# Decolorization of Acid Black 194 using certain species of *Aspergillus*

Amany* A.A. and Sally A.A.

*Department of Botany and Microbiology, Faculty of Science, Helwan University, Egypt.
Sally_science@yahoo.com

**Abstract:** Decolorization of textile dye acid black 194 by *Aspergillus flavus* **LinkAUMC 9060**, *Aspergillus tamarii* **Kita AUMC 9061** and *Aspergillus parasiticus* **Speare AUMC 9062** has been investigated. The maximum dye decolorization has been achieved at 24℃, pH 7, 250 mls flask and shaking at150 rpm. Also aspergilli mixture showed high ability to decolorize acid black 194.
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**Key Words:** Decolorization, Acid black 194, *Aspergillus flavus* Link AUMC 9060, *Aspergillus tamarii* Kita AUMC 9061, *Aspergillus parasiticus* Speare AUMC 9062.

## 1. Introduction

The textile industry is a substantial consumer of water and produces enormous volumes of contaminated water; the most important contaminants are azo dyes. Microbial processes for the treatment of textile wastewater have the advantage of being cost-effective and environmentally friendly and producing less sludge. The most promising microorganisms for wastewater treatment are those isolated from sites contaminated with dyes because they have adapted to survive in adverse conditions (Myrna *et al*., 2012).

Dyes are synthetic chemical compounds having complex aromatic structures which are extensively used in the textile, cosmetic, plastic, food, and pharmaceutical industries (Forgacs *et al*., 2004). The dye-containing wastewater discharged from the industries can adversely affect the aquatic environment by impeding light penetration. Moreover, most of the dyes are toxic, carcinogenic and harmful to human health. Even at low concentration (1 mg $L^{-1}$), dyes could be highly noticeable, and could cause anaesthetic pollution and disturbance to the ecosystem and water source (Vimonses *et al*., 2010). Therefore, there is an increasing demand of efficient and economical technologies for removing dyes from water environment in the world. Wastewater dyes are usually treated by physical or chemical treatment processes. These include flocculation combined with flotation, electroflocculation, membrane filtration, ion-exchange, irradiation, precipitation, ozonation and katox treatment method involving the use of activated carbon and air mixtures (Banat *et al*., 1996). However, these technologies are generally ineffective in color removal, expensive and less adaptable to a wide range of dye wastewaters (Banat *et al*., 1996).

The aim of the present study is to remove acid black 194 from aqueous solution using both new three local fungal species as single and mixture. Statistical analysis of data was carried out by using one way analysis of variance (ANOVA) followed by homogenous subsets (Duncun[a]) at confidence level of 5 % (0.05). Each experiment was conducted in triplicate and mean ± SD values were taken.

## 2. Materials and Methods

**Dye:**

Acid black 194 dye was metal complex its product name was Lanasyn Black M-DL P 170 kindly supplied by" Moket Mac" Company in industrial zone B1, **10th** of Ramadan City, El-Sharqyiah, Governerate, Egypt.

**Isolation of fungi:**

Water samples were collected from wastewater of an Egyptian company for artificial carpet at 10th of Ramadan city, in sterile clean glass bottles then, stored at 4°C. Wastewater samples were taken from the surface.

Fungi were isolated from water samples by using 1ml of sample, to which 9ml of sterilized distilled water were added. The contents were mixed by shaking. Serial decimal dilutions (Benson,2002) were made from the original concentration to reach dilutions up to 1/1000.The media used for fungal isolation and purification were potato-dextrose agar, sabouraud's glucose agar, czapek-dox´s agar and malt extract agar. Three replicates were prepared for each dilution and incubated at 28˚C for 3-5 days.

**Fungal identification and maintenance:**

*Aspergillus sp1, Aspergillus sp2, Aspergillus sp3* were isolated from surface. Identification was carried out according to the following references: John and Pitt (1979); Gilman (1957); Domsch and Gams (1980). Then identification of *Aspergillus sp1*, *Aspergillus sp2* and *Aspergillussp3* were confirmed by Mycological Center, Faculty of Science, Assiut University, Egypt to be *Aspergillus flavus* **Link**

**AUMC 9060,** *Aspergillus tamarii* **Kita AUMC 9061** and *Aspergillus parasiticus* **Speare AUMC 9062**.

These fungal isolates were maintained and sub-cultured on potato dextrose agar (PDA) and Czapek's Yeast (autolysate) extract Agar media (CYA).

