

MICROBIAL DECOLOURIZATION OF CONGO RED DYE BY *Pseudomonas spp.*

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Abstract: The present study focused at evaluating the ability of *Pseudomonas spp.* to decolourize and degrade Congo Red dye. *Pseudomonas spp.* could tolerate Congo Red dye up to 400mg⁻¹. A bacterium identified as *Pseudomonas spp.* was isolated from soil that is contaminated by dye. This strain expeditiously decolourizes a Congo red azo dye solution. The decolourizing process related to biodegradation and biosorption were also studied. The dye was efficiently decolourized in static compared to shake cultures. The bacterium showed a phenomenal colour removal capability over a wide range of dye concentration (50-300 mg/l), pH (5-9) and temperatures (35-45°C). The *Pseudomonas spp.* decolourized the repeated addition of Congo Red dye up to four cycles with variable decolourization rate (15-90%).

Keywords: Congo red, pseudomonas, temperature, pH, biodegradation

1. INTRODUCTION

Rapid industrialization has made the manufacturing and use of different chemicals in our daily life. The textile industry is one of the industries which mostly use synthetic chemicals as dyes. The wastewaters from textile industries are dangerous to the environment, because great amount of dyes that are chemically different are used. Through wastewater notable proportion of these dyes enter the environment. Approximately 10,000 different dyes and pigments are used industrially and over 0.7 million tons of synthetic dyes are produced annually, worldwide [24]. In recent years due to textile industry effluent pollution is increased. Moreover, it is very difficult to treat textile industry effluents because of their high BOD, COD, heat, colour, pH and the presence of metal ions [4]. The textile finishing generates a large amount of waste water containing dyes and represents one of the largest causes of water pollution [6], as 10-15% of dyes are lost in the effluent during the dyeing process [35]. The traditional textile finishing industry consumes about 150 liters of water to process about 1.5 Kg of textile material. The new closed-loop technologies such as the enzymatic treatment of effluents from dyeing industry could help reducing this gigantic water pollution [1]. Azo dyes is most common dye that has been used in industries and its use is increasing in industries because of their effortlessness and cost effectiveness in synthesis compared to natural dyes. However, most azo dyes are toxic, carcinogenic and mutagenic [22]. In these compounds the azo bonds that is present are resistant to breakdown, with the possibilities for their stability and accumulation in the environment [29]. However, they can be degraded by bacteria under aerobic and anaerobic conditions [33]. Many techniques have been suggested for 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 [25]. These all methods either physical or chemical are very expensive and has drawback as large amounts of sludge is created, which creates the lower level of land pollution. Therefore, effective and safe removal of these polluting dyes is still an important issue. Bioremediation by microorganisms technique has been determined as a remunerative and environment friendly for discarding of textile effluent [9, 23]. In past few years a number of studies has been done and they have focused on some microorganisms that are capable of degrading and fascinating dyes from wastewater. A wide variety of microorganisms are reported to be capable of decolonization of dyes [2,3,7-8,11-15,18,26,31,34,18]. This study deals with the separation of textile dyes degrading bacterium from the environment contaminated by dye and its ability to degrade reactive dyes.

2. MATERIALS & METHODS

2.1. Screening of Decolourizers

Soil was collected from textile industrial outlet and was used as source for enrichment and separation of decolourizers. The screening medium (SM medium) contained: peptone, 9g; meat extract, 9g; NaCl, 4g; in 1 liter of distilled water with 0.4 g of Congo Red. Congo Red dye was sterilized by passing it through a 0.45-µm pore size filter, while other components were sterilized at 121°C for 30 min. Five grams of soil was then added to a 500-ml Erlenmeyer flask containing 110 ml of SM medium. The cultures were incubated at 37°C on a rotary shaker at 200 rpm. Next, the broth of the decolourized flask was transferred to fresh SM medium 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 medium and it was incubated at 37°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) (Chen et al., 1999; and Syed et al., 2008).

