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Research Study

Microbial decolourization of methyl orange dye by *Pseudomonas spp.*

MP Shah*, KA Patel, SS Nair, AM Darji

Abstract

Introduction

We present a study aimed at assessing the ability of *Pseudomonas spp.* to decolourize and degrade methyl orange dye. *Pseudomonas spp.* could tolerate methyl orange dye for up to 500 mg^{-1}.

Materials and methods

A bacterium identified as *Pseudomonas spp.* was isolated from dye-contaminated soil. This strain rapidly decolourized a methyl orange azo dye solution. Features of the decolourizing process related to biodegradation and biosorption were also studied.

Results

The dye was efficiently decolourized in static compared with shaken cultures. The bacterium exhibited a remarkable colour-removal capability over a wide range of dye concentrations (50–200 mg/l), pH (6–10) and temperatures (30–40˚C). The *Pseudomonas spp.* decolourized the repeated addition of methyl orange dye for up to four cycles with variable decolourization rates (10–94%).

Conclusion

The strain can tolerate and decolourize azo dyes at high concentrations, making it an advantage for treatment of textile industry wastewaters. However, the strain needs to be tested on the treatment of real dye-bearing wastewaters using appropriate bioreactors.

* Corresponding author
Email: shahmp@uniphos.com

Applied & Environmental Microbiology Lab, Enviro Technology Limited (CETP), Plot No: 2413/2414, GIDC, Ankleshwar 393 002, Gujarat, India

Introduction

Rapid industrialization has necessitated the manufacture and use of different chemicals in day-to-day life[1,2]. The textile industry extensively uses synthetic chemicals as dyes. Wastewaters from textile industries pose a threat to the environment, as large amount of chemically different dyes are used. A significant proportion of these dyes enter the environment via wastewater[1]. Approximately 10,000 different dyes and pigments are used industrially, and over 0.7 million tons of synthetic dyes are produced annually, worldwide[3]. Pollution, due to the textile industry, effluent has increased during recent years. Moreover, it is very difficult to treat textile industry effluents because of their high BOD, chemical oxygen demand (COD), heat, colour, pH and the presence of metal ions[4]. The textile finishing generates a large amount of wastewater containing dyes and represents one of the largest causes of water pollution[5], as 10–15% of dyes are lost in the effluent during the dyeing process[6]. The traditional textile finishing industry consumes about 100 l of water to process about 1 kg of textile material. The new closed-loop technologies such as the re-use of microbial or enzymatic treatment of dyeing effluents could help in reducing this enormous amount of water pollution[7]. Azo dyes have been used increasingly in industries because of their ease and cost-effectiveness in synthesis compared with natural dyes. However, most azo dyes are toxic, carcinogenic and mutagenic[8]. Azo bonds present in these compounds are resistant to breakdown, with the potential for persistence and accumulation in the environment[9]. However, they can be degraded by bacteria under aerobic and anaerobic conditions[10]. Several physicochemical techniques have been proposed for the treatment of coloured textile effluents. These include adsorption on different materials, oxidation and precipitation by Fenton's reagent, bleaching with chloride or ozone photo degradation or membrane filtration[11]. All these physical or chemical methods are very expensive and result in the production of large amounts of sludge, which creates the secondary level of land pollution. Therefore, economic and safe removal of the polluting dyes is still an important issue. Bioremediation through microorganisms has been identified as a cost-effective and environment-friendly alternative for disposal of textile effluent[12,13]. In recent years, a number of studies have focused on some microorganisms capable of degrading and absorbing dyes from wastewater. A wide variety of microorganisms are reported to be capable of decolonization of dyes[14–26]. The current study evaluated the potential of the isolated bacterial strain from textile effluent for its decolourization efficiency of the textile dye methyl orange (MO).

Materials and methods

The protocol of this study has been approved by the relevant ethical committee related to our institution in which it was performed.

