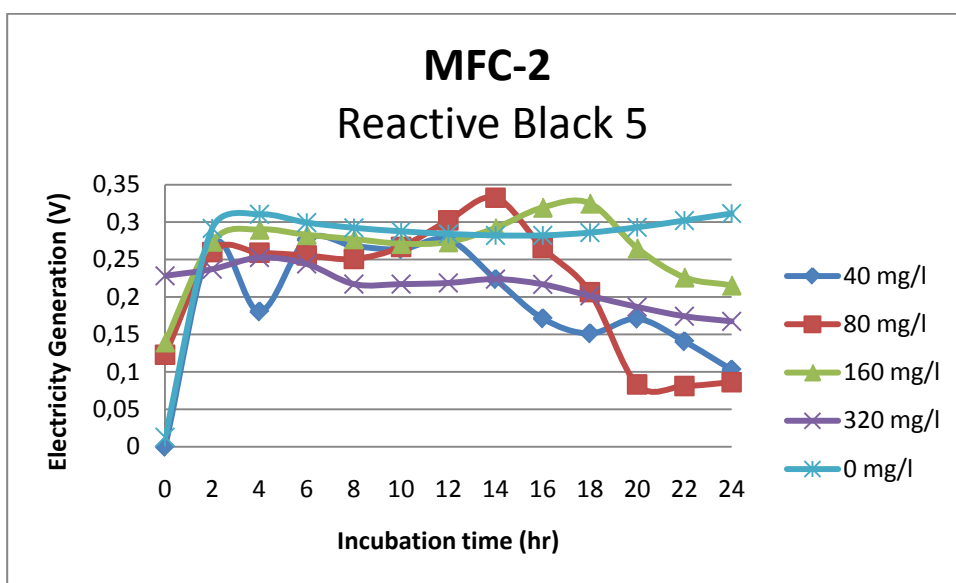


Figure 3.17 : Energy generation in MFC 2 and decolorization of Reactive Black 5

MFC 3 had the most stable electricity production curve with/without addition of dyes.

