DECOLORIZATION OF REMAZOL BLUE AND REMAZOL RED USING ASPERGILLUS NIGER ISOLATED FROM TEXTILE WASTEWATER

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Abeer, M. Mohamed [(1)]; Dalia, A. M. Abduo [(2)]; Al-zahraa, A. Karam Al- Dien [(2)]; Elshhat, M. Ramadan [(3)] and Taha M. Abd Elrazek[(4)]

1) Department of Occupational Environment, National Institute of Occupational Safety and Health (NIOSH).2) Department of Microbiology, Faculty of Science, Ain Shams University 3) Department of Microbiology, Faculty of Agriculture, Ain Shams University 4) Department of Basic Sciences Institute of Environmental Studies and Research, Ain Shams University.

ABSTRACT

Discharge of textile dyes in wastewater of textile factories represents a major environment problem threatening the aquatic life. The decolorization of such harmful chemicals is of paramount importance. In this work, Aspergillus niger, a brown rot fungi, isolated from the effluent samples around the textile industry of El- Baragil, Giza, Egypt was evaluated for its efficiency in decolorization of two textile dyes; Remazole blue and Remazol red. Effluent samples were also analyzed for their physiochemical properties. The optimum conditions for decolorization were found to be 4 days incubation, 30 ° C and pH4 in Sabouraud liquid medium supplemented with dextrose (10 g/l) and ammonium sulfate (2 g/l) as a carbon and nitrogen source, respectively. The response of the isolate to the increase in dye concentrations was found to be growth inhibitory. Decolorization extent and facile conditions showed the potential for the A.niger to be used in biological treatment of textile dyes.

Keywords: A.niger, Decolorization, Physiochemical, Textile dyes.

INTRODUCTION

Wastewater from textile industry is considered as one of the major sources of pollution due to the great demand for textile products resulting proportional increase in production and application of synthetic dyes (Balapure *et al*., 2014). Azo dyes represent the most common group of synthetic dyes constituting 60–70% of more than 10,000 dyes used in textile industry (Ong *et al*., 2010). It is estimated that about 2% and 10–15% of azo dyes are lost during manufacture and dyeing processes, respectively (Pearce *et al*., 2003). Release of theses dyes into the environment causes an adverse impact on the aquatic ecosystem because that they are considered to be toxic to aquatic biota and are reported to be carcinogenic to humans (Tauber *et al*., 2008). Although their contribution to organic load may sometimes be not significant, even the presence of low concentrations of dyes would impart an intense color to these wastewaters (Singh *et al*., 2007). Therefore, effective treatment of azo-dye-containing wastewaters before discharge into the environment should be emphasized. Compared with physical and chemical processes, biological methods are generally considered as better alternatives for treatment of azo-dye-containing wastewaters due to their lower cost, higher efficiency and less secondary pollution (dos Santos *et al*., 2007). Highly efficient microorganisms are the most important factors for effective biological treatment processes. For a long time, the corresponding researches are mainly focused on bacteria and fungi (de Miranda *et al*., 2013). Decolorization generally occurs by the adsorption of dyestuffs on bacteria, rather than oxidation in aerobic systems. Some bacteria can biodegrade

dyestuffs by azoreductase activity. However, the effluent at the end of biotransformation of dyestuffs could be toxic (Chung and Stevens, 1993). These problems limit large-scale application of bacterial decolorization.

Several fungi are capable of mineralizing pollutant compounds through their highly oxidative and non-specific ligninolytic enzymes, which are also responsible for the decolorization and degradation of many different dyes (Dos Santos et al., 2004). Recently, there is a growing interest in studying the brown rot fungi; Aspergillus niger, for the decolorization and degradation of many different dyes because their biomass can be used as an adsorbent and serve as a part of a technical solution in water pollution control (Fu and Viraraghavan, 2000 and Srividhya et al., 2012). The present study aimed at using a newly screened, Aspergillus niger, a brown rot fungus, isolated from effluents of textile industry for decolorization of two azo-based synthetic dyes; Remazol blue and Remazol red. Various conditions required for decolorization have been optimized.