Screening of decolourizers

Soil near to textile industrial outlet was used as a source for enrichment and isolation of decolourizers. The screening medium (SM) contained peptone, 10g; meat extract, 10g and NaCl, 5g in 1 l of distilled water with 0.5 g of MO. MO dye was sterilized

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by passing it through a 0.45-µm pore size filter, while other components were sterilized at 121˚C for 20 min. Ten grams of soil was then added to a 500-ml Erlenmeyer flask containing 100 ml of SM. The cultures were incubated at 32˚C on a rotary shaker at 120 rpm. Next, the broth of the decolourized flask was transferred to fresh SM to screen the strain having colour-removing ability. The screening procedure in the liquid culture was conducted repeatedly until a decolourized culture occurred. A small amount of decolourized broth was then poured into an agar plate containing SM, and it was incubated at 32˚C. Colonies surrounded by decolourized zones were selected. Isolates were then tested for their colour-removal ability in a submerged culture, and the best isolate was selected. Finally, identification of the isolate was done by Bergey's Manual of Determinative Bacteriology (2000)[27,28].

Dyes

MO was procured from a local dye industry (Ankleshwar, Gujarat, India). Dye was checked for its colour, solubility in water, ethanol and absorption maximum. Stock solution of 6,000 ppm was prepared by dissolving the dye in distilled water and was filter sterilized and kept at 4˚C. Dyes at different concentrations (50, 100, 150, 200, 400 and 500 ppm) were used to study their effect on bacterial growth and adsorption after adding to the culture media.

Decolourization experiments

All decolourization experiments were performed in three sets. The culture with OD 0.699 at 540 nm at a concentration of 4% was inoculated in a 250 ml Erlenmeyer flask containing 100 ml SM and incubated at 32˚C for 24 h. After 24 h of incubation, the dye was added at a concentration of 150 mg/l, and 3 ml of the culture media was withdrawn at different time intervals. Aliquot was centrifuged at 6,000 rpm for 10 min to separate the bacterial cell mass, and the clear supernatant was used to measure the decolourization at the absorbance maxima of the dye. Abiotic controls (without microorganism) were always included[29].

The percentage decolourization was calculated as follows:

$$\%\ \text{Decolourization} = \frac{\text{Initial OD} - \text{Observed OD}}{\text{Initial OD}} \times 100$$

Effect of dye concentration

The various concentrations of dye (50, 100 150, 250 and 400 mg/l) were added into the culture medium in order to examine the effect of initial dye on the decolourization in static conditions at various time intervals[29].

Effect of temperature

The inoculated SM was incubated at various temperatures (10, 32, 37 and 50˚C) in static conditions for 48 h. The effect of temperature on dye decolourization was checked spectrophotometrically after 48 h[30].

Effect of pH of culture medium

The pH of the inoculated SM was adjusted to 2, 4, 6, 7, 8 and 10 with 1 M HCl or 1M NaOH. The effect of pH on dye decolourization was checked spectrophotometrically after 48 h[30].

Decolourization at static and shaking conditions

Decolourization ability of bacterial isolate was tested in shaking and static conditions at optimum pH (7.0) and temperature (32˚C) using SM with 150 mg/l of MO. The supernatant was withdrawn at intervals of 24 h for 4 days and was used for analysis of COD and decolourization. Decolourization was monitored spectrophotometrically and COD was determined according to the standard method[31].

Effect of glucose and peptone on dye decolourization

To study the effect of carbon and nitrogen sources on decolourization of MO, mineral medium with trace element addition and varied concentrations of glucose/peptone from 1% to 5% and 150 mg/l of dye were used[29].

Change in absorption spectra during dye decolourization

The change in peak in absorption spectrum reveals the dye adsorption or biodegradation during decolourization by isolate. Variation of UV-visible spectra of azo dye solution at a concentration of 150 mg/l MO with *Pseudomonas spp.* was checked at 0, 24 and 48 h intervals spectrophotometrically[27].

Assimilation of dye

An attempt was carried out to test the isolate ability to decolourize 150 mg/l MO in mineral medium depleted of carbon or nitrogen or both. The decolourization was read spectrophotometrically after 48 h[30].

Fed batch decolourization of MO by isolate

The fed batch decolourization of MO dye was also studied; in this study 150 mg/l dye was added into the 24-h grown culture of bacterial isolate. After decolourization, 150 mg/l dye was added into the decolourized broth without supplement of additional nutrient. Dye was added continuously until the culture did not lose decolourization ability. The dye concentration was determined by monitoring the absorbance of dye spectrophotometrically[31].

Decolourization of azo dyes in consortium

Different azo dyes viz. methyl red, MO and Congo red at the final concentration of 150 mg/l was used in SM with isolate, and dye reduction was checked spectrophotometrically for 4 days at 24-h intervals.

