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Original Research Paper

Study on Biological Decolorization of Textile Sewage in Kermanshah by Microbial Isolates from Environmental Samples

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ABSTRACT

Nowadays, drug, pulping, textile and other industries are developed in most of the countries and their non save sewages makes many problems for environment. One of the most important dangerous sewage is pigmented sewage, which has complex structures such as azo compounds. In Iran one of the main sources of pigmented sewage is textile manufacturing. Hence, in order to remove colour of this sewage, the present study was conducted to evaluate potential of some bacteria for dye decolourising. To perform this investigation 30 samples from soil and 10 from sewage were collected. All bacteria with potential of decolorization were isolated and identified using biochemical tests. Out of seven different isolated bacteria three were Gram positive and four were Gram negative. Optimization of decolorization for all bacteria was carried out in second step with respect to evaluation of bacterial activity at different temperatures (25-45°C), pHs (5-9) and dye concentrations (0.01-0.04g/L). The results obtained indicated that the best temperature for all bacteria tested was 35°C, pH 8.0, and dye concentration 0.02 g/L. Therefore, decolorization of pigmented sewage for elimination of dangerous compounds could be possible using bacteria.

INTRODUCTION

Nowadays, the chemical materials and compounds are widely used in the whole world. Among them pesticides, chemical materials, used in farming and textile factories, and heavy metals, acids, bases and dyes are most important compounds which enter in the environment (Chang et al. 2001b, Zhao & Hardin 2007, Saratale et al. 2009a, Hogan et al. 2010).

Therefore, the role of human in controlling and maintaining the environment is so noticeable. Currently, scientists showed that annually 28000 kinds of textile dyes are discharged into the environment (Jin et al. 2007) and among them azo-dyes constitute almost 70% of the basic dyes used in the world (Telke et al. 2008). Therefore, biological decolorization in comparison with the chemical processes, is more economical and time consuming (Verm & Madamwar 2003, Rai et al. 2005) but it should be noted that the efficiency of microbial decolorization method depend on the selected microorganisms (Jadhav et al. 2007, Dawkar et al. 2008, Kalyani et al. 2008, Telke et al. 2008, Saratale et al. 2009b). Hence, the present study was conducted to reduce azo dyes, which are released by textile sewage from Kermanshah Textile Company (the greatest textile company in the west of Iran) into the Qare sou's river located near the factory using microbial isolates from environmental samples.

MATERIALS AND METHODS

Sample collection from wastewater and soil: For isolation of microorganisms, thirty soil samples were collected from the margin of the Qare sou's river which is located at 47°10'E and 34-47°24'N. The samples were taken in sterile polythene bags. In addition, the textile effluent was collected from the discharge tanks of a textile mill located in Kermanshah, Iran. The effluent was sampled in dry, sterile plastic cans and stored in the incubator at 15°C (Ramamurthy et al. 2011).

Isolation and identification of bacteria from the samples: The serial dilution technique using Brain heart infusion agar was performed to isolate bacteria from the soil as well as sewage. Afterward different morphological colonies were characterized using biochemical tests and selected for further analysis (Butani et al. 2011).

Dyes and chemicals: The 167 dispersed red dye were obtained from Hamadan Alvan Dye Factory, Iran which is consumed by Kermanshah Textile Company.

Evaluation of dye decolorization: Dye decolorization by isolated bacteria was experienced in 250 mL Erlenmeyer flasks with 250 mL Zhu and Zimmerman specific medium (g/L; 5-yeast extract, 5-glucose, 0.5-$(NH_4)_2SO_4$, 2.66-KH_2HPO_4, 4.32-Na_2HPO_4, 1000 mL distilled water) containing concentration of 0.01mg/L dispersed-Red dye 167. Then, the concentration increased to 0.02, 0.03 and 0.04 in the same conditions and the test was repeated. The inoculated media were incubated at 30°C on rotary shaker at 150 rpm for 6 days.

Spectrophotometric assessment of microbial dye decolorization: For evaluation of microbial decolorization, 10 mL

Table1: physiological and biochemical characterization of the isolated Gram positive bacteria.

Biochemical	Gram-stain test	Morphology	Sporformation	Catalase	Oxidase	Citrate	Urease	Starch Hydrolysis	Casein Hydrolysis	Gelatin Hydrolysis	6.5% NaCl	Arabinos	Mannitol	Nitrate reduction	Oxidativ/ Fermentative
S1	+	Bacill	+	-	-	-	-	+	+	+	+	+	+	+	+
S2	+	Bacill	+	-	-	-	-	+	+	+	+	+	+	+	+
S5	+	Bacill	+	-	-	-	-	+	+	-	+	+	+	+	-

Sw = sewage, S = soil

Table 2: Physiological and biochemical characterization of the isolated Gram negative bacteria.