MATERIALS AND METHODES

Sampling: Effluent samples were collected from textile factory in El-Baragil, Giza, Egypt. Samples were collected in sterile air tight bottles with filtering through the ordinary filter paper to remove large suspended particles. Standard procedures were followed during sampling and samples were transported to the laboratory and stored at 4°C.

Media and chemicals: Two textile dyes, Remazol blue and Remazol red (kindly provided from Giza Spinning & Weaving, El- Baragil) were used for the decolorization in the present investigation. All media components and

chemicals used in the present study were of analytical grade. The chemical structure of Remazol red is shown in figure-1. The structure of the Remazol blue is not published.

Physiochemical analysis of effluents: Color, smell, temperature and pH of the various wastewater samples were recorded on the spot. Samples collected from the discharge sites were filtered through Whatman no.1 filter paper and their chemical oxygen demand (COD), biological oxygen demand (BOD), total suspended solids (TSS) and total dissolved solids (TDS) were determined using standard procedures according to (APHA, 2012).

Determination of absorption maxima (λmax) of Remazol blue and Remazol red: The absorption maximum was determined by using a spectrophotometer. Optical density of dye solution in water was observed at different wavelength between visible regions (340- 700nm). The wavelength where, the dyes showed maximum absorbance is taken as absorption maximum of the dye; for Remazol blue and for Remazol red it was observed 600 nm and 530 nm respectively. .

Isolation and Identification of dye decolorizing fungi: Fungal strains native to the sampled area were isolated on Sabouraud Dextrose Agar (SDA) using a dilution plate technique (Harley and Prescott, 1993). The following composition of a medium was used (Peptone, 10 g/l; Dextrose, 40g/l; Agar, 12g/l). The isolated fungal species were identified by Lactophenol cotton blue staining and microscopic analysis. Strains were preserved on SDA slants at 4°C in a refrigerator and were served as stock cultures.

Dye decolorization experiment: From 6-day-old fungal culture on SDA, three mycelia disks (each 8 mm diameter) were cut from actively growing regions and inoculated to Erlenmeyer flasks containing 50 ml of Sabouraud liquid medium (SLM) (Pancreatic digest of casein, Peptic digest of fresh meat, Glucose. pH5.7) amended with 100mg/l experimental dye. Incubation was carried out at 30 °C in static condition for 5days, the culture broth was filtered then the filtrate was centrifuged at 10,000 rpm for 15 min. Absorbance of the supernatant was recorded at the corresponding λmax (600 nm) for Remazol blue and λmax (530 nm) for Remazol red. The decolorization activity in terms of (%) decolorization was calculated from standard curve of the dye according to the formula given by Olukanni *et al*., (2006):

$$\text{Decolorization \%} = \frac{Ao - At}{Ao} \times 100$$

Where, Ao is initial absorbance of sample and Atis the absorbance at different time intervals.

Optimization: Decolorization of Remazol blue and Remazol red by selected fungal isolate was optimized with respect to different factors including:

- Time course (2,4,6, 8 and 10 days)
- Temperature (27, 30, 37, 40 and 45 °C)
- pH (3, 4, 5, 6, 7 and 8)
- Dye concentrations (100, 200, 300,400,500 and 1000mg/ L)
- Carbon sources (10 g/ L): (dextrose, sucrose and starch)

- Nitrogen sources (2 g/ L): peptone, ammonium chloride and ammonium sulfate) using the experimental procedures as same as those for dye decolorization assay.

Statistical analysis: Data were analyzed using the mean of triplicates ± standard deviation (SD).

RESULTS AND DISCUSSION

Physiochemical characterization of the textile effluent: The physiochemical analysis of sampled textile effluent helped us to measure the pollution level. Thus, the physiochemical parameters for effluents were conducted and examined (Table-1).