Results

Data in Figure 1 depict that at the lowest dye concentration (50–200 mg/l), the dye was decolourized more than 84% after 4 days incubation. As the dye concentration increased in the

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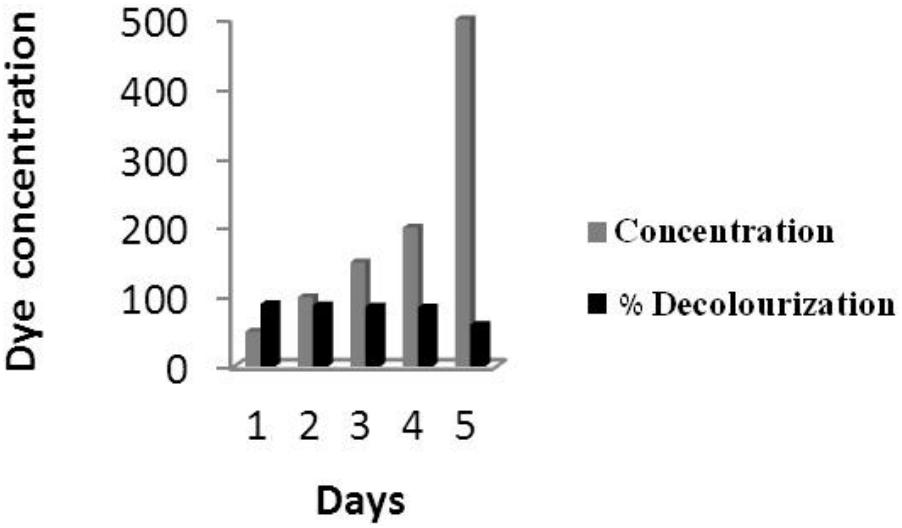

Figure 1: Effect of dye concentration on decolourization performance of *Pseudomonas spp.*

culture medium, a decline in colour removal was attained. This might be attributed to the toxicity of dye to bacterial cells through the inhibition of metabolic activity, saturation of the cells with dye products, inactivation of transport system of the dye or the blockage of active sites of azo reductase enzymes by the dye molecules. Under given experimental conditions, 60% decolourization was attained upon using 500 mg/l of the dye after 4 days. Bacterial growth in the presence of MO was also studied using control without dye, which showed the dye has an inhibitory effect on growth of bacteria as the number of bacteria was decreased in the presence of MO as compared with the control without dye (data not shown). The rate of chemical reaction is a direct function of temperature. Bacteria require optimum temperature for growth. The operating temperature of the incubation process varied between 10˚C, 30˚C, 37˚C and 50˚C, to study the effect of temperature on the decolourization process (Figure 2). At temperatures below 37˚C, due to slow growth of the bacteria, it took more days for decolourization, and at temperatures above 37˚C, the activity of *Pseudomonas spp.* and hence percentage of decolourization decreases. The variation in pH of the growth medium results in change in activity of bacteria and hence the bacterial growth rate as well as decolourization. Bacteria are active over a certain range of pH. In contrast with other decolourizing microbes like fungi with a narrow pH range, *Pseudomonas spp*. cells proved to be of desirable characteristics, removing MO colour over a wide range of pH (6–10) with the optimum pH being 8 (80% dye decolourization). A large decrease in decolourization occurs at high acidic pH (2–4) (Figure 3). This is an advantage of this bacterium for developing a practical bioprocess in treating dyeing mill effluents. Hence the bacteria are preferred over fungi for dye decolourization. The pattern of MO decolourization in static as well as in shaken cultures was elucidated in the medium. Figure 4 shows that lower decolourization percentages were exhibited in shaken cultures compared with static ones. Maximal efficiency of MO decolourization (86%) was achieved in 4 days when incubated statically. These observations suggest that the decolourization performance of *Pseudomonas spp.* was better in the presence of low oxygen content. The reason could be due to competition of abundant oxygen and the azo compounds for the reduced electron carriers under aerobic conditions. The cell growth in shaking condition was higher than static conditions, but there was less decolourization (65%) with more COD removal (48%) under shaking conditions and more decolourization (86%) with less COD removal (25%) under static condition within 4 days (data not shown). These findings are