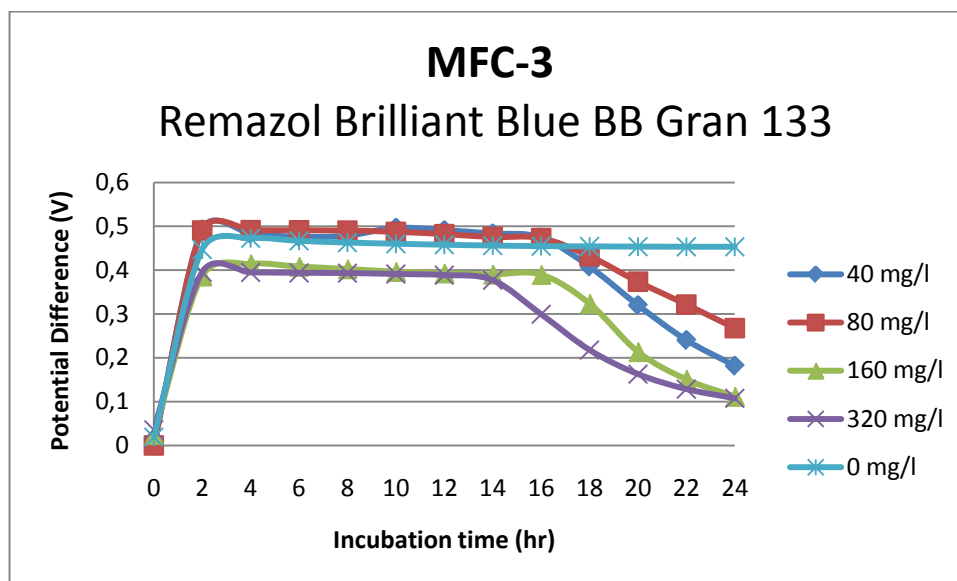


Figure 3.18 : Energy generation in MFC 3 and decolorization of Brilliant Blue

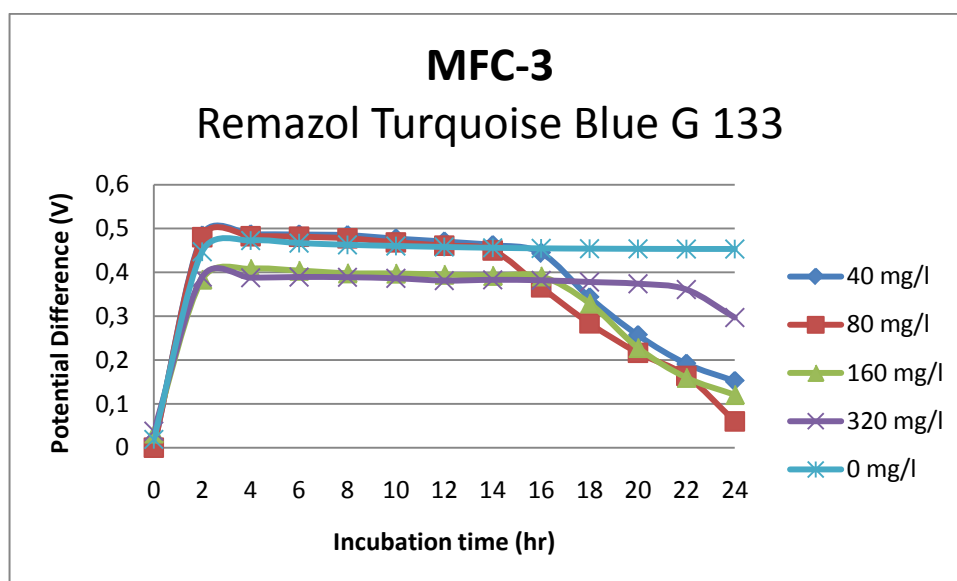


Figure 3.19 : Energy generation in MFC 3 and decolorization of Turquoise Blue

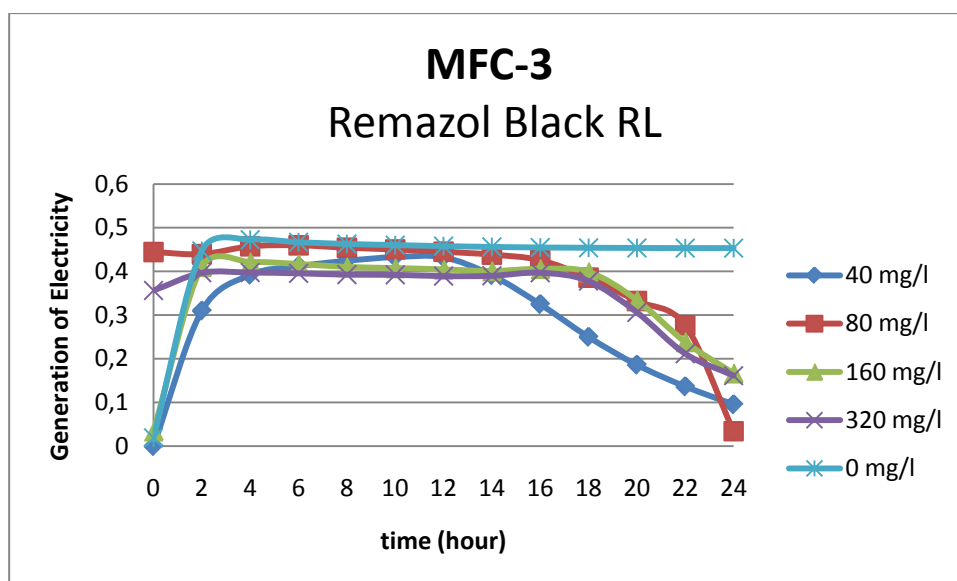


Figure 3.20 : Energy generation in MFC 3 and decolorization of Remazol Black RL

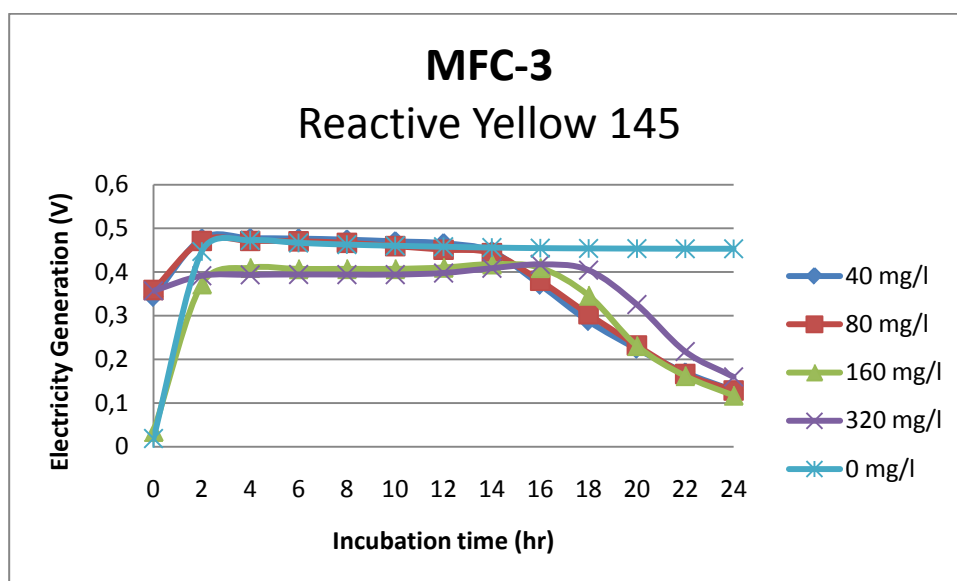


Figure 3.21 : Energy generation in MFC 3 and decolorization of Reactive Yellow 145

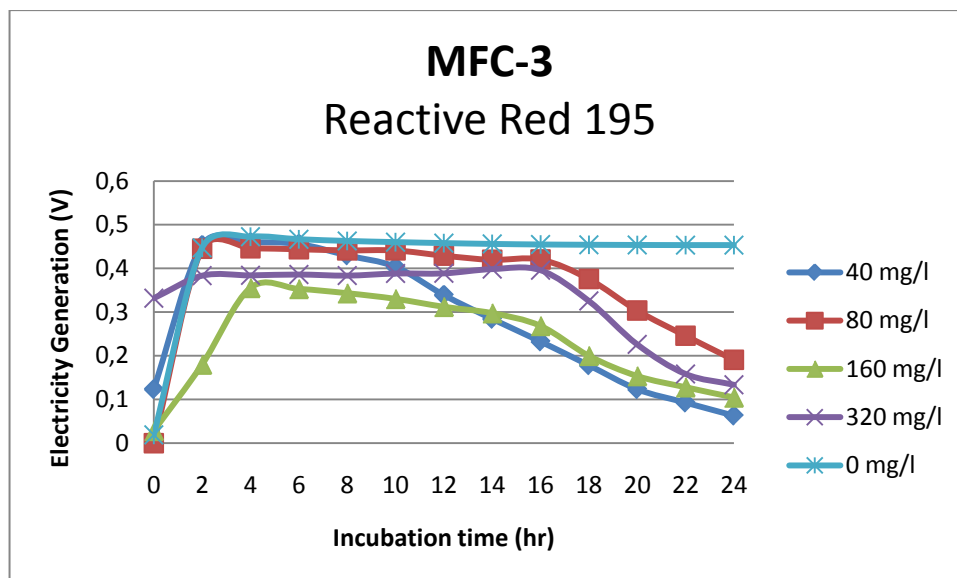


Figure 3.22 : Energy generation in MFC 3 and decolorization of Reactive Red 195

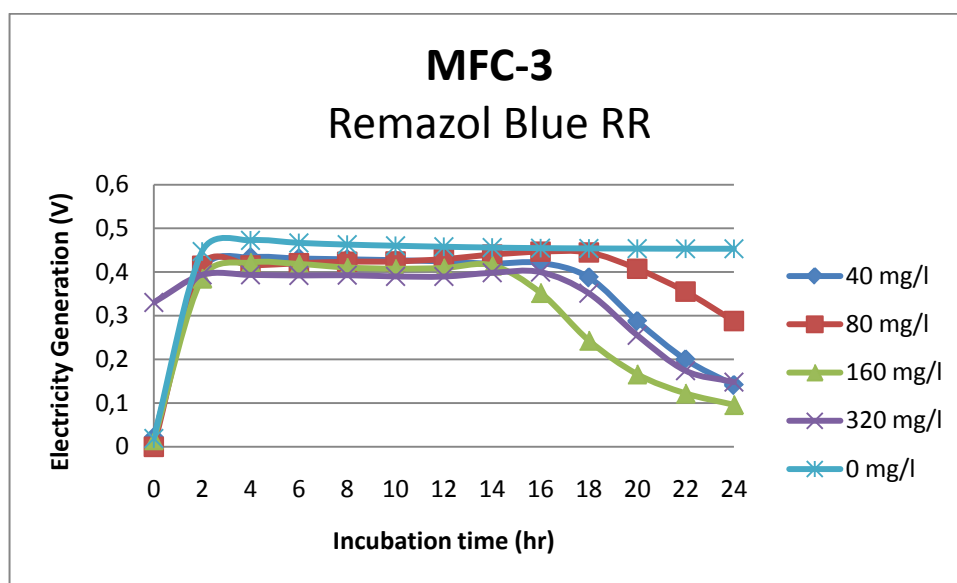


Figure 3.23 : Energy generation in MFC 3 and decolorization of Remazol Blue RR

For MFC 1, MFC 2, MFC 4 and MFC 5, electricity production curves began decreasing at the 12-16th hour, while this decrease began after the 16th hour in MFC 3 during the decolorization experiments. However, decrease in electricity production was independent from addition of high concentrations of textile dyes.