Biochemical test	SW2	SW1	S4	S3
Gram stain	-	-	-	-
Morphology	rod	rod	rod	rod
Catalase				
Oxidase	-	-	-	-
Indol	-	-	-	-
Mr/Vp	-/+	-/+	-/+	+/-
Citrate	+	+	+	+
Urease	-	-	-	+
Lysin decarboxylase	-	-	+	+
Ornitin decarboxylase	-	+	+	-
Motility	-	-	-	-
Gelatin hydrolysis	+	-	-	-
Glucose	+	+	+	+
Lactose	+	+	+	+
Mannitol	-	+	+	+
surbitol	-	+	-	-
Arabinose	+	+	-	+
Xaylose	+	-	+	+
TSI	Alk/Ac	Alk/Ac	A/A+G	A/A
Oxidative/Fermentative	Fermentative	Fermentative	Fermentative	Fermentative

Sw = sewage, S = soil, Alk = Alkalin, Ac = Acid

of sample was drawn and centrifuged at 4000 rpm for 20 minutes (Ramamurthy et al. 2011). Then the decolorization was examined by measuring the absorbance of the supernatant at 600 nm using UV-VIS spectrophotometer. In addition, uninoculated medium was checked as a control and the experiment was performed in triplicate. Finally, decolorization efficiency was expressed as percentage of decolorization and calculated using equation (Ponraj et al. 2011).

Decolorization % = OD initial **-** OD final/OD final × 100

Effect of pH and temperature on decolorization: Effect of different pHs (5-9) and temperatures (25, 30, 35, 40 and 45°C) was evaluated on decolorization by growing the selected isolates in the ZZ medium containing specific dye. Samples were withdrawn after 6 days and decolourising activity was determined as described above.

RESULTS AND DISCUSSION

Isolation and identification of the bacteria from soil and wastewater samples: Totally, 5 and 2 different bacteria were isolated from the soil and wastewater, respectively. Out of seven bacteria, three were Gram positive, and four Gram negative (Tables 1 and 2).

Microbial dye decolorization: The results obtained from this study indicated that all of bacteria have ability to decolorize the colour (Fig. 1). With increasing the dye concentration to 0.02 mg/L percentage of decolorization has increased (Fig. 3), but with further increasing the dye concentration up to 0.04 mg/L the isolated bacteria were not able to grow in the same medium. Furthermore, the best bacterium for dye decolorization was the isolated bacterium from soil which belongs to the genus *Bacillus.*

Effect of different pH and temperature ranges on bacterial decolorization: As shown in Fig. 3, with increasing the pH from 5 to 8 decolorization has increased, and then at pH 9.0 it decreased, hence, the optimum pH for decolourisation was 8.0. In addition, the results obtained from effect of different temperatures on dye decolorization showed that the

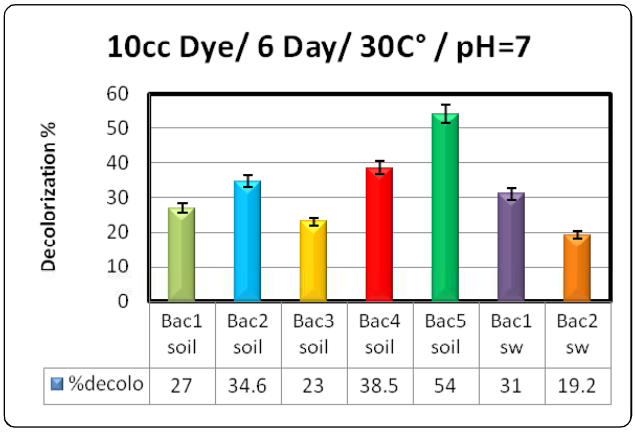

Fig. 1: Decolorization ability of isolated bacteria
Bac: Bacterium; Sw: Sewage

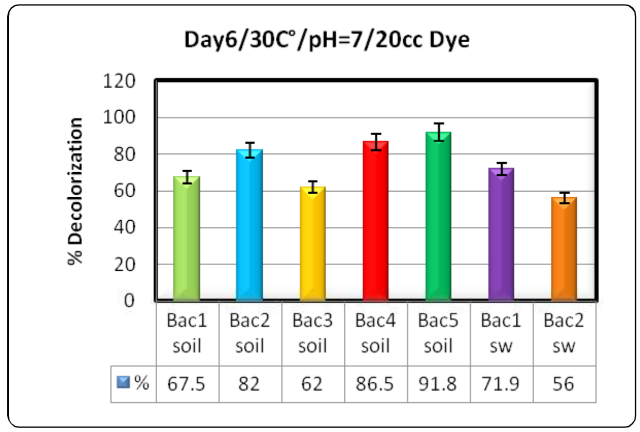

Fig.2: Decolorization ability of isolated bacteria with increasing of dye concentration (0.02 mg/L); Bac: Bacterium; Sw: Sewage