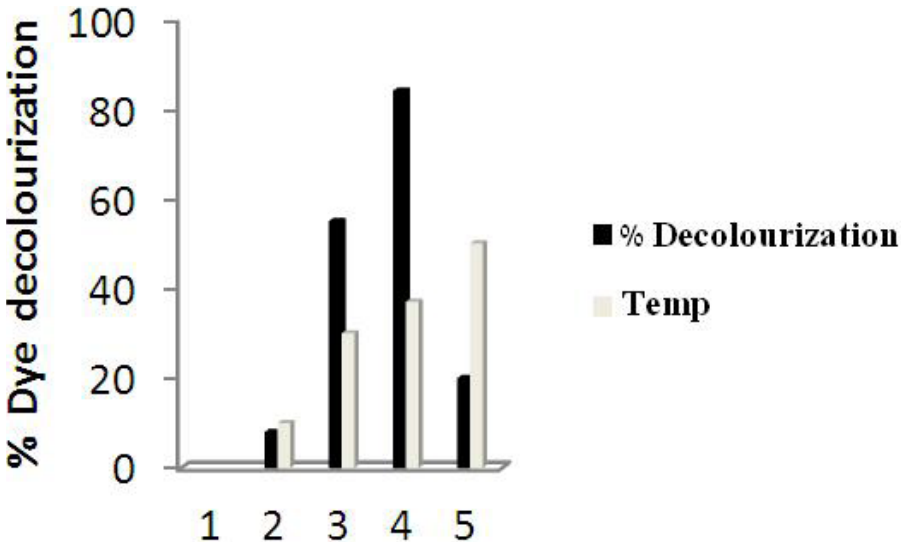

Figure 2: Effect of temperature on decolourization by *Pseudomonas spp*.

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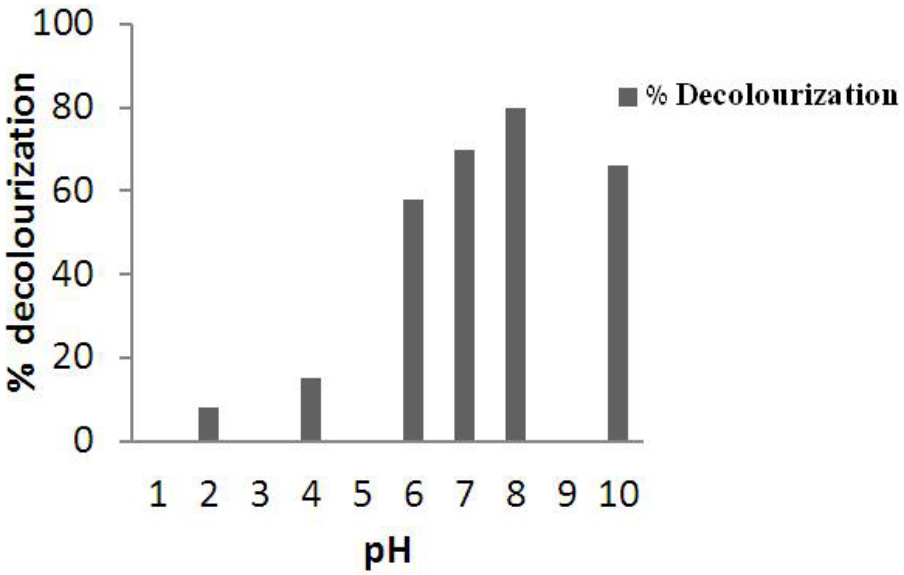

Figure 3: Effect of pH on decolourization by *Pseudomonas spp*.