2.2. Dyes

Congo Red (CR) was acquired from local dye industry, Ankleshwar, Gujarat, India. Dye was checked for its colour, solubility in water, ethanol, and absorption maximum. Stock solution of 6500 ppm was prepared by dissolving the dye in distilled water and was filter sterilized and kept at 5°C. Dye at different concentrations (50ppm, 100 ppm, 200 ppm, 300 ppm, 400 ppm) were used to study their effect on bacterial growth and adsorption after adding to the culture media.

2.3. Decolourization Experiments

All decolourization experiments were carried out in three sets. The culture with OD 0.7 at 560 nm at concentration of 5% was inoculated in 250 ml Erlenmeyer flask containing 110ml Screening medium and incubated at 37°C for 24 h. After 24h of incubation, dye was added at concentration of 160 mg/l and 3.5 ml of the culture media was withdrawn at different time intervals. Aliquot was centrifuged at 7000 rpm for 15 minutes to separate the bacterial cell mass, clear supernatant was used to measure the decolourization at the absorbance maxima of the dye. Abiotic controls (without microorganism) were always included (Parshetti et al., 2006).

The percentage decolourization was calculated as follows-

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

2.4. Effect of Dye Concentration

The various concentrations of dye (50, 100, 200, 300, 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 (21).

2.5. Effect of Temperature

The inoculated SM medium was incubated at various temperatures (8, 21, 37 and 57°C) in static conditions for 48hrs. The effect of temperature on dye decolourization was checked spectrophotometrically after 48hrs [19].

2.6. Effect of pH of Culture Medium

The pH of the inoculated screening medium was adjusted to 3, 5, 7, 8, 9 and 11 with 1M HCL or 1M NaOH. The effect of pH on dye decolourization was checked spectrophotometrically after 48 hrs [19].

2.7. 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 (37°C) using screening medium with 160 mg/l of Congo Red. The supernatant was withdrawn at interval of 24 hrs for four days and was used for analysis of COD and decolourization.

Decolourization was monitored by spectrophotometrically and chemical oxygen demand (COD) was determined according to standard method [30].

2.8. Effect of Glucose and Peptone on Dye Decolourization

To study the effect of carbon and nitrogen sources on decolourization of Congo Red, Mineral medium with trace element addition and varied concentration of glucose / peptone from 1-6% and 160 mg/l of dye was used [21].

2.9. 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 concentration of 160 mg/l Congo Red with *Pseudomonas spp.* ETL-M was checked at 0, 24 and 48 hrs intervals spectrophotometrically [10].

2.10. Assimilation of Dye

An attempt was carried to test the isolate ability to decolourize 160 mg/l Congo Red in mineral medium depleted from carbon or nitrogen or both. The decolourization was read spectrophotometrically after 48 hrs [19].

2.11. Fed Batch Decolourization of Congo Red by Isolate

The fed batch decolourization of Congo Red dye was also studied, in this study 160 mg/l dye was added into the 24hrs grown culture of bacterial isolate. After decolourization 160 mg/l dye added into the decolourized broth without supplement of additional nutrient. Dye was added continuously until culture does not lose decolourization ability. The dye concentration was determined by monitoring the absorbance of dye spectrophotometrically [30].

2.12. Decolourization of Azo Dyes in Consortium

Different Azo dyes viz. Methyl red, Methyl orange, Congo red, Tatrazine at the final concentration of 160 mg/l was used in screening medium with isolate and dye reduction was checked spectrophotometrically for five days at 24 hrs intervals.

