

## Bioelectricity Generation and Dye Decolorization by *Aspergillus niger* and *Trichoderma harzianum*

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### Abstract

This study explored the ability of *Aspergillus niger* and *Trichoderma harzianum* to decolorize azo dye acid black 172 and bioelectricity generation. Both filamentous fungi oxidized acid black 172 in the anode chamber, the electrons resulting from this oxidation pass through an external circuit to the cathode chamber, thus producing current. Bioelectricity generation in double chamber microbial fuel cells (MFCS) were investigated under variable dye concentration of acid black 172, external resistance, co-substrate, and dye as a sole carbon source.

**Keywords:** Decolorization; Bioelectricity; Acid black 172

### Introduction

Dyes are widely used in the textile industry and 10-15% of these dyes are lost in effluent during the dyeing process [1]. Azo dyes are a group of poorly biodegradable synthetic colorants that are often found in the textile wastewater [2]. These compounds are highly resistant to aerobic biodegradation by microorganisms but can be easily reduced by anaerobic microorganisms to form aromatic amines, which are a group of carcinogens that are stable under anaerobic conditions and must be returned to aerobic conditions before they can be further degraded and mineralized [3]. A range of physicochemical methods exists to remove color from dye containing effluents [4]. The most extensively used are coagulation and flocculation processes. They require significant quantities of chemicals and produce notable amounts of sludge, requiring further handling and disposal. Enzymatic decolorization is now widely used for the decolorization of dye wastewater. However, this method is also facing several problems such as cost of enzymes, enzymes stability and product inhibition [5]. On the other hand, biological processes provide a low-cost and efficient alternative for simultaneous color removal. In recent years, microbial fuel cell (MFC) technology has explored extensively for their innovative features and environmental benefits [6]. This work presents an efficient and cost effective approach to establish an MFC-assisted electrochemical oxidation process for the decolorization of azo dye.

### Methodology

#### Dyes

Five model textile dyes (obtained from Mocket Mac Company at 10<sup>th</sup> of Ramadan City, Egypt) were used for testing decolorization capacity of *Aspergillus niger* and *Trichoderma harzianum* including: Acid red 399, Acid yellow 235, Acid yellow 218, Acid blue 296, and Acid black 172.

### Sample collection and cultivation conditions

A fungal species was collected from wastewater of an Egyptian Company for artificial Carpet at 10<sup>th</sup> of Ramadan City, in sterile clean glass bottles then, stored at 4°C, cultivated in petri-dishes containing Dox's agar medium at 25°C and stocked in slant tubes containing Czapek's yeast agar solid medium under refrigeration at 4°C. Initial selection was based on maximum voltage production and dye decolorization. For submerged cultures, a pre-inoculum of young mycelium disk of 0.5 cm of diameter was obtained from 2 days-old colonies for *Aspergillus niger*, while in case of *Trichoderma harzianum* two young mycelium disks of 0.5 cm of diameter were obtained from 4 days-old colonies in solid medium. Disks were inoculated into flasks with 50 ml of fresh medium containing 15 g/L glucose, 2 g/L NaNO<sub>3</sub>, 1 g/L KH<sub>2</sub>PO<sub>4</sub>, 0.5 g/L KCL and 5 g/L yeast extract in 50 mM phosphate buffer (pH 7) and incubated at 25°C for 96 h for *Trichoderma harzianum*, while *Aspergillus niger* was incubated at 35°C for 48 h. After this period of time, flasks were used for inoculation of 250 ml flasks (bottles) containing 50 ml Acid black 172 dye (which has been prepared using 10 mg/L) of anodic compartment of fungal microbial fuel cells. Each culture was incubated under maximum incubation temperature.

### Construction of fungal microbial fuel cell

The dual chambered MFC was constructed using two air tight laboratory glass bottles (anode and cathode) of 250 ml capacity connected via a glass tube (Length 11 cm, width=6.6 and diameter 0.5 cm) that is heated and bent into a u-shaped, filled with 2% agar gel in saturated KCL solution and inserted through the lid of each bottle. The anodic and cathodic electrodes were graphite rods (surface area 5.5 cm). Copper (Cu) wires were soldered to the electrodes by using a conductive epoxy. A multimeter with a data logger system (MT-1820 Pros'Kit, Taiwan) was used as the automated data acquisition system.

### Operation of a fungal microbial fuel cell

The fuel cell compartments were cleaned and the electrodes were autoclaved for 20 min. The anode solution (50 mL) contained the following components (per liter) 15 g C<sub>6</sub>H<sub>12</sub>O<sub>6</sub>, 2 g NaNO<sub>3</sub>, 1 g

$\text{KH}_2\text{PO}_4$ , 0.5 g KCL, 5 g yeast extract in 50 Mm phosphate buffer (pH 7) and fungal biomasses. The compartment was inoculated with 50 ml acid black 194 which prepared as mentioned previously, finally anode volume (100 ml).