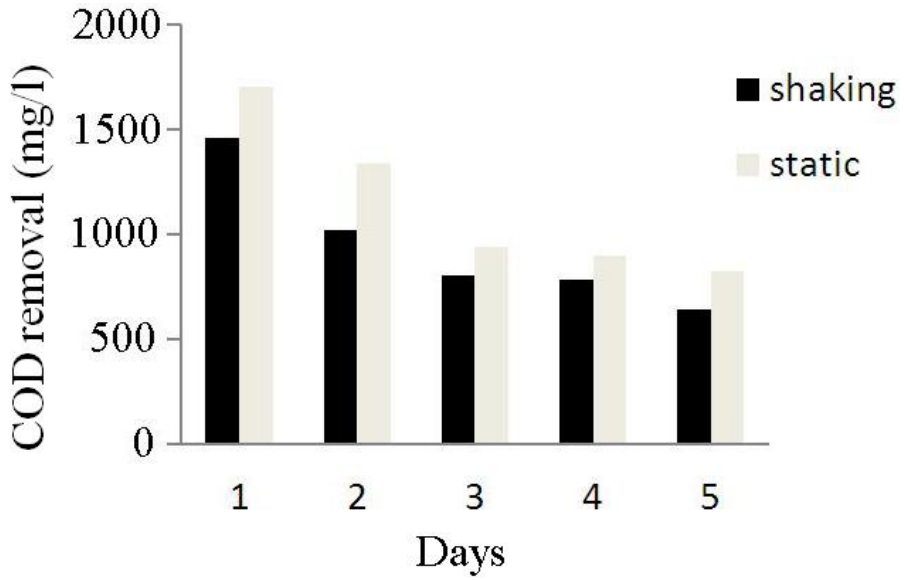

Figure 4: Effect of static and shaking condition on decolourization by *Pseudomonas spp*.

consistent with the results shown by Ozdemir et al.[33] who suggested COD removal is more under shaking conditions. Addition of a carbon source such as glucose at different concentrations has an effect on the percentage of decolourization (data not shown). The concentration of glucose varied from 1% to 5%, and it was found that the percentage of decolourization increases with the increase in concentration of glucose due to decrease in lag period. The percentage decolourization decreases with the increase in concentration of peptone up to a maximum peptone concentration of 1% (75% dye decolourization) after which there is a decrease in percentage of decolourization. Results obtained in the presence of different heavy metals are shown in Figure 5. Data indicate that the process of colour removal is significantly inhibited by the presence of mercuric chloride (11%) and potassium dichromate (12%), especially during the initial period (1–2 days) of the incubation. Marginal inhibition in colour uptake is noticed by the presence of silver nitrate, zinc sulphate and cadmium chloride. Hence the bacteria are able to tolerate the toxic effect of silver nitrate, zinc sulphate and cadmium chloride to achieve decolourization. Slow rates of colour uptake in the presence of chromium and mercury may be related to heavy metal inhibition of enzymes and metabolic pathways. Similar data were reported by Sumathi et al. (2001)[34] who studied the effects of Cr^{+6} on *Aspergillus foetidus* in the decolourization of Procion dyes. Results display the change of UV-visible spectra of MO, using the supernatant fluid of the culture at 0, 24 and 48 h (data not shown). The absorbance peak at 440 nm disappears after cultivation. The fed batch decolourization study was carried out to check the ability of isolate for the decolourization of repeated added dye. The *Pseudomonas spp*. decolourized the repeated addition of MO dye for up to four cycles (each 24 h) with variable decolourization rates (10–94%). In the first cycle, 93% decolourization occurred and in the second cycle 70% decolourization occurred, and the decolourization percentage keeps decreasing (up to 10% at fourth cycle) as the number of cycles increases (data not shown). Our isolate also has the ability to decolourize the following azo dyes viz. MO, methyl red, Congo red and tartrazine in consortium. Figures 6 and 7 depict that it can decolourize these mixed dyes up to 75 % in 4 days.

Discussion

Industrial effluent is not stable, and it varies often in a wide range depending on the process practiced. South

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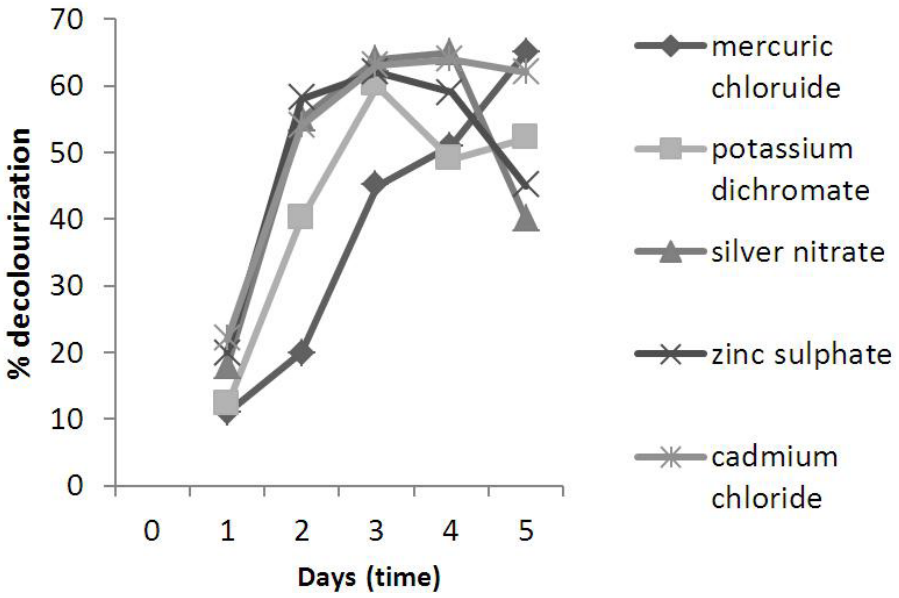