3. RESULTS & DISUSSION

Industrial effluent is unstable and it varies and is dependent on the process practiced. South Asian countries are experiencing severe environmental problems due to rapid increase in industrialization and urbanisation. This happening is very common where the industries like textile dyeing, leather tanning, paper and pulp processing, sugar manufacturing, etc. thrive as clusters. Among these industries the Textile industries are largest consumers of waters and also they produce large amount of wastewater. The sewage discharged by this industry leads to serious pollution of groundwater and soils and eventually affects the living of the poor [17]. The textile industry is one of the industries that produce highest volume of waste water. The most serious problem of the textile waste effluent is the strong colour of textile waste water. The disposal of these waste water into flowing or static waters causes severe damage to the environment. Dyes may notably influence photosynthetic activity in aquatic life because the light penetration will decrease and it may also be dangerous to some aquatic life due to the presence of many aromatics compounds, metals, chlorides, etc. In the textile and printing industries synthetic dyes are mostly used. Azo dyes are the most important group of synthetic colourants and are the largest class of dyes. Azo dyes are the dyes that are mostly used. Due to various synthetic origin of dyes from textile industry or dye stuff industry the waste water of these industry is one of the difficult to treat. And due to presence of complex aromatic molecular structures in them, which make them more stable and more difficult to be degraded. By physical & chemical methods, such as flocculation, membrane filtration, electrochemical techniques, ozonation, coagulation adsorption etc is being carried out to remove the dyes from textile waste water. In recent years, a number of studies have been done and is focused on some microorganisms which are capable to biodegrade and biosorb dyes in waste waters. A wide variety of microorganisms are able to decolourize a wide range of dyes include some bacteria, fungi and algae. It offers many advantages by using microorganisms for removal of synthetic dyes from textile or

dyestuff or any industrial effluent. The process is relatively not expensive and it is simple method and its running costs are not much high and the end products of absolute mineralization are not poisonous. This study was planned to test decolourization of azo dye by bacteria that were isolated from textile effluent drainage site. The soil was collected from place that was near to textile effluent drainage site & enrichment and separation for azo dye decolourizing bacteria was carried out in screening medium containing azo dye. Microorganism that showed maximum decolourization in less time was chosen & using Bergy's Manual of Determinative Bacteriology (2000) was identified as *Pseudomonas spp.* The time course of congo red decolourization was studied at different initial concentrations (50 – 400 mg /L) in static cultures. Data in Figure 1 depict that at the lowest dye concentration (50- 400 mg/L) the dye was decolourized more than 82 % after four days incubation. As the dye concentration increased in the culture medium, there was a decrease in colour removal. This might be assigned to the toxicity of dye to bacterial cells through the hindrance of metabolic activity, saturation of the cells with dye products, inactivation of transport system of the dye or the blockage of active sites of azoreductase enzymes by the dye molecules. Under given experimental conditions, 65% decolourization was attained upon using 400 mg /L of the dye after four days. Bacterial growth in presence of congo red was also studied using control in which the congo red dye was not added, which showed the dye has hindering effect on growth of bacteria as in presence of congo red the number of bacteria were decreased reaction is the direct function of temperature. Bacteria require ideal temperature for growth. However dye decolourization is metabolic process hence shift in temperature from optimal results into decrease in dye decolourization as high temperature causes thermal inactivation of proteins & possibly of such cell structures such as membrane. The operating temperature of the incubation process varied between 8°C, 21°C, 37°C, 57°C to study the effect of temperature on the decolourization process (Figure 2). At temperature 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 & hence the bacterial growth rate as well as decolourization. Bacteria are active over certain range of pH. The optimum pH for the growth is the same for the dye decolourizing activity as it is mainly the metabolic process. In contrast with other decolourizing microbes like fungi with narrow pH range, *Pseudomonas spp.* cells proved to be of desirable characteristic, removing congo red colour over a wide range of pH (6 – 11) with optimum at pH 8 (90 % dye decolourization). There is a decrease in decolourization at high acidic pH (2- 4) (Figure 3). In case of fungi increase in pH greater than 5.5 resulted in the fragmentation of mycelia pellets & below 5.5 there is no appreciable growth of fungi hence percentage of decolourization decreases. Hence the bacteria are chosen over fungi for dye decolourization. The pattern of congo red decolourization in static as well as in shaken cultures was elucidated in medium. Figure 4 shows that lower decolourization percentages was shown in shaken cultures compared to static ones. Maximal efficiency of congo red decolourization (90%) was achieved in four days 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 condition. Yan et al., 2004, also revealed that to achieve an effective colour removal, agitation and vigorous aeration should be avoided. The cell growth in shaking condition was higher than static condition but there was less decolourization (60%) with more COD removal (45 %) under shaking condition, while 80 % decolourization with less COD removal (20%) under static condition within four days (Data not shown). These findings are consistent with result shown by Guven Ozdemir et al., who suggested COD removal is more under shaking condition. 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 was varied from 1% to 6 % 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 maximum peptone concentration of 1% (70% dye decolourization) and after which there is decrease in percentage of decolourization. The decrease 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, the textile industry effluents often contain heavy metals which generally affect the uptake and metabolism of azo dyes. Results obtained in the presence of different heavy metals are shown in Figure 5. Data indicates that the process of colour removal is significantly