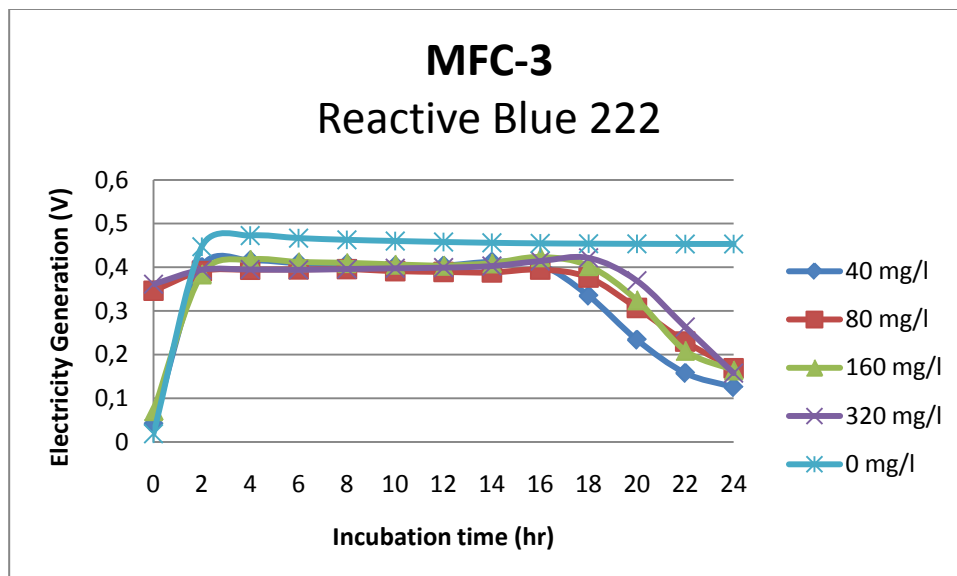


Figure 3.24 : Energy generation in MFC 3 and decolorization of Reactive Blue 222

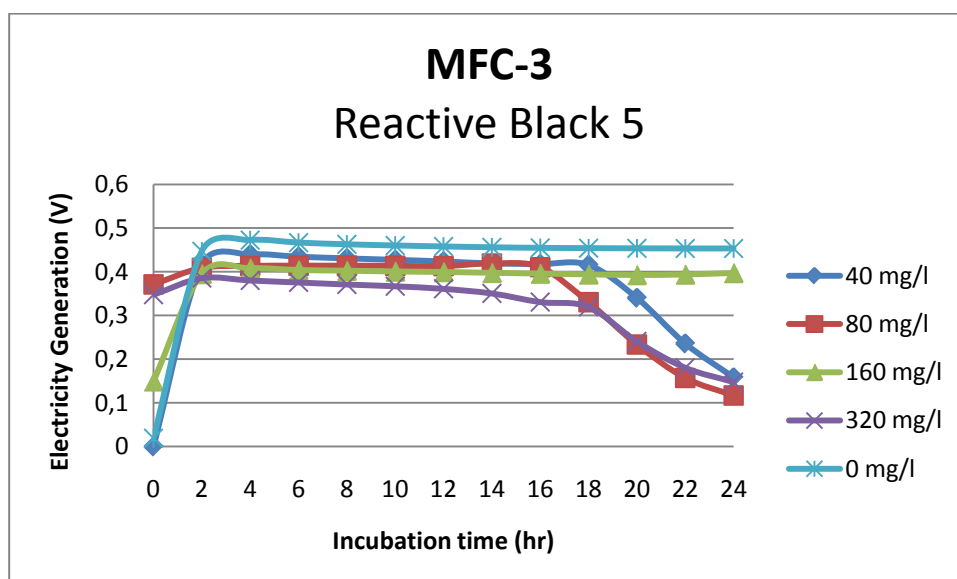


Figure 3.25 : Energy generation in MFC 3 and decolorization of Reactive Black 5

The highest voltage production was monitored in MFC 4 regardless of dye addition. The decrease in power generation was earlier and more pronounced in the presence of the dyes. (Figure 3.26, Figure 3.27, Figure 3.28, Figure 3.28, Figure 3.29, Figure 3.30, Figure 3.31, Figure 3.32 and Figure 3.33).

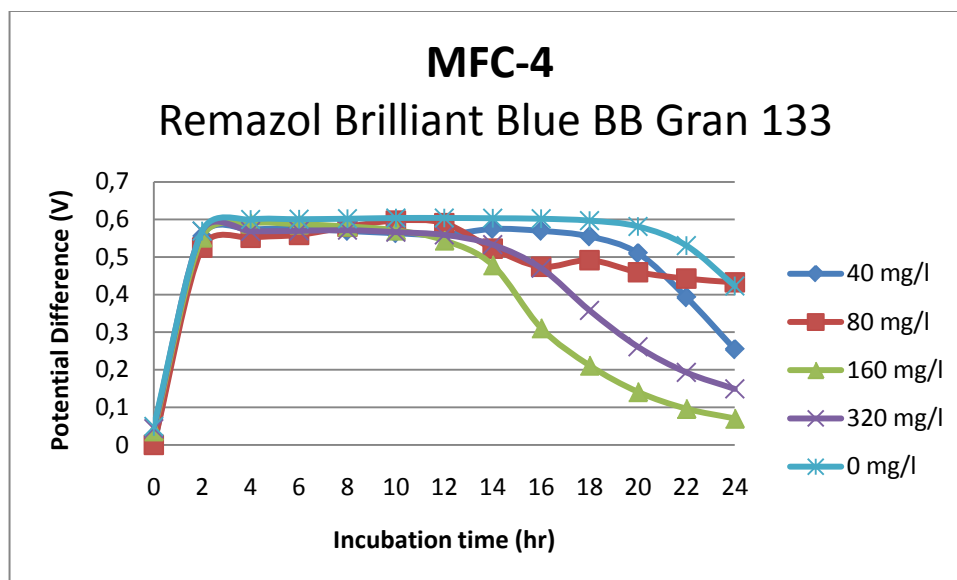


Figure 3.26 : Energy generation in MFC 4 and decolorization of Brilliant Blue

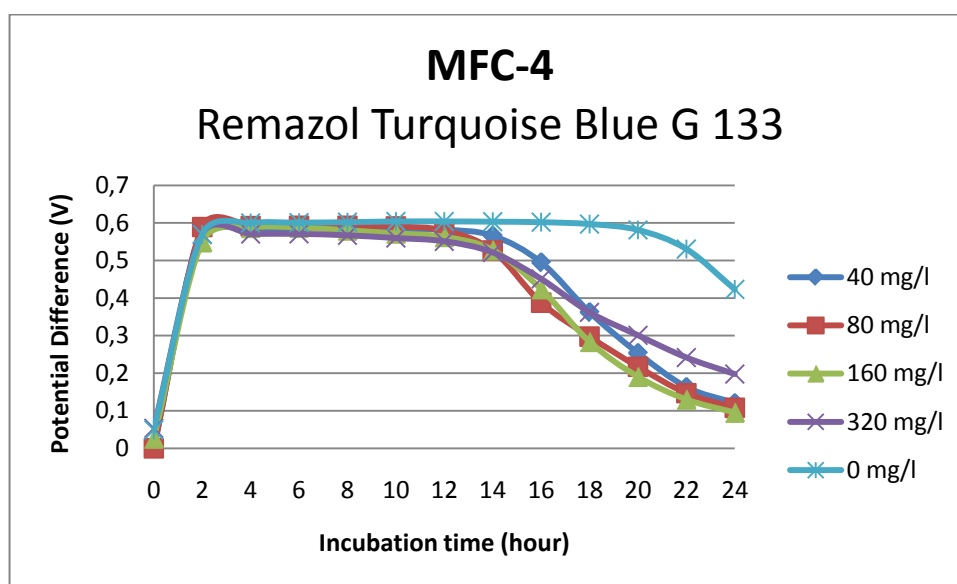


Figure 3.27 : Energy generation in MFC 4 and decolorization of Turquoise Blue

Being exposed to the higher concentrations of textile dyes, decrease in electricity production became more distinct. This phenomenon was seen in every set of dyes except Remazol Black RL. Low concentrations of this dye caused severe decrease in electricity generation while high concentrations made a stimulating effect and caused a delay in decrease (Figure 3.28).