Figure 5: Effect of heavy metals on rate of uptake of dye during decolourization by *Pseudomonas spp.*

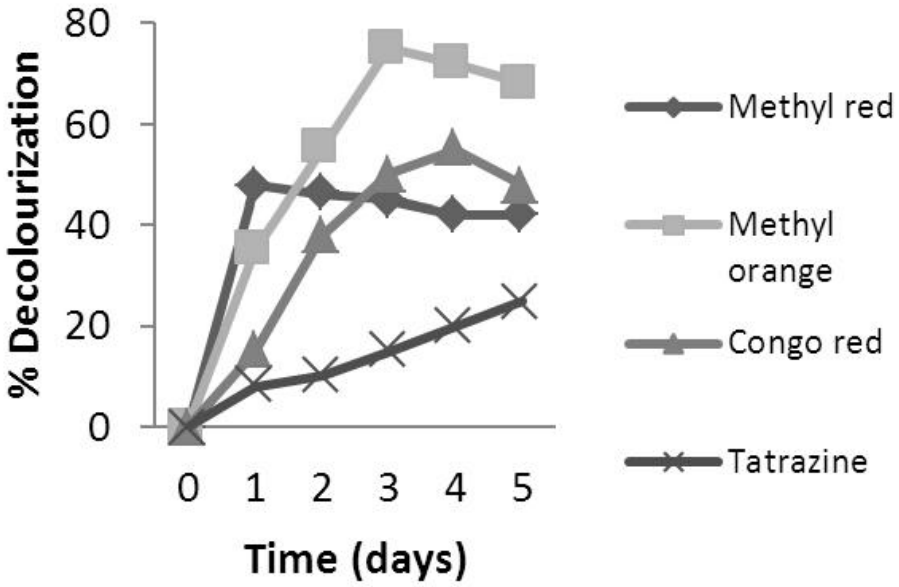

Figure 6: Decolourization of different azo dyes by *Pseudomonas spp*.

Asian countries are experiencing severe environmental problems due to rapid industrialization. This phenomenon is very common where the polluting industries like textile dyeing, leather tanning, paper and pulp processing, sugar manufacturing, etc. thrive as clusters. Among these, the textile industries are large industrial consumers of waters as well as producers of wastewater. The effluent discharged by this industry leads to serious pollution of groundwater and soils and ultimately affects the livelihood of the poor[32]. The textile industry is an industry that generates a high volume of wastewater. Strong colour of the textile wastewater is the most serious problem of the textile waste effluent. The disposal of these wastes into receiving waters causes damage to the environment. Dyes may significantly affect photosynthetic activity in aquatic life because of reduced light penetration and may also be toxic to some aquatic life due to the presence of aromatics, metals, chlorides, etc. Synthetic dyes are extensively used in the textile and printing industries. Azo dyes are the most important group of synthetic colourants. These are the largest class of dyes and more than half of the annually produced dyes. Dye wastewater from textile or dye stuff industry is one of the most difficult to treat because dyes have various synthetic origins and they contain complex aromatic molecular structures, which make them more stable and more difficult to be degraded. The removal of dyes from the textile waste effluent has been carried out by physical and chemical methods, such as flocculation, membrane filtration, electrochemical techniques, ozonation, coagulation and adsorption. In recent years, a number of studies have focused on some microorganisms, which are able to biodegrade and biosorb dyes in wastewaters. A wide variety of microorganisms capable of decolourizing a wide range of dyes include some bacteria, fungi and algae. The use of microorganisms for the removal of synthetic dyes from industrial effluents offers considerable advantages. The process is relatively inexpensive, it is a simple method, the running costs are low and the end products of complete mineralization are not toxic. The present study was designed to test decolourization of azo dye by bacteria isolated from the textile effluent drainage site. For this purpose, soil near textile effluent outlets was collected, and enrichment and isolation for azo dye-decolourizing bacteria was carried out in SM containing azo dye. Microorganism showing maximum decolourization in less time was selected and using Bergy's Manual of Determinative