inhibited by the presence of Mercuric chloride (10%) & Potassium dichromate (12%) especially during the initial period (1-2 days) of the incubation. Marginal inhibition in colour uptake is noticed in the presence of Silver Nitrate, Zinc Sulphate & Cadmium Chloride. Hence the bacteria are able to tolerate the toxic effect of Silver Nitrate, Zinc Sulphate & Cadmium Chloride to achieve decolourization. Slow rates of colour uptake in the presence of Chromium & Mercury may be related to heavy metal inhibition of enzymes and metabolic pathways. Similar data were reported by Sumathi et al., (2001) who studied effect of Cr^{+6} on *Aspergillus foetidus* in decolourization of portion dyes. Decolourization of the dye solution by bacteria could be due to adsorption to microbial cells or to biodegradation. 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. Result displays the change of UV-visible spectra of congo red, using the supernatant fluid of the culture at 0, 24, and 48 hrs (Data not shown). Decolourizing cultivation with *Pseudomonas spp.* The absorbance peak at 450nm 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 congo red dye up to four cycles (each 24h) with variable decolourization rate (20 – 90%). In first cycle 90% decolourization occurred, 75% decolourization in second cycles & the percent decolourization goes on decreasing (up to 10% at 4th cycle) as the number of cycle increases (Data not shown). Our isolate also has the ability to decolourize following azo dyes viz. Methyl orange, Methyl red, Congo red & Tartrazine in consortium. Figure 6 & Figure 7, depict that it can decolourize these mixed dye up to 65 % in four days.