The cathode solution on the other hand, consisted of 100 ml of Potassium Ferricyanide ( $\text{K}_3(\text{Fe}(\text{CN})_6)$ ) solution (10 mmol/L) prepared in distilled water. The initial pH of Potassium Ferricyanide solution (pH 2) at cathode was adjusted using 0.5 M HCL which acted as the electron acceptor.

The MFCs with inoculation were first incubated with electrolytes described above for 4 days, then evaluated the decolorization rate of Acid black 172 and electricity generation. The external resistance was fixed at 1000  $\Omega$  and in some case the MFCs were operated under different external resistances to obtain the polarization curves. The temperature of MFCs was kept at 35°C for *Aspergillus niger* and at 25°C for *Trichoderma harzianum* using the laboratory incubators both the two inoculated MFCs and the two control sets were operated under identical conditions respectively, and average results were reported.

### Electrochemical analyses

Cell voltages were recorded every 20 minutes after a stable open circuit potential was achieved using a data logger system. Current (I) was calculated at a resistance(R) from the voltage (V) by  $I=V/R$ , and current density ( $I/A_2$ ), where A is the projected anode surface area.

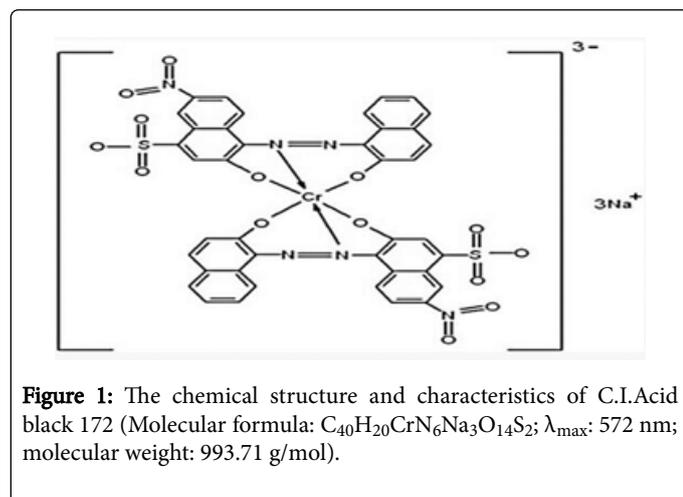
### Acid black 172 decolorization

The absorption of A. black 172 in the visible light range peaks at a wavelength of 572 nm was measured using a T60 UV-VIS spectrophotometer and used to calculate A. black 172 from Eq. (1) where  $A_0$  and A are the initial and final absorptions, respectively.

$$\text{Decolorization (\%)} = \frac{A_0 - A}{A} \times 100$$

### Results and Discussion

Figure 1 shows the chemical structure and characteristics of C.I.Acid black 172:



### Decolorization-efficiency of *Aspergillus niger* and *Trichoderma harzianum*

*Aspergillus niger* and *Trichoderma harzianum* were selected to investigate their effect on decolorization rate of the following dyes: (1) Acid red 399, Acid yellow 235, Acid yellow 218, Acid blue 296, and Acid black 172. The initial concentration was (10 mg/L) amended in flasks containing 100 ml of Dox's broth medium. The flasks were inoculated as previously mentioned. The results in Figure 2 show variation in decolorization abilities. *Aspergillus niger* was able to remove more than 50% of these acid dyes. On other hand, *Trichoderma harzianum* was able to remove less than 50% of acid red 399, acid yellow 235, acid yellow 218 and acid blue 296 dyes. The results showed that, *Aspergillus niger* and *Trichoderma harzianum* were effectively able to remove more than 85% of acid black 172. Accordingly, it is selected for further investigations. These results may be due to chemical structures of dyes significantly affecting the performance of dye biodecolorization. For similar structures of azo dyes, monoazo dyes are easily biodecolorized than polyazo dyes [7]. Also, our results agree with Bor et al. [8] who state that color removal percentages of Reactive Blue 160 for *Proteus hauseri* still achieved above 90%.

### Effect of acid black 172 concentration on dye decolorization and voltage output (OCV)

Comparison on performance of voltage output in a fungal microbial fuel cell at various concentrations of acid black 172 (i.e., 5, 10, 30 and 50 mg/L; Table 1) showed that the decolorization percent and voltage output (OCV) for *Aspergillus niger* and *Trichoderma harzianum* reached its maximum value (95%) and (890 mV) respectively at dye concentration 10 mg/L. Also showed that increasing in the dye concentration resulted in a decrease in the decolorization percent and voltage output (OCV). The results are in agreement with the findings of Bor et al. [8] that observed the decreased peak output voltage at high loading of Reactive Blue 160 might explain that decolorization and bioelectricity generation of *Proteus hauseri* are competitive to each other as both reactions all utilized electrons released from bacterial oxidation of organic matter. In addition, possibly due to accumulation of the decolorized intermediates of Reactive Blue 160 as mediators, the steadystate (SS) cell-voltages were stabilized at slightly higher levels than the SS-voltage in Reactive blue 160 free cases. In general, the performance of an MFC depends on the concentration and the type of dye used; Mu et al. [9] showed that during closed-circuit operation, decolorization efficiency decreased from 78.7% to 35% with an increase in influent dye concentration. Sun et al. [10] reported that the percent decolorization decreased with increase in Active Brilliant Red X-3B concentration.