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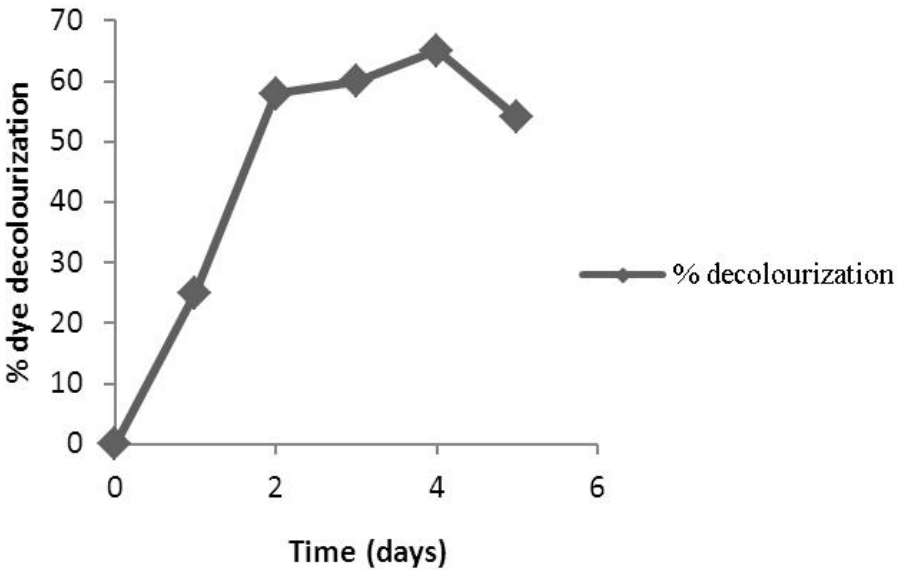

Figure 7: Decolourization of azo dyes in consortium by *Pseudomonas spp.*

Bacteriology (2000) was identified as *Pseudomonas spp.* The time course of MO decolourization was studied at different initial concentrations (50–500 mg/l) in static cultures. Dye decolourization is a metabolic process that causes a shift in temperature from optimum results into a decrease in dye decolourization as high temperature causes thermal inactivation of proteins and possibly of cell structures such as the membrane. Also, to achieve an effective colour removal, agitation and vigorous aeration should be avoided. The decreased decolourization results from nitrate or nitrite, a reducing equivalent that cells generated from peptone consumption. These metabolites of nitrate/nitrite may compete with the azo dye and result in less decolourization (data not shown). In addition to a complex mixture of dyes, the textile mill effluents often contain heavy metals, which generally affect the uptake and metabolism of azo dyes. Decolourization of the dye solution by bacteria could be due to adsorption to microbial cells or to biodegradation[35]. In adsorption, examination of the absorption spectrum would reveal that all peaks decreased approximately in proportion to each other. If dye removal is attributed to biodegradation, either the major visible light absorbance peak would completely disappear or a new peak would appear. Dye adsorption would result in cell mats, which are deeply coloured because of adsorbed dyes, whereas those retaining their original colours are accompanied by the occurrence of biodegradation.

Conclusion

In this study, a decolourizing bacterial strain, *Pseudomonas spp.*, was isolated from dye-contaminated soil. *Pseudomonas spp.* showed decolourizing activity through a degradation mechanism rather than adsorption, and it could tolerate high concentrations (up to 500 mg^{-1}) of MO. With high degradative and decolourizing activity against various reactive dyes commonly used in the textile industries, it is proposed that *Pseudomonas spp.* has practical application potential in the biotransformation of various dye effluents. The effects of oxygen, pH, temperatures and dye concentration on the decolourization of MO were investigated. Examination of the mechanism of the decolourization process indicated that it proceeded primarily by biological degradation associated with a minor portion of the dye adsorbing onto the cell surface. Identification and toxicity study of products from the degradation of MO dye by *Pseudomonas spp*. is now in progress. This observation has established that the bacteria are adaptive in nature and can degrade contaminants. The ability of the strain to tolerate and decolourize azo dyes at high concentration gives it an advantage for treatment of textile industry wastewaters. However, the potential of the strain needs to be demonstrated for its application in the treatment of real dye-bearing wastewaters using appropriate bioreactors.

Abbreviations list

COD, chemical oxygen demand; MO, methyl orange; SM, screening medium

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