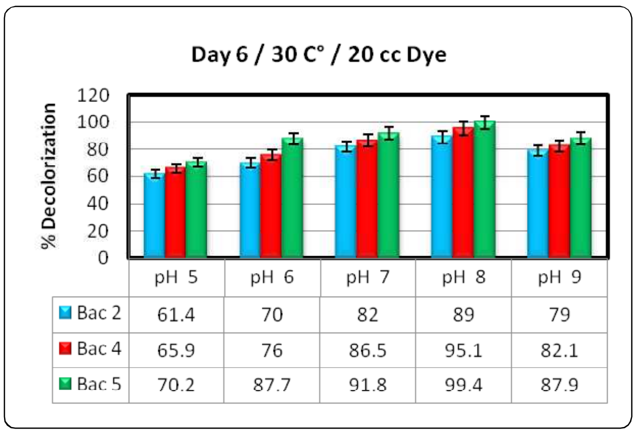

Fig. 3: Microbial decolorization at different pH ranges (5-9).

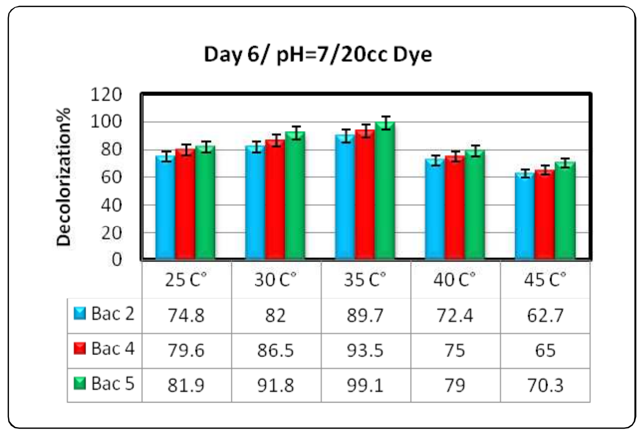

Fig. 4: Microbial decolorization at different temperature ranges (25-45ºC).

optimum temperature for selected isolates (Bac 2, 4 and 5) was 35°C.

Natural environment has an interaction between the human lives, therefore, any pollution or destruction may affect this environment. In the recent years, different types of industrial units and their wastewaters have resulted in many destructive effects on the environments (Mandapa et al. 2004, Petrus & Warchol 2005), for example, in south of Asia including India which has an advanced textile and dye industry, there are many environmental problems because of non-standard release of wastewater into the environment (Kumar et al. 1999, Grag & Kaushik 2008).

It should be noted that the physico-chemical structure of the wastewater released from Kermanshah textile factory shows a high concentration of pollution factors. The wastewater is dark pink with pH 6.1, which may cause many changes in water (Vandevivre et al. 1998). Therefore, although these pollutants have threaten the environment and people's life, nowadays biological methods have attracted the scientists' attention. The advantage of these methods, compared with the physical and chemical methods, is that they produce less sludge with low water consumption, and low cost (Forgacs et al. 2004, Zhang et al. 2004, Eichlerova et al. 2007). Hence, in the present study, using microbial isolates from soil and sewage, an evaluation on dye decolorization under aerobic condition was made. The results showed that all isolated microorganisms have more or less decolorization characteristics. Indeed, under aerobic conditions, mono-and di-oxygenase enzymes catalyse the oxygen bond in the loop of aromatic of initial organic compounds (Sarayu & Sanhya 2010, Lin et al. 2010). Another important factor, which is obvious, is environmental pH concerning with decolorization. pH can have considerable effect on decolorization (Chen et al. 2003, Kilic et al. 2007), which is confirmed by this work. Generally, researchers believe that the effect of pH is probably concerned with transferring the dye molecules into the cellular membrane of the organisms (Chang et al. 2001b, Kodam et al. 2005).

Furthermore, effect of different temperatures was evaluated on microbial dye decolorization. Scientists have shown that the physiological changes of microbial temperature may result in sudden change in the activation energy (Yu et al. 2001). In addition, the temperature has also affect the speed of growth, biomass products and mechanism (Blaga et al. 2008). Therefore, decolorization speed increase up to optimum temperature and then there is a slight decrease in decolorization activity, and the decrease might be due to the reduction of cellular life or denaturation of reductase enzyme (Chang et al. 2001a, Saratale et al. 2009c), which is in parallel with the present results. Overall, it should be noted that in each environment there are microorganisms which are adapted to the harsh environment and we can use them for bioremediation and biodegradation.

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