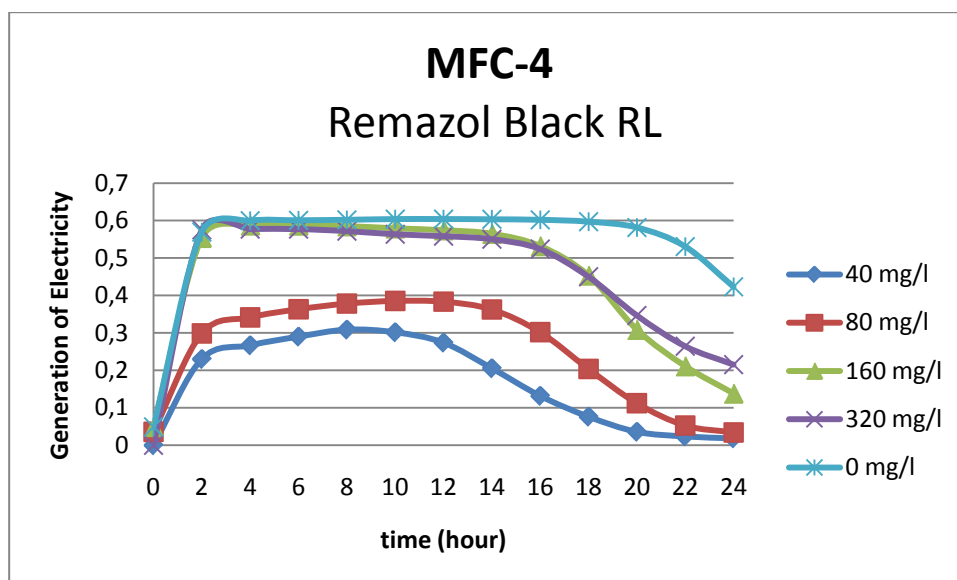


Figure 3.28 : Energy generation in MFC 4 and decolorization of Remazol Black RL

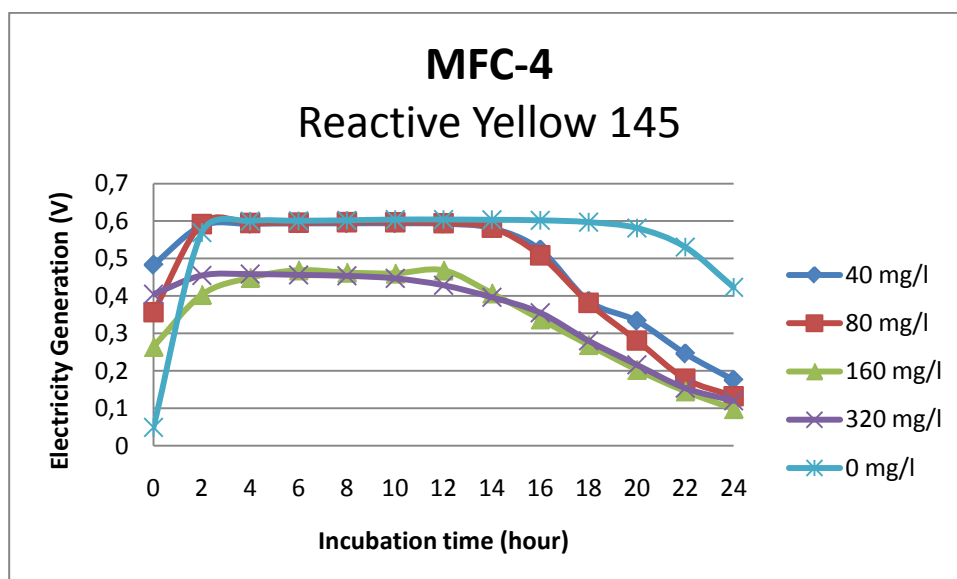


Figure 3.29 : Energy generation in MFC 4 and decolorization of Reactive Yellow 145