Table (1): Physiochemical characterization of effluents

Parameter	Unit	Effluent
Color	-	max570nm)(λdark blue
Smell	-	Pungent
Temperature	°C	39.8
pH	-	8.7
BOD	mg/L	676
COD	mg/L	2360
TSS	mg/L	144
TDS	mg/L	2500

Effluents color is dark blue due to the mixture of various dyes and chemicals used in the dyeing process (Devi and Kaushik, 2005). The pH of the effluent alters the physiochemical properties of water which in turn adversely affects the biodiversity. High pH is mainly due to the use of carbonate, bicarbonate andNaOH during bleaching process in the textile (Wood and Kellogg, 1988). Soil permeability gets affected, which results in

polluting the underground resources of water (Buckley, 1992). Elevated temperature tends to decrease the solubility of gases in water, which is ultimately expressed as high BOD/COD. High TSS and TDS values reduce the light penetration into the water and ultimately decreases the photosynthesis in aquatic flora. This cause reduction in dissolved oxygen level of water bodies, which results for extremely low purification of wastewater by microorganism (Namdhari*et al*., 2012).

Isolation and Identification of dye decolorizing fungi: The three fungal strains were identified as Aspegillus niger, Aspergillus famigatus and Aspergillus terreus. The occurrence of fungi in the polluted water depends on the availability of nutrient, oxygen, biological, physical and chemical characteristics of the pollutants .Aspergillus species are well adapted to textile waste water and are frequently isolated from effluents and dye contaminated soils (Devi and Kaushik, 2005 and Ponraj *et al*., 2011).

Optimization of culture conditions for maximum dye decolorization

Effect of time course on decolorization: Decolorization for Remazol blue and Remazol red was studied (Fig. 2), The two dyes were decolorized after 4 days of incubation at 30 °C. The dyes wereabsorbed by the biomass and the visual observation of decolorization of the dyes was from the 3rd-6th day of incubation. The color of the biomass of fungus changed to the color of the tested dye. The color on the biomass was reduced gradually from the 6th until 10th day of incubation. Bergsten-Torralba *et al*. (2009) and Yu and Wen (2005) reported that the decolorization of dyes by yeast and fungi can be due to adsorption of the dye to microbial cells or to biodegradation. The present experiments demonstrated the efficiency of A. niger to decolorize the two

kinds of dyes, with differences in the decolorization ability. The mechanism of decolorization may be due to biosorbtion, which is dependent on functional groups in the dye molecule and in fungal biomass, which may also play role in the biosorption of dye (Raju *et al*., 2007; Fu and Viraraghavan, 2002).

Effect of temperature on dye decolorization: The extent of dye decolorization increased with an increase in temperature from 25 to 30 °C, and highest degree of decolorization was reported at 30°C (Fig. 3).

The maximum decolorization at this temperature may be due to high rate of respiration, substrate metabolism and production of dye degrading enzymes. However, the decolorization extent found to be decreased as the incubation temperature was set beyond 30 °C. This might have occurred due to adverse effect of high temperature on the fungal growth and enzymatic activities (Cetin and Donme, 2006).

Effect of initial pHon decolorization: Decolorization maxima were recorded at pH4.0. This was supported by the fact that slightlyacidic environment was preferable for metabolic activity of A.niger, hence the greatest extent of decolorization which tends to fall sharply below and beyond pH4.0 (Fig. 4).

This may be due to the electrostatic attraction between negatively charged dye anions and positively charged cell surface at lower pH values (Mahony *et al*., 2002).