**Decolorization assay**

The experiments were carried out in 250 mls Erlenmeyer flasks containing 100 mls of broth dox's medium to which the dye was added at concentration 0.05g/L for both *A.tamarii* and *A.parasiticus* but in case of *A.flavus* the concentration was at 0.025g/L.

The pH was adjusted at 7±0.2 using phosphate buffer solution.The autoclaved flasks were left to cool then, separately inoculated with 1disc (1cm in diameter) for *A.parasiticus* while in case of *A. flavus* and *A.tamarii*, flasks were inoculated with 2 discs (1cm in diameter). The flasks were then incubated for 8 days and at the end of this period, 10 ml of the residual culture medium were centrifuged at 5000 rpm for 15min (Hettich Zentrifugen Mikro22 R D-78532 Tuttlingen).

Then measured spectrophotometrically at $\lambda_{max}$ (570 nm) for acid black 194 dye using T60 UV VIS spectrophotometer. The decolorization percent calculated as the following.

% Decolorization = $A_o - A_t / A_o \times 100$

Where $A_o$= is the absorbance at zero time and $A_t$= is the absorbance after some time (Olukanni *et al*., 2006). Decolorization percentage is representing the mean of three replicates.

Note. Determination of the maximum wavelength of dye has been carried out at the central lab of Faculty of Science, Helwan University, Egypt using UV/Vis spectrophotometrically (Jasco-V-530).

## 3. Results and Discussion

Effect of incubation temperature on decolorization of acid black 194. The results in table 1 show that the decolorization ability of *A.flavus* was less than 50% when incubated at 20℃. The increase in temperature resulted in the increase in decolorization ability. No significant difference was shown between incubation at 24℃ and 28℃. In case of *A. tamarii* the level of decolorization showed no significant difference under all tested temperatures. However, in case of *A.parasiticus*, the increase in temperature resulted in a significant decrease in decolorization percent. So the optimal incubation temperature for decolorization was considered 24℃. The abrupt increase of decolorization of *A.flavus* by the rise in temperature from 20℃ to 24℃ may be due to its fast growth at that temperature. Ponraj *et al*., 2011 found that *Aspergillus niger* and *Mucor sp* were found to be most effective as decolorizer at 27 and 37℃, respectively.

**Table1.Effect of incubation temperature on decolorization of acid black 194**

| *Aspergillus spp* / Temp(˚C) | *A.flavus* **(% D)** ± SD | *A.tamarii* **(% D)** ± SD | *A.parasiticus* **(% D)** ± SD |
|---|---|---|---|
| 20 | $35.6^{a}$ ± 7 | $80.7^{c}$ ± 5 | $82.9^{d}$ ± 1.5 |
| 24 | $82.8^{b}$ ± 3 | $88.7^{c}$ ± 0.5 | $80.3^{d}$ ± 3.1 |
| 28 | $85.9^{b}$ ± 0.5 | $85.4^{c}$ ± 1.5 | $60.7^{e}$ ± 3 |
| 32 | $72.5^{b}$ ± 8 | $84.2^{c}$ ± 2 | $69.7^{e'd}$ ± 3 |
| 36 | $81.3^{b}$ ± 2 | $82.5^{c}$ ± 2.5 | $76.3^{d}$ ± 8 |
| 40 | $78.1^{b}$ ± 3 | $81.1^{c}$ ± 3 | $58.4^{e}$ ± 4 |

Means in the same column with different letters have significant difference between each other.
% D: Decolorization percent

**Effect of initial pH values on decolorization of acid black 194**

Hydrogen ion concentration of the solution bearing acid black 194 (azo dyestuff/chromium complex anionic) has been recognized as key factor in determining the decolorization percent. The results recorded in table 2 show that pH 7 was the optimum for maximum decolorization (89.0, 75.9 and 89.9%) by *A. flavus* **Link** *A.tamarii***Kita** and *A. parasiticus* **Speare,** respectively. The recorded decolorization percent at acidic pH was less than that observed at pH 7. In contrast to the present results Yuyi *et al*. (2011), reported that acidic conditions could be favorable for the biosorption of dyes by the fungal biomass, and this could be attributed to electro-static attraction which might exist between the positively charged surface of the adsorbent under acidic conditions and the anionic dyes. On other hand, Khalaf (2008) mentioned that, there is an electrostatic attraction between the dye and the biosorbent during the biosorption process. That could explain this response at high and low pH values.