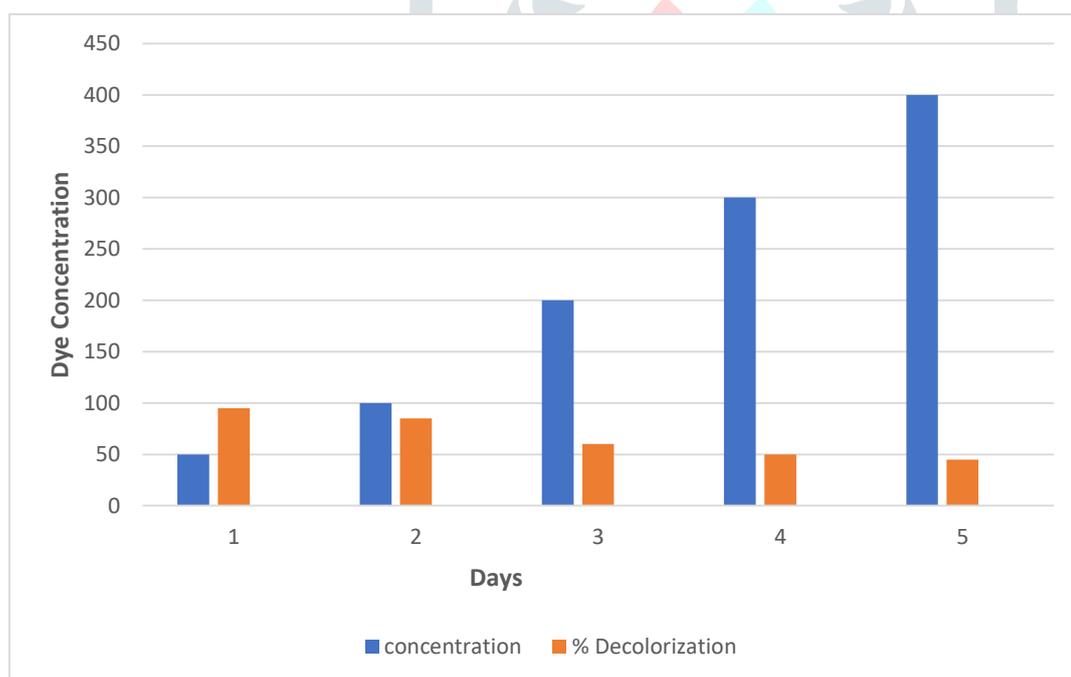


Figure 1. Effect of Dye concentration on decolourization performance of *Pseudomonas spp.*

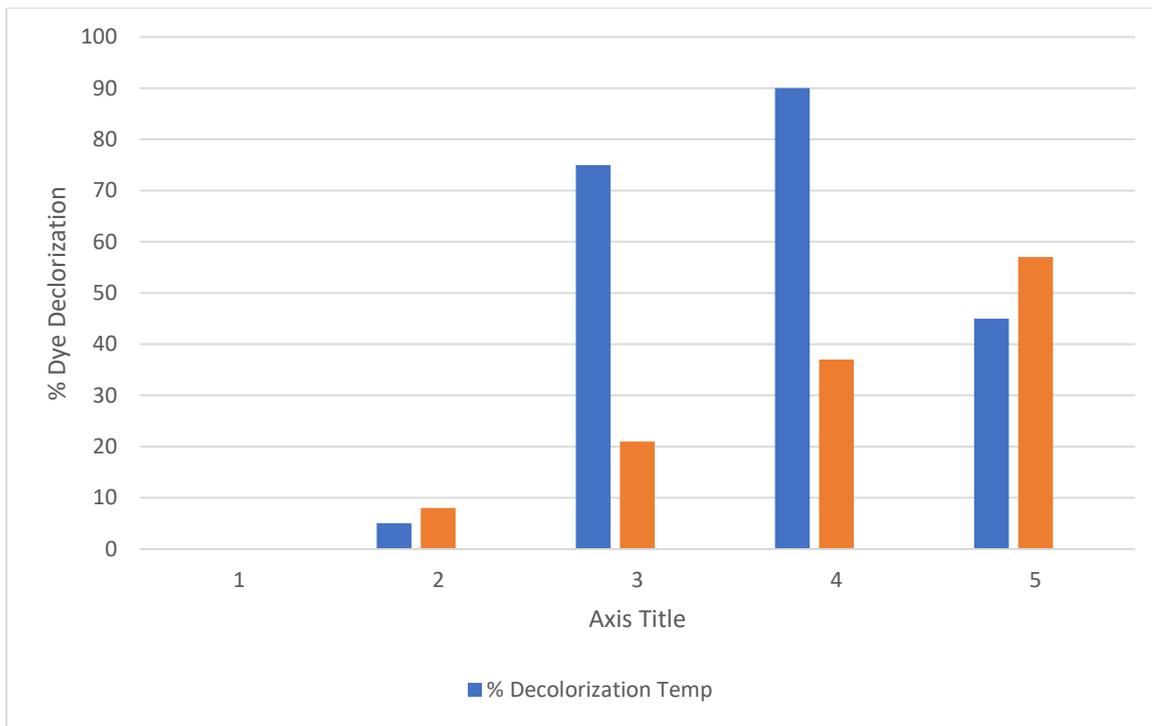