Effect of dye concentrations on dye decolorization: For industrial applications, the tolerance of microorganism to higher concentrations of the dyes is an important factor since the dye concentration in a typical lindustrial effluent can vary between 60 and 250mg/ L (Pierce, 1994). The effect of

initial dye concentrations on decolorization of Remazole blue is shown in Fig. (5), where a decrease in dye removal was observed with increasing initial dye concentrations. Kumar *et al*. (2009) tested various initial concentrations of dye ranging from 25 to 300 ppm and found that percentage removal of dye decreased with an increase in dye concentration. A slower decolorization rate was attributed to higher molecular weight, structural complexity and the presence of inhibitory functional groups like –NO2 and –SO3Na in the dyes (Hu and Wu, 2001).

Effect of carbon sources on dye decolorization Among the carbon sources tested, maximum decolorization was recorded with dextrose (Fig. 6), however, starch is more cheaper makes it a better economic choice. Jin et al. (2007) reported that improved decolorization was observed with potato starch and sucrose during the decolorization of a mixture of reactive dyes. Readily usable carbon sources are mandatory for fungal growth, and subsequent production of secondary metabolites and extracellular enzymes for biodegradation and/or adsorption (Khelifi *et al*., 2009).

Effect of nitrogen sources on dye decolorization: A.niger exhibited high growth and decolorization percentage of Remazol blue and Remazol red with all utilized nitrogen sources. However, organic nitrogen source was found to be less satisfactory in decolorization process than the inorganic ones. Use of ammonium sulfate resulted in the highest decolorization among the nitrogen sources tested (Fig. 7). The possible explanation behind ammonium salt assimilation by fungi is that, by directly using ammonia, fungal cell would likely to convert nitrogen into the glutamate and glutamine amino acids that

serve as nitrogen donors for all other nitrogen-containing compounds in the cell (Eelko *et al*., 2000).

CONCLUSION

To comply with the strict environmental legislation, it is necessary to explore innovative environmental technologies for the treatment of colored dying effluents. The present study focuses on the isolation, identification of indigenous fungus A.niger for decolorization of a textile dyes Remazol blue and Remazol red. Results showed that A.niger has the ability to remediate the dyes from the effluent and decolorization of the tested dyes can be improved by providing appropriate culture conditions and maintaining microbial inoculants at active physiological state. Further, it can be suggested that dye contaminated sites can potentially be reclaimed by a low cost bioremediation process with native fungal species isolated from the dye disposal sites.

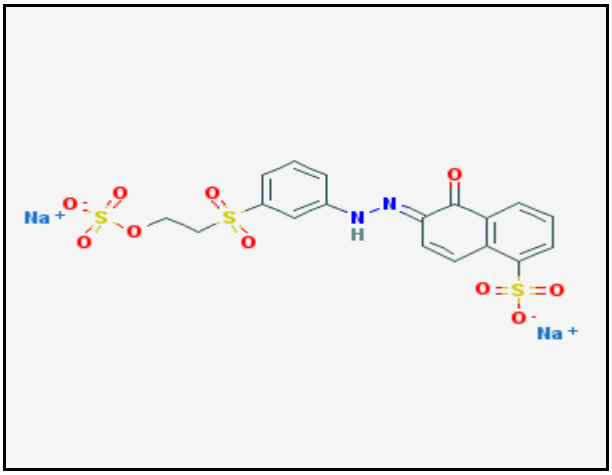

Fig. (1): Chemical structure of RemazolRed(IUPAC name:disodium;(6E)-5-oxo-6-[[3-(2-sulfonatooxyethylsulfonyl)phenyl] hydrazinylidene] naphthalene-1-sulfonate)