If there is a negative charge on the dye and a positive charge on the biosorbent they would attract each other. But at low pH, there is an excess of $H^+$ ions so attraction could be disrupted because of the attraction between the $H^+$ and the dye. A similar but

opposite reaction would happen at alkaline pH, as there is an excess amount of $OH^-$ ions in the solution**.**

**Table2**.Effect of initial pH on decolorization of acid black 194

| *Aspergillus spp* / pH | *A. flavus* **(% D)** ± SD | *A.tamarii* **(% D)** ± SD | *A.parasiticus* **(% D)** ± SD |
|---|---|---|---|
| 6 | $49.2^{a}$ ± 0.7 | $79.1^{c}$ ± 2 | $79.6^{d}$ ± 0.6 |
| 7 | $89^{b}$ ± 2 | $75.9^{c}$ ± 0.4 | $89.9^{a}$ ± 0.7 |
| 8 | $87.6^{b}$ ± 0.8 | $75.3^{c}$ ± 3 | $82.8^{d}$ ± 1.5 |
| 9 | $42^{a}$ ± 2 | $73.1^{c}$ ± 2 | $69.7^{e}$ ± 0.4 |
| 11 | $48.7^{a}$ ± 1.5 | $61.5^{d}$ ± 2 | $67.7^{e}$ ± 0.7 |

Means in the same column with different letters have significant difference between each other.
% D: Decolorization percent

**Effect of air volume on decolorization of acid black 194**

Air volume may have a role in decolorization level so different flask volumes had been used (ranging between 250 - 1000 mls) to study this factor. The results in table 3 show that maximum decolorization was achieved using 250 mls flasks (90.65%, 95.07% and 96.89%) as well as 500 mls (95.8%, 94.4% and 95.66%) for *A,flavus* **Link**, *A.tamarii* **kita** and *A.parasiticus* **Speare,** respectively. Also, the results showed that the *Aspergillus* species could get their oxygen requirement from the air content in 250 mls flasks and larger volume has no significant effect on decolorization.

Belsare and Prasad, 1988 observed that intermittent aeration for a period of three days stimulated lignin breakdown and that color removal was between 82% on the first day and 90% on the third day. Glenn and Gold (1983) also observed that, decolorization of dyes by *Phanorocheate chrysosporium* was strongly dependent on the oxygen concentration in culture.

**Table3.** Effect of air volume on decolorization of acid black 194

| *Aspergillus spp* / Air volume (ml) | *A.flavus* **(% D)** ± SD | *A. tamarii* **(% D)** ± SD | *A.parasiticus* **(%D)** ± SD |
|---|---|---|---|
| 250 | $90.65^{a}$ ± 2.8 | $95.07^{c}$ ± 0.34 | $96.89^{e}$ ± 0.4 |
| 500 | $95.8^{a}$ ± 0.6 | $94.4^{b,c}$ ± 0.1.1 | $95.66^{d,e}$ ± 0.5 |
| 1000 | $87.01^{a}$ ± 0.8 | $92.06^{b}$ ± 0.3 | $94.28^{d}$ ± 0.8 |

Means in the same column with different letters have significant difference between each other.
% D: Decolorization percent

**Effect of shaking speed on decolorization of acid black 194**

The results in table 4 show that decolorization was more efficient under shaking condition. Also, the results clearly indicating that there was no significant difference between the used shaking speeds. The present results are agreement with that of Raj*et al*.,2012**,** who studied the effect of static/shaking on decolorization and observed that *Aspergillus sulphureus* was more efficient in decolorization in shaking condition (93.04±1.86%) than static (77.33 ± 0.76%) and also in agreement with that observed by Hazrat Ali and Shah Khalid (2008)**,** who found that the extent of decolorization percent of this dye by *Alternaria solani* may be increased by treatment under shaking conditions because shaking facilitates the transfer and distribution of materials and oxygen between the medium and the microbial cells.

In contrast to our results, Husseiny (2008) found that, the static conditions are more efficient than the shaking for both *Aspergillus niger* and *Penicillium spp*. These results are similar to those obtained by Daneshvar *et al.* (2007) using another type of microorganisms and can be discussed in terms of the high rate of the agitation decreases the fungal growth and the activities of some biological substances such as enzymes which play an important role in the decolorization of the dye, Faison and Kirk (1985); Ge *et al.* (2004).