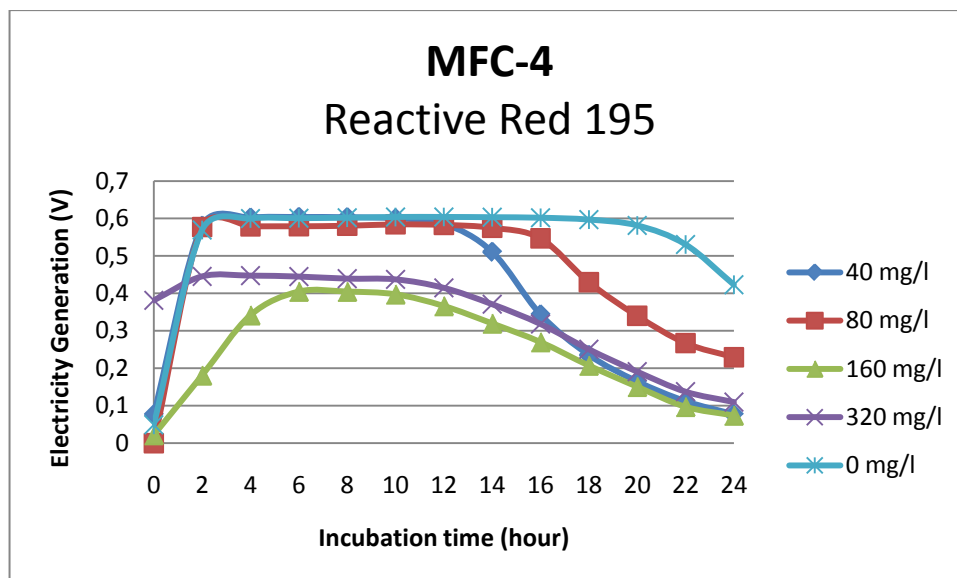


Figure 3.30 : Energy generation in MFC 4 and decolorization of Reactive Red 195

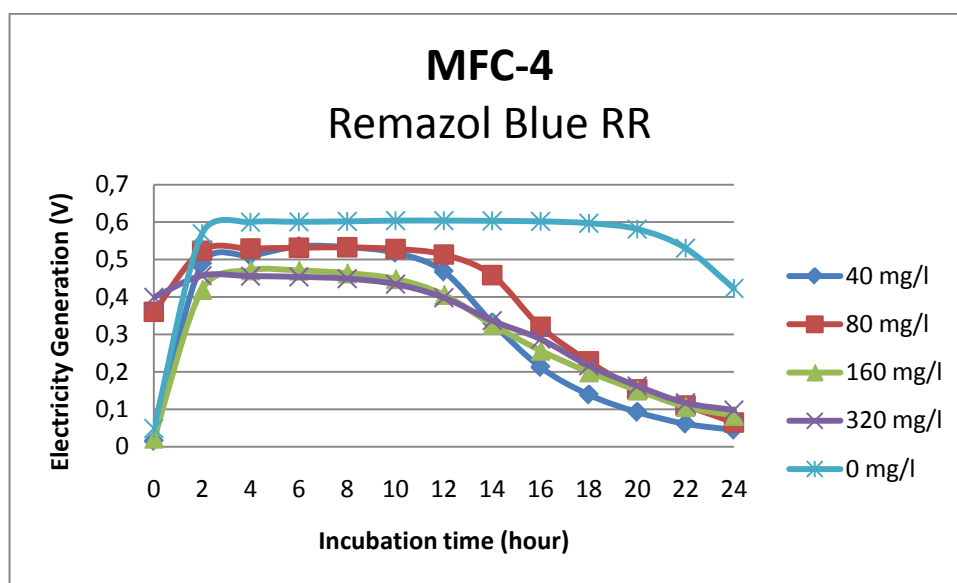


Figure 3.31 : Energy generation in MFC 4 and decolorization of Remazol Blue RR

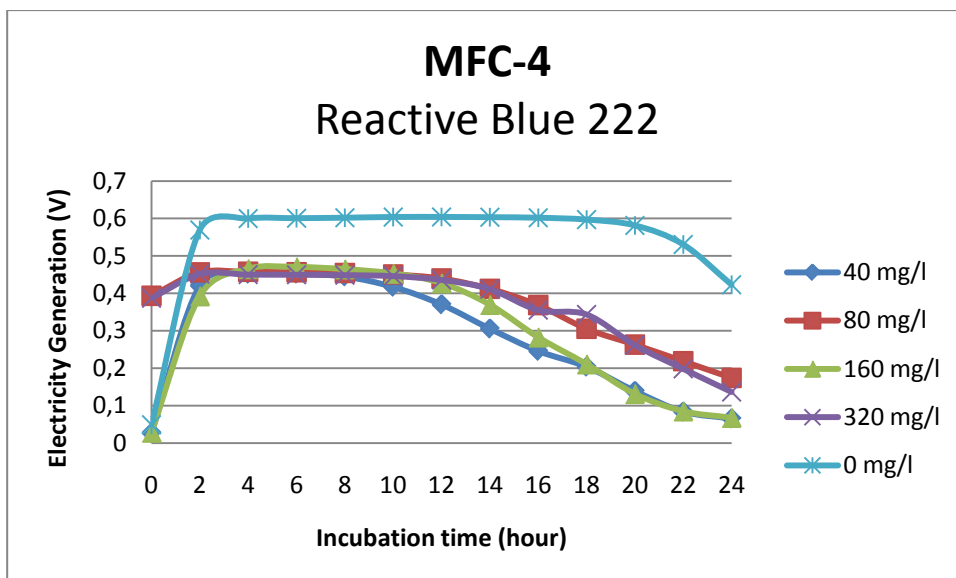


Figure 3.32 : Energy generation in MFC 4 and decolorization of Reactive Blue 222

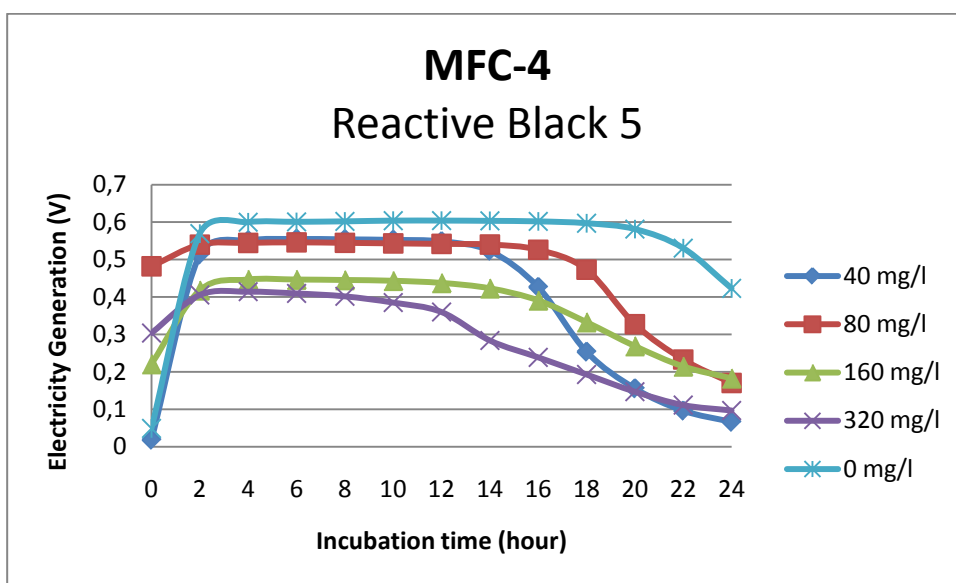


Figure 3.33 : Energy generation in MFC 4 and decolorization of Reactive Black 5

The lowest decolorization efficiency was observed in MFC 5. In this reactor, electricity generation in the absence of dye was lower than that in the presence of 40 and 80 mg/L dye, while it was higher than that in the presence of 160 and 320 mg/L of dye (Figure 3.34, Figure 3.35, Figure 3.36, Figure 3.38 and Figure 3.39).