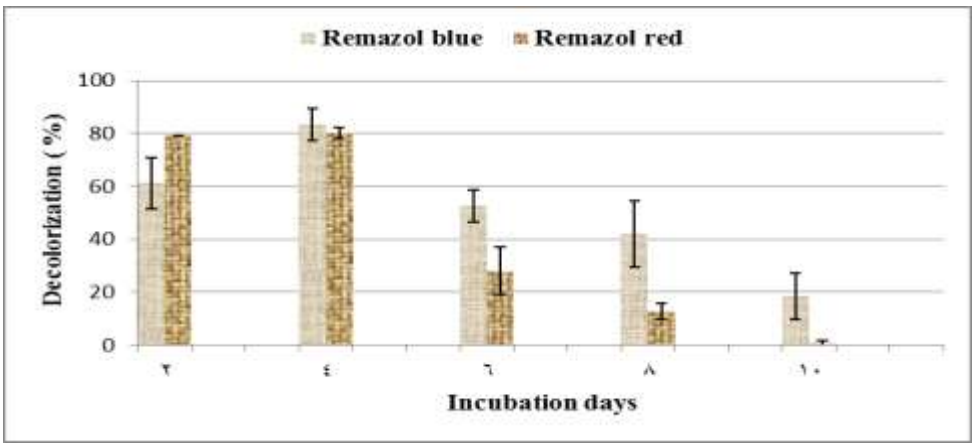

Fig. (2): Effect of time course on decolorizationof Remazol blue and Remazol red by A.niger

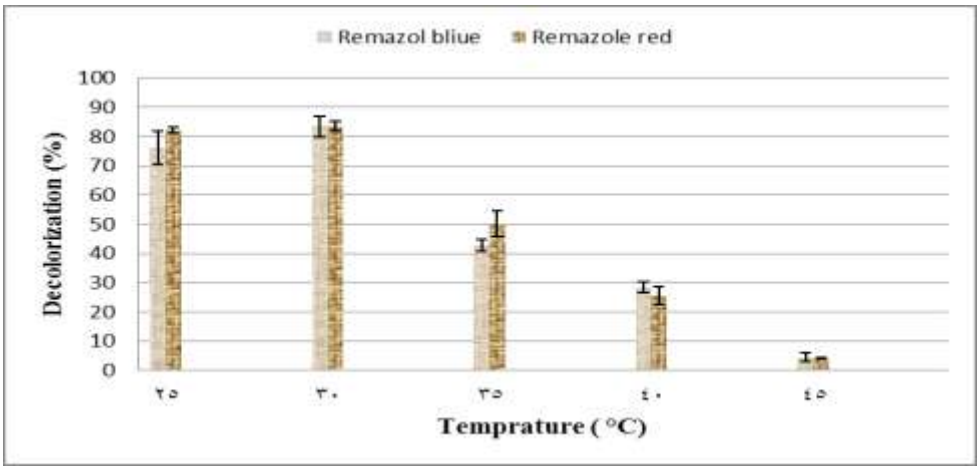

Fig. (3): Effect of temperature on decolorization of Remazol blue and Remazol red by A.niger

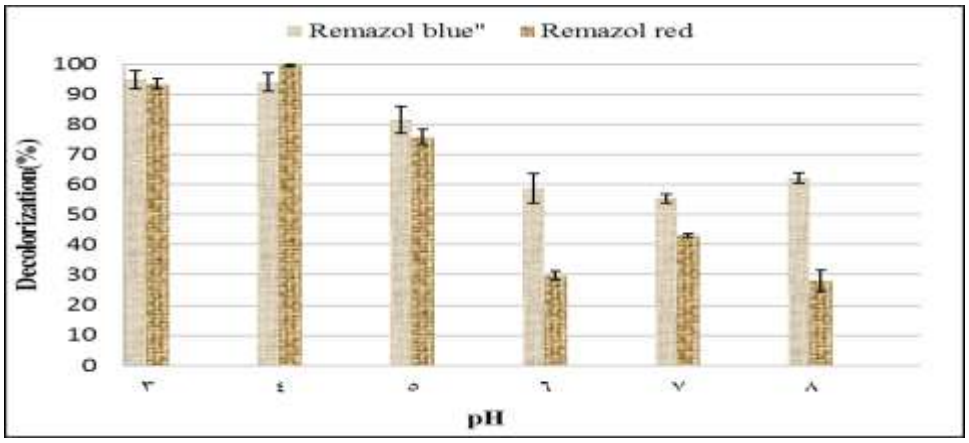

Fig. (4): Effect of pH on decolorization of Remazol blue and Remazol red by A.niger