Dye concentration (mg/L)	Decolorization (%)		Voltage(OCV)	
	<i>Aspergillus</i>	<i>Trichoderma</i>	<i>Aspergillus</i>	<i>Trichoderma</i>
5	93	94	850 mV	870 mV
10	95	95	890 mV	890 mV
30	80	75	810 mV	800 mV
50	65	61	750 mV	720 mV

**Table 1:** Effect of acid black 172 concentration on dye decolorization and voltage output (OCV).

### Effect of co-substrate on decolorization of acid black 172 and voltage output (OCV)

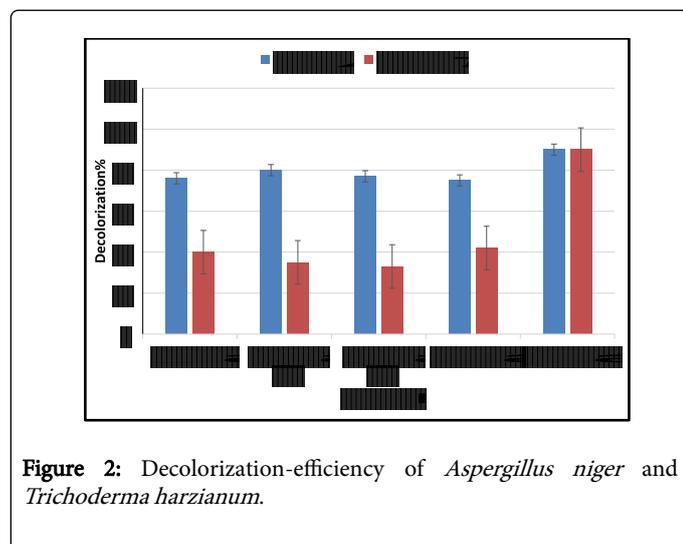
Different co-substrates such as glucose, and sucrose were individually added as equimolecular to 30 g sucrose per liter were tested to determine the optimum co-substrate which supported the maximum decolorization and voltage output of the tested fungi. The results in Table 2 showed that, the decolorization percent for *Aspergillus niger* and *Trichoderma harzianum* reached its maximum value (95% and 98%) respectively using glucose as co-substrate, also showed voltage output value reached its maximum value (899 mV and 895 mV) for *Aspergillus niger* and *Trichoderma harzianum* respectively. The results similar to those obtained by Sun et al. [10] who stated that, the maximum decolorization rate of ABRX3 observed with glucose, also in agreement with our results Cao et al. [11] found that, the power density was highest for glucose during decolorization of Congo red.

Co-substrate	Decolorization (%D)		Voltage (OCV)	
	<i>Aspergillus</i>	<i>Trichoderma</i>	<i>Aspergillus</i>	<i>Trichoderma</i>
Glucose	95	98	899 mV	895 mV
Sucrose	90	92	820 mV	800 mV
Control	80	75	700 mV	620 mV

**Table 2:** Effect of co-substrate on decolorization of Acid black 172 and voltage output (OCV).

### Simultaneous dye decolorization and current generation

Dye decolorization rate and current generation were measured after operation for three days. The results in Table 3 showed that decolorization rate and current density were decreased after sixty hours of operation. These results in contrast with Chen and Chen et al. [12,13] who mentioned that impulse additions of C.I. reactive red 141 significantly enhanced increasing the rate of oxidative phosphorylation of *P. hauseri* to accelerate electron transport in the respiratory chain of immobilized cells on the anodic biofilm for bioelectricity production in MFC.



**Figure 2:** Decolorization-efficiency of *Aspergillus niger* and *Trichoderma harzianum*.

Time (h)	Decolorization (%)		Current density (mA/m <sup>2</sup> )	
	<i>Aspergillus</i>	<i>Trichoderma</i>	<i>Aspergillus</i>	<i>Trichoderma</i>
0	95	94	163	160
12	93	94	149	154
24	92	93	147	154
36	92	92	145	145
48	90	90	145	136
60	90	90	136	131
72	88	85	133	129

**Table 3:** Simultaneous dye decolorization and current generation.

### Conclusion

The microbial fuel cell (MFC) with a fungal anode achieved more than 84% decolorization of azo dye acid black 172 in three days of continuous operation with current density more than 128 MA/m<sup>2</sup>. Also a maximum open circuit voltage(OCV) was obtained with 10 g/L of dye concentration was 890 mV and maximum open circuit voltage(OCV) was obtained with Co-substrate with glucose was more than 894 mV for the two fungal species.

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