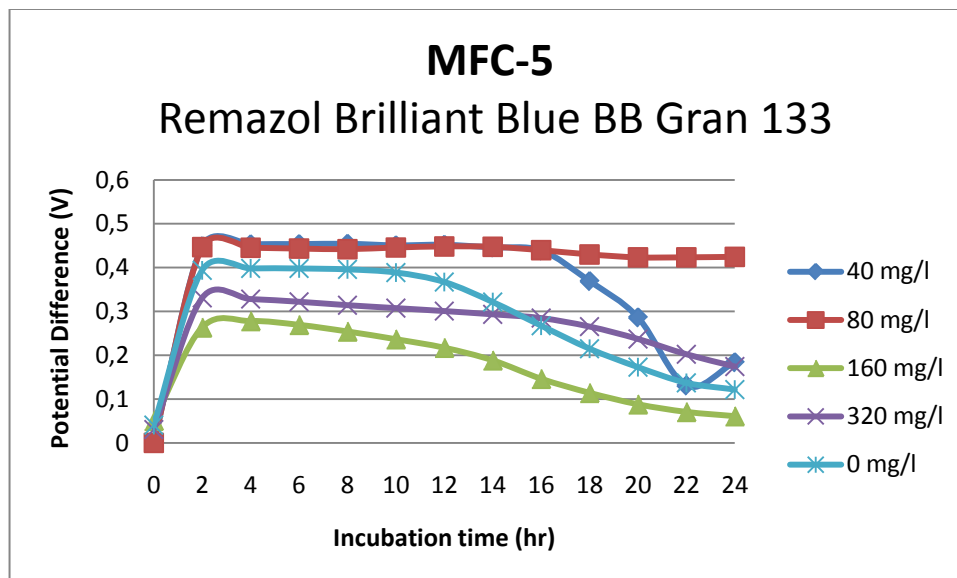


Figure 3.34 : Energy generation in MFC 5 and decolorization of Brilliant Blue

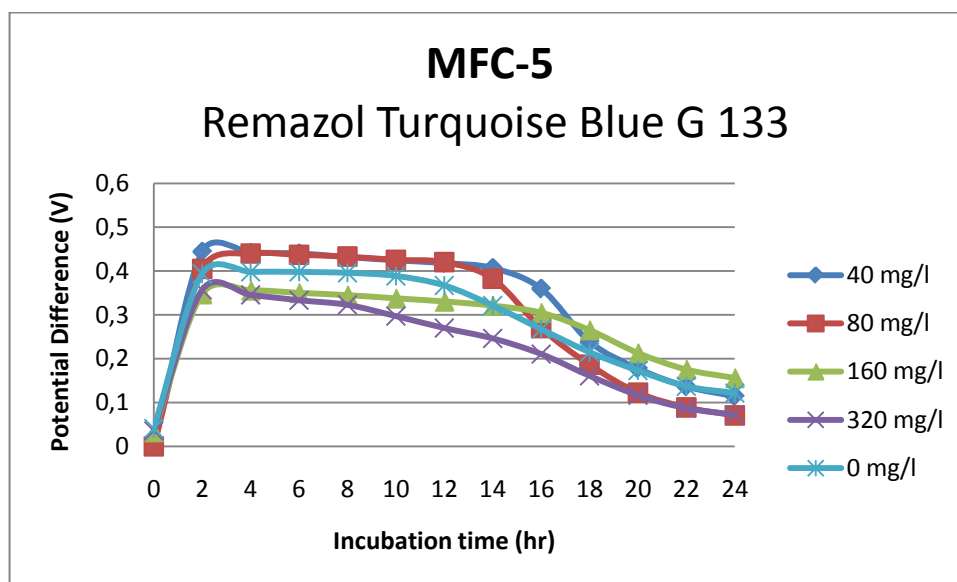


Figure 3.35 : Energy generation in MFC 5 and decolorization of Turquoise Blue

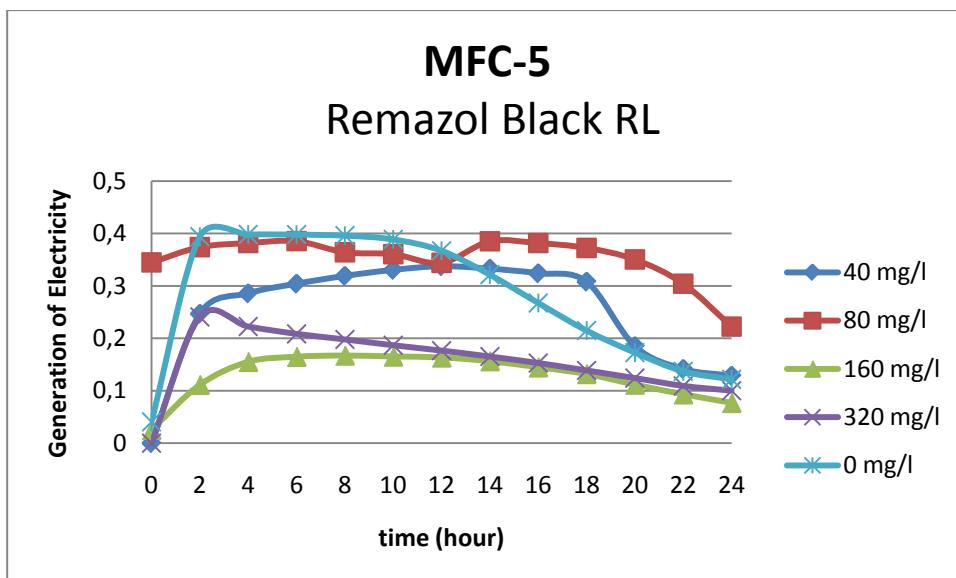


Figure 3.36 : Energy generation in MFC 5 and decolorization of Remazol Black RL

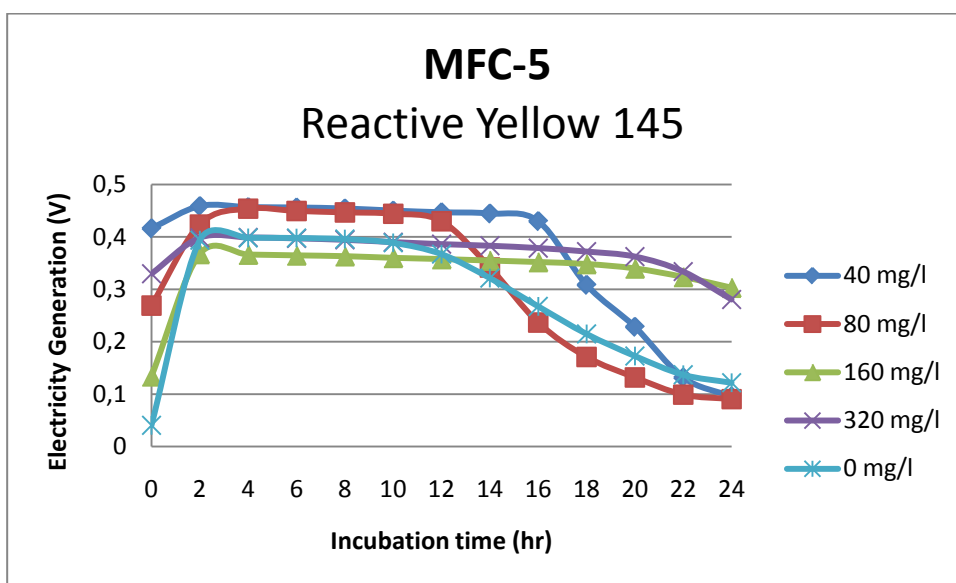


Figure 3.37 : Energy generation in MFC 5 and decolorization of Reactive Yellow 145

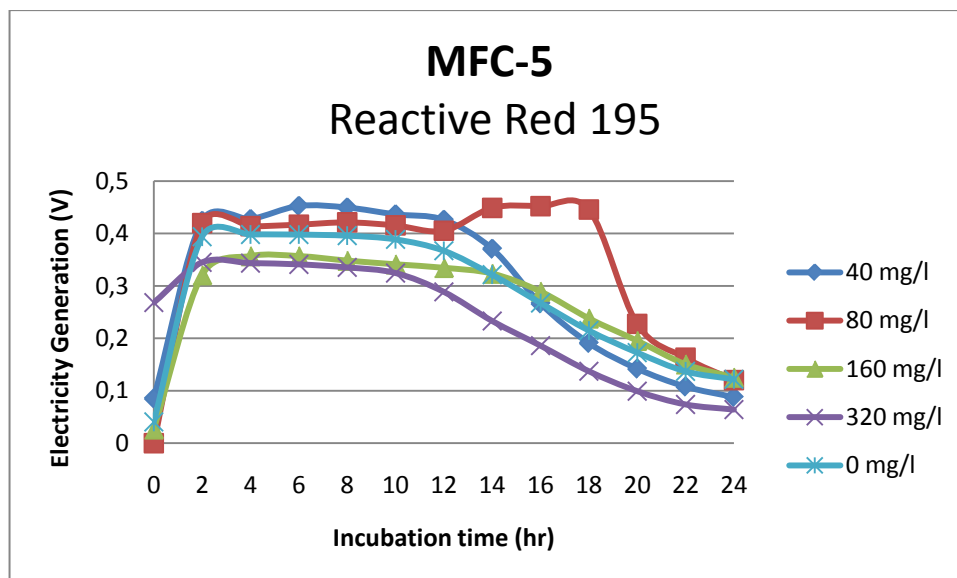


Figure 3.38 : Energy generation in MFC 5 and decolorization of Reactive Red 195

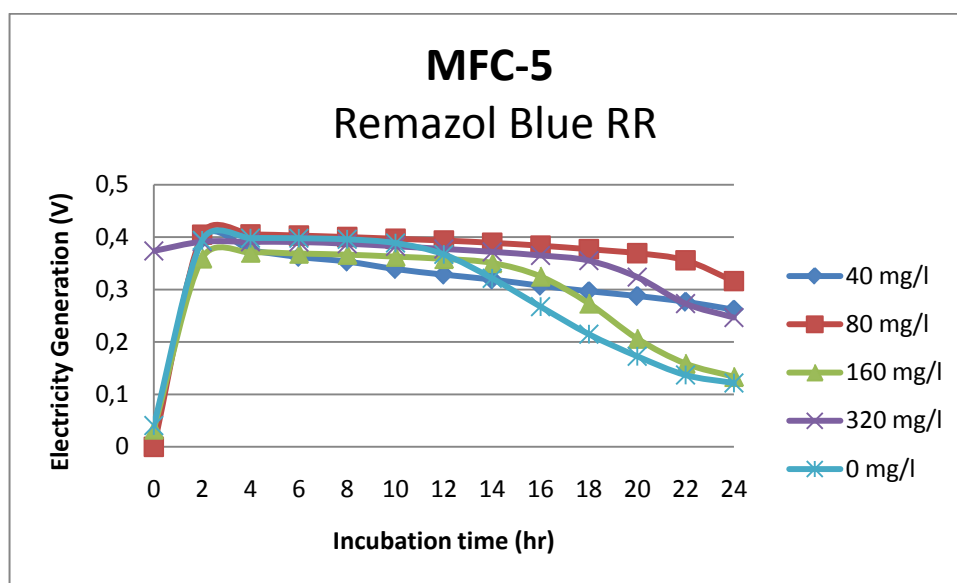


Figure 3.39 : Energy generation in MFC 5 and decolorization of Remazol Blue RR

Different from other MFCs, sometimes, electricity production was induced with the addition of dyes especially in the low concentrations. According to the type of the dye, induction in electricity generation became more distinct (Figure 3.34 and Figure 3.36) or remained very close (Figure 3.39 and Figure 3.40).

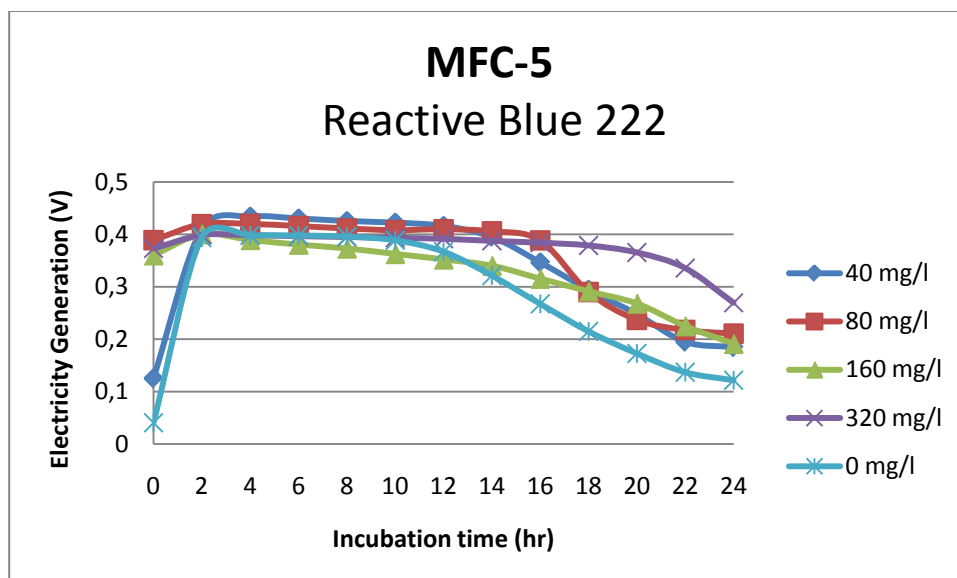


Figure 3.40 : Energy generation in MFC 5 and decolorization of Reactive Blue 222

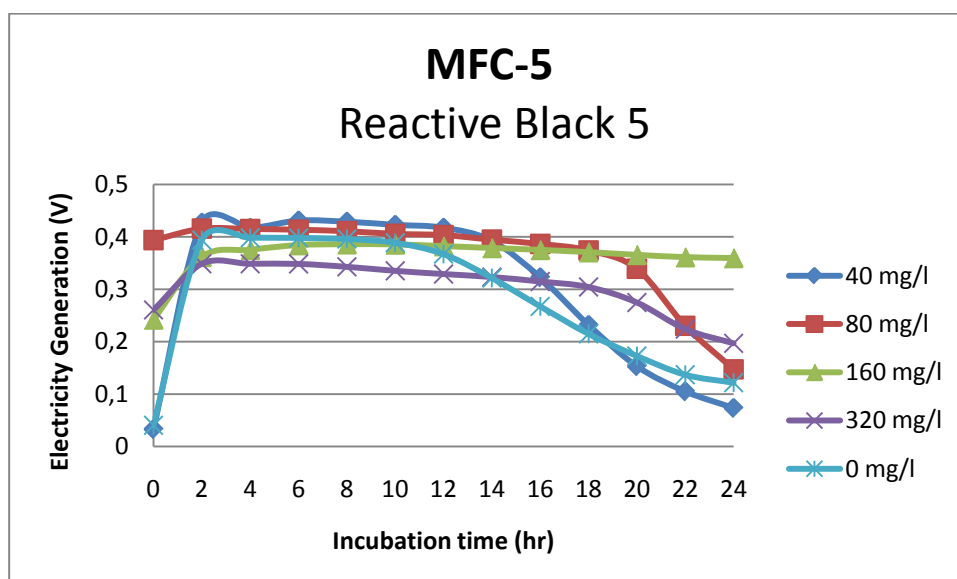


Figure 3.41 : Energy generation in MFC 5 and decolorization of Reactive Black 5

In general, while the dye addition created somehow inhibitory effect in electricity generation, however, in some of the reactors, some increase in voltages were monitored in the presence of low dye concentrations such as 40 or 80 mg/L. In the

current study, reason of this phenomenon was not investigated; yet, this might be attributed to the capability of some dyes to act as electron shuttling mediators. This hypothesis may also offer a solution to the question of why the electricity generation lasts shorter. It might be possible that while the dyes help electron transfer, they also stimulate the cell growth, consequently, which increases the oxidation rate of the carbon source as were stated previously (Chen, 2006; Chen *et al.*, 2009; Chen *et al.*, 2010).

According to the current results, it is not yet possible to determine whether or not the dyes themselves were used as energy sources in the MFCs. This is also the subject of further studies. Ideally, if the waste material, i.e., the dyes in our case, can also be consumed in benefit of the organism, the applicability of MFC technology could be much greater.

Although the maximum power production remained similar throughout the decolorization assays, higher concentrations of dyes inhibited the power generation, and caused significant drops in shorter time periods. These results are also similar to the previous studies with decolorization of a textile dye with MFCs [Sun, J. *et al.*, 2009, Cao *et al.*, 2009]. Yet, with an appropriate dilution, the negative effect of the dyes on their own bioremediation and electricity generation may be neglected, when designing an industrial setup. The opposite can also be postulated from the results of some of our experiments. As could be seen with some dyes, 320 mg/L dye application resulted in higher power generation profiles than did 160 mg/L. This could be due to the better adaptation/acclimatization of the bacterial community to certain dyes, following the consequent dye addition sessions.

3.3 Repetitive Dye Application Studies

In an industrial application, one of the most important concerns is the continuity of the process. Therefore, we have applied repetitive batch dye application studies in order to monitor the changes in both electricity production and decolorization rates (Figure 3.12, 3.13, 3.14, 3.15).

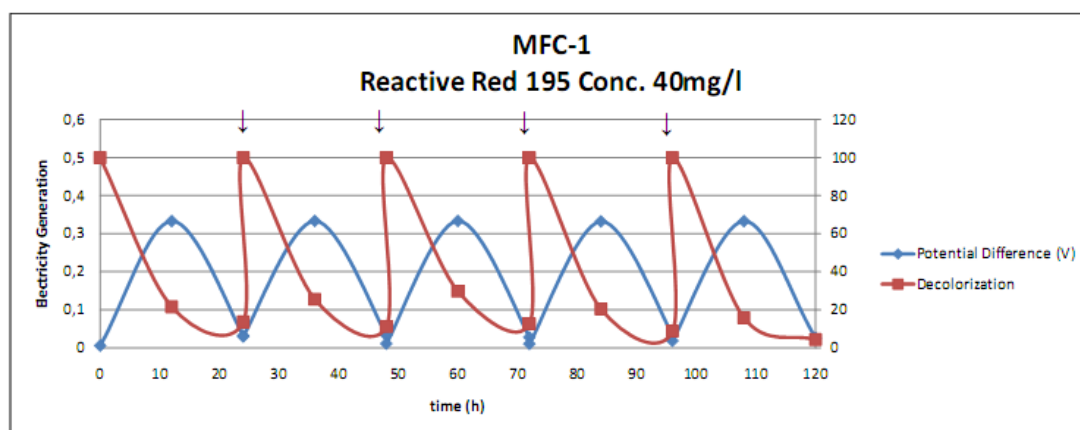


Figure 3.42 : Electricity Generation of MFC 1 and Decolorization of Reactive Red 195 during Repetitive Dye Addition Experiment

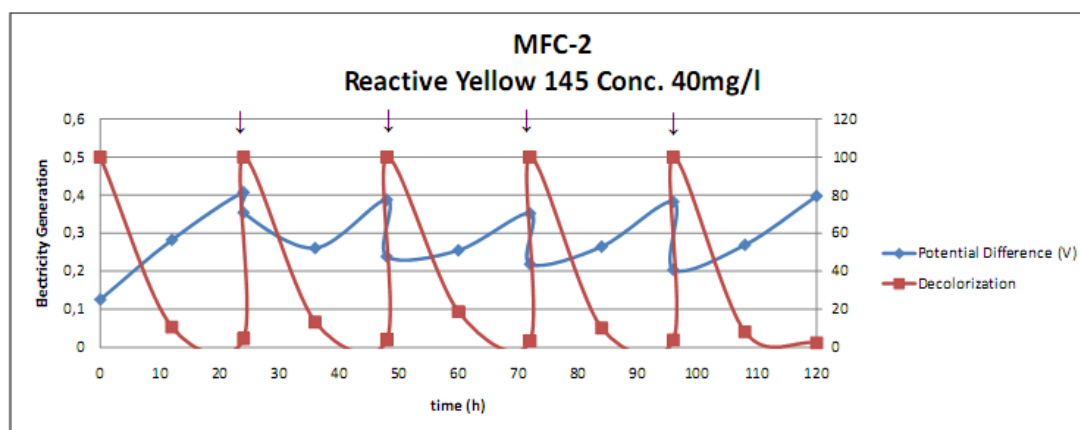


Figure 3.43 : Electricity Generation of MFC 2 and Decolorization of Reactive Yellow 145 during Repetitive Dye Addition Experiment

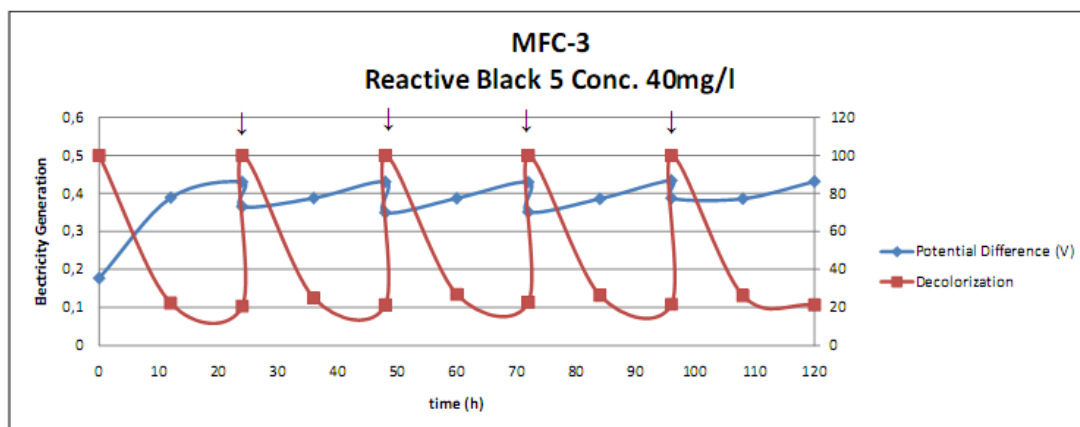


Figure 3.44 : Electricity Generation of MFC 3 and Decolorization of Reactive Black 5 during Repetitive Dye Addition Experiment

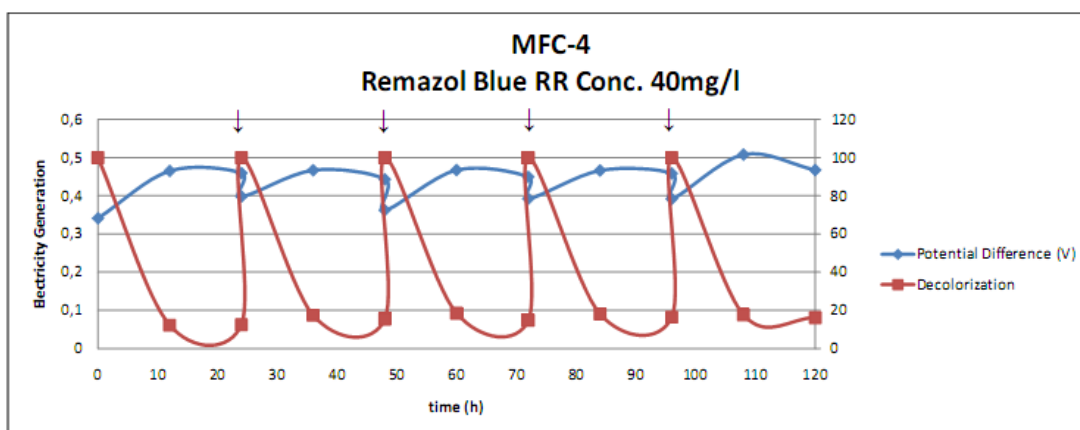


Figure 3.45 : Electricity Generation of MFC 4 and Decolorization of Remazol Blue RR during Repetitive Dye Addition Experiment

Throughout the experiment, dyes were added to MFCs in the concentration of 40 mg/L in a batch mode and all of these were decolorized to a certain extent. As seen in experiments with Remazol Blue RR, there were no significant changes in overall electricity generation and decolorization rates for five days. Repetitive dye addition of the low concentrations (40 mg/L) of different dyes did not effect the performances of different MFCs. Besides, in following batches, no decrease in decolorization performance or electricity production was observed. Higher concentrations of dyes must be investigated in a similar manner, to observe possible changes in dye tolerance of the bacteria in a repetitive batch application.

4. CONCLUSION AND FUTURE STUDIES

To the knowledge, this is the first comprehensive study of biodegradation of various textile dyes in air-cathode single chamber MFCs. Electricity production in the presence of a readily available carbon source and the dyes were evaluated. Previously, dye decolorization using MFCs containing pure bacterial cultures were studied, yet, dye decolorization for a single dye was studied in MFCs inoculated with mixed cultures. It is therefore very important to evaluate the capabilities of MFCs in decolorization of various dyes. Dyes are mostly recalcitrant and highly resistant against biodegradation. Mixed-culture containing MFCs with their structure and micro-ecosystem, may provide both aerobic and anaerobic degradation environments for various chemical and biochemical compounds. Besides, the cooperative action of various microbes might enable recalcitrant compounds to be degraded more easily. These mixed cultures have a dynamic structure which can easily adapt to the changing environment, in this case, to the addition of different textile dyes.

In such a bioreactor setup, decolorization of varying concentrations of eight azo dyes (Remazol Brilliant Blue BB G 133, Remazol Black RL, Remazol Turquoise Blue G 133, Reactive Yellow 145, Reactive Red 195, Reactive Black 5, Reactive Blue 222 and Remazol Blue RR) was achieved in air-cathode single chamber MFCs. Each dye exhibited different levels of resistance against decolorization, however, even the dyes known as most recalcitrant were degraded to a certain extent. In addition to that, decolorization and electricity generation performances of MFC remained intact during repetitive dye addition studies. These results indicate a stable microbial consortia in MFCs which was not effected by being exposed to continuous dye addition. The nature of degradation/decolorization has not been studied; however, it is clear that the azo bond is destroyed, since color removal was clearly monitored by means of spectrophotometry. Optimization studies for color removal should be undertaken in order to increase dye removal efficiency.

This study clearly demonstrated for the first time that the same microbial consortium can possibly be utilized for treatment of various textile dye effluents, and electricity

generation, although somehow decreased, can still be a simultaneous process along with the decolorization. Further work must focus on: 1) the mechanism of decolorization, 2) optimization of conditions and 3) studies with real effluents instead of pure dye solutions.

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6. APPENDICES

APPENDIX A : Laboratory Equipment

APPENDIX B : Chemicals

APPENDIX C : Culture Medium

APPENDIX A

Laboratory Equipment

Balance	Presica BJ 610 C
Computer	QUAKE
Magnetic Stirrer	Heidolph MR-Hei Standart
Microfuge	Sigma 1-14
Microplate	Santa Cruz
Microplate Reader	Benchmark Plus, Bio-Rad
Multimeter	KEITHLEY 2700
pH meter	HANNA Instruments Combo
Pipettes	Eppendorf 10 ml, 1ml, 300 µl
Safety Thermostat	Nüve EN 400
UPS	GERMEN

APPENDIX B

Chemicals

Ammonium Chloride	CARLO ERBA Reagents
Na₂HPO₄·2H₂O	Riedel-de Haën
NaH₂PO₄·2H₂O	Riedel-de Haën
Potassium Chloride	CARLO ERBA Reagents
Reactive Black 5	Setaş Kimya
Reactive Red 195	Setaş Kimya
Reactive Yellow 145	Setaş Kimya
Remazol Black RL	Dystar
Remazol Blue RR	Dystar
Remazol Brilliant Blue BB Gran 133 %	Dystar
Remazol Turquoise Blue G 133	Dystar
Reactive Blue 222	Setaş Kimya
Sodium Acetate Anhydrous	Fluka

APPENDIX C

Culture medium

Table C.1 : MFC medium (pH 7.0)

	g/L
Sodium acetate	1 g or 20 mM
NH₄Cl	0.31
KCl	0.13
NaH₂PO₄·H₂O	5.84
Na₂HPO₄·7H₂O	15.47
minerals solution	12.5ml
vitamins solution	1.25 for concentrated solution (x10)
Distilled Water	Up to 1 l

Table C.2 : Minerals and vitamins solutions

Vitamins (mg/l)		Minerals (g/l)	
biotin	2.0	NTA	1.5
folic acid	2.0	MgSO₄	3.0
pyridoxine HCl	10.0	MnSO₄•H₂O	0.5
riboflavin	5.0	NaCl	1.0
thiamin	5.0	FeSO₄•7H₂O	0.1
nicotinic acid	5.0	CaCl₂•2H₂O	0.1
pantothenic acid	5.0	CoCl₂•6H₂O	0.1
B-12	0.1	ZnCl₂	0.13
p-aminobenzoic acid	5.0	CuSO₄•5H₂O	0.01
thioctic acid	5.0	AlK(SO₄)₂•12H₂O	0.01
		H₃BO₃	0.01
		Na₂MoO₄	0.025
		NiCl₂•6H₂O	0.024
		Na₂WO₄•2H₂O	0.025

7. CURRICULUM VITAE



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