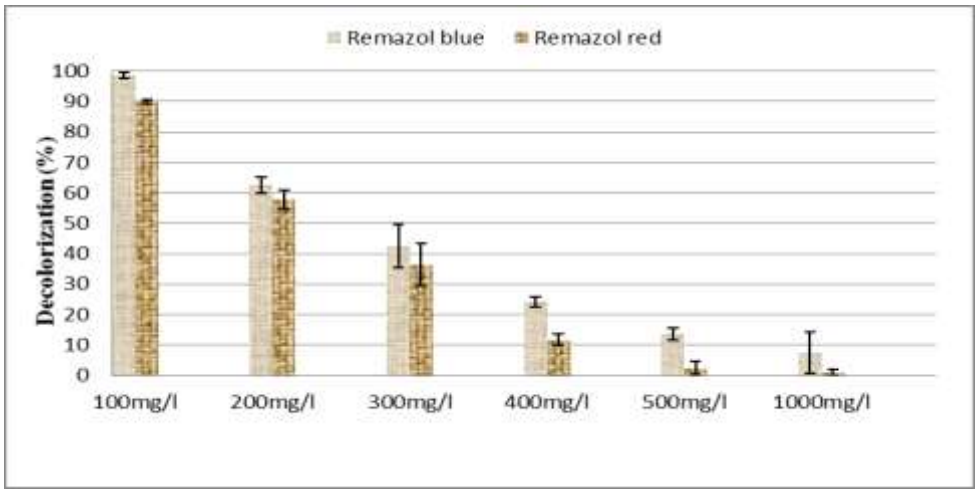

Fig. (5): Effect of different dye concentrations on decolorization of Remazol blue and Remazol red by A.niger

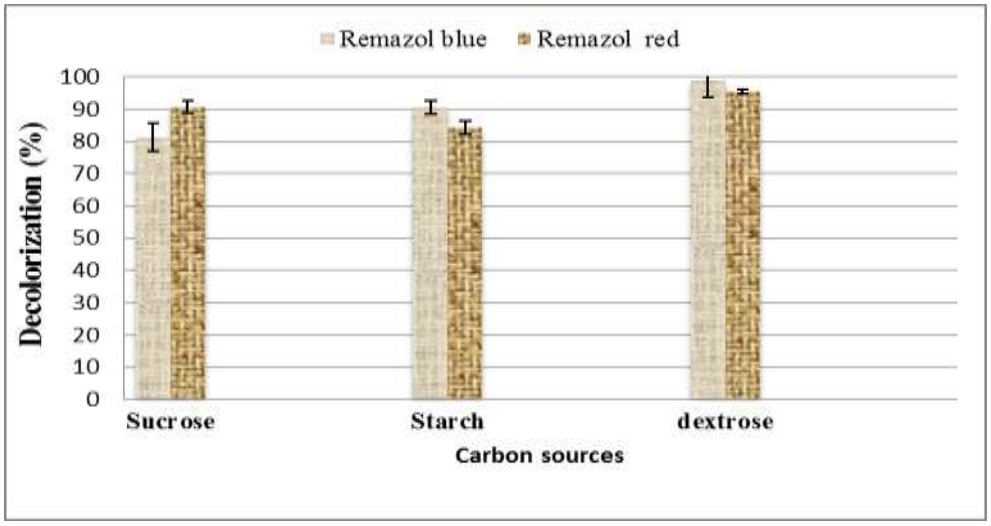

Fig. (6): Effect of different carbon sources on decolorization of Remazol blue and Remazol red by A.niger