**Table 4.**Effect of shaking speed on decolorization of acid black 194

| *Aspergillus spp* / Shaking speed(rpm) | *A.flavus* **(%D)** ± SD | *A.tamarii* **(%D)** ± SD | *A.parasiticus* **(%D)** ± SD |
|---|---|---|---|
| Static (control) | $82.3^{a} \pm 3$ | $84^{c} \pm 1.3$ | $94.4^{e} \pm 0.5$ |
| 100 | $98^{b} \pm 0.6$ | $100^{d} \pm 0.1$ | $99.4^{c} \pm 0.2$ |
| 150 | $98.9^{b} \pm 0.1$ | $98.6^{d} \pm 0.3$ | $100^{c} \pm 0.1$ |
| 200 | $99.4^{b} \pm 0.2$ | $99.1^{d} \pm 0.2$ | $99.6^{c} \pm 0.2$ |

Means in the same column with different letters have significant difference between each other.
% D: Decolorization percent

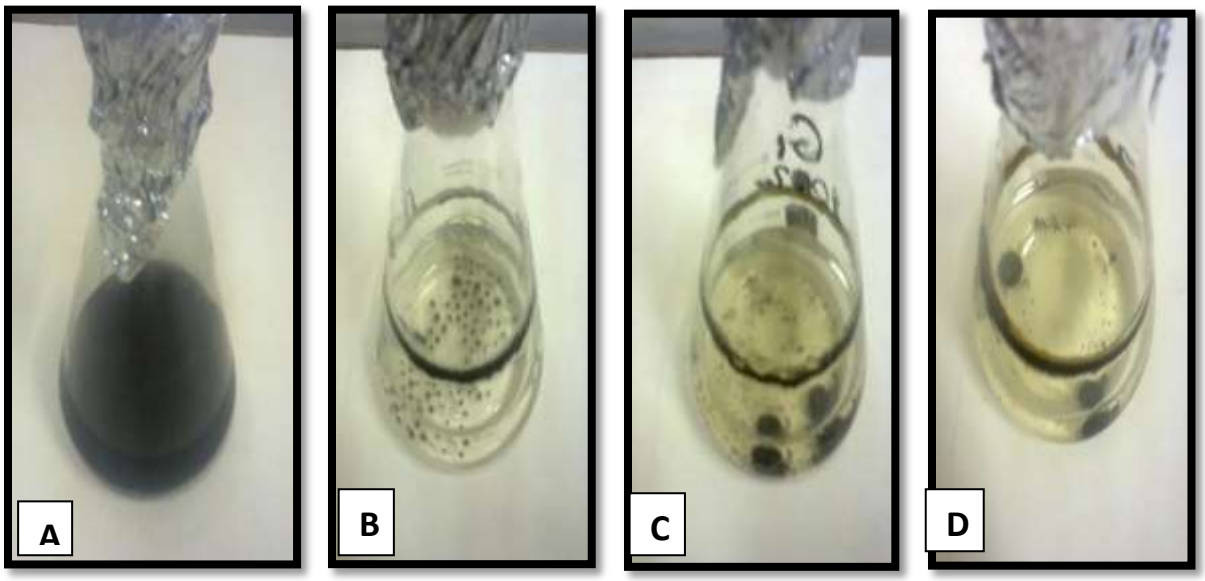


**Fig 1**. Effect of shaking speed on decolorization of acid black 194 (A) Control (B) *A.parasiticus* **Speare**(C) *A.flavus* **Link** (D) *A.tamarii* **kita**

**Effect of aspergilli mixture on decolorization of acid black 194**

The present experiment was carried out to test the effect of tested aspergilli on decolorization percent.

Results obtained in table 5 indicated in case of aspergilli mixture, decoloriztion percent reached 95% which was more or less the same as that obtained by the use of each aspergillus as single culture.This result insured the efficiency of aspergilla mixture to be applied in wastewater treatment.

**Table 5.**Effect of aspergilli mixture on decolorization of acid black 194

| ***Aspergillus spp*** | **%D of acid black 194 ± SD** |
|---|---|
| Mixture | 96 ± 0.1 |
| *A.flavus***Link** | 98.9 ± 1.7 |
| *A.tamarii***Kita** | 99 ± 1 |
| *A.parasiticus* **Speare** | 94.7 ± 5.3 |

%D: Decolorization percent

**Conclusion**

Microbial decolorization of dyes has received much attention, as these are cost-effective methods for dye removal. Our investigation for decolorization of textile dye by new local fungal species (*A.flavus* **Link**, *A.tamarii* **Kita** and *A.parasiticus* **Speare**) showed maximum decolorization of acid black 194 which reached 100%. Also, showed that mixture of aspergilli achieved decoloriztion percent more than 95%. Further investigation will be required to eliminate or minimize the discharge of toxic chemicals for reuse safely the treated wastewater.

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