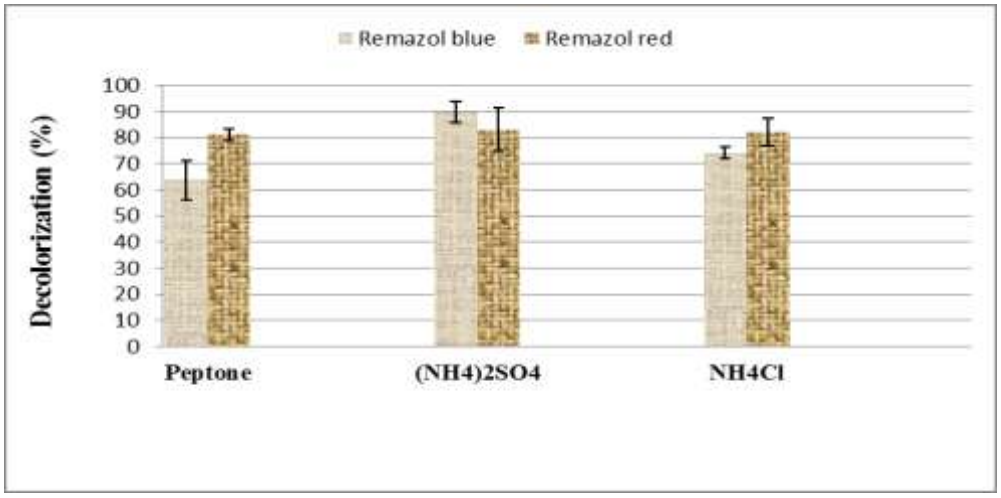

Fig. (7): Effect of different nitrogen sources on decolorizationof Remazol blue and Remazol red by A.niger

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إزالة صبغات (ازرق الريمازول وأحمر الريمازول) باستخدام فطر الاسبرجلس نيجر المعزول من المخلفات السائلة لصناعة النسيج

[٢]

عبير مدحت محمد[١]– داليا على محمود عبده[٢]– الزهراء أحمد كرم الدين[٢]
الشحات محمد رمضان[٣]– طه عبد العظيم محمد عبد الرازق[٤]

١) قسم البيئة المهنية، المركز القومي لدراسات السلامة والصحة المهنية ٢) قسم الميكروبيولوجي، كلية العلوم، جامعة عين شمس ٣) قسم الميكروبيولوجي، كلية الزراعة، جامعة عين شمس ٤) قسم العلوم الأساسية البيئية، معهد الدراسات والبحوث البيئية، جامعة عين شمس

المستخلص

يمثل وجود أصباغ النسيج في مياه الصرف الصناعي لمصانع الصباغة تهديدا للحياة المائية. في هذه الدراسة تم تقييم كفاءة الفطر Aspergillus niger – فطر أسمر بني، والذي تم عزله من عينات المخلفات السائلة من مصنع نسيج في البراجيل، الجيزة ، مصر– في إزالة اثنين من الأصباغ النسيجية؛ أزرق الريمازول وأحمر الريمازول. كما تم تحليل الخصائص الفيزيوكيميائية لعينة الصرف الصناعي. وقد تبين من خلال الدراسة أن الظروف المثلى لأعلى إزالة اللون للنوعين من الصبغات

هي ٤ أيام تحضين.و عند درجة حرارة ٣٠ درجة مئوية ودرجة الحموضة ٤ مع إضافة دكستروز (١٠ جم / لتر) وكبريتات الأمونيوم (٢ جم / لتر) كمصدر للكربون والنيتروجين، على التوالي. كما تبين أن لزيادة تركيزات الصبغة تأثيرا مثبطا لنمو الفطر. وقد أظهرت النتائج إمكانية استخدام فطرالاسبرجلس نيجر في المعالجة البيولوجية لازالة أصباغ النسيج.

كلمات دالة: فطر الاسبرجلس نيجر، إزالة اللون، الخصائص الفيزيوكيميائية، أصباغ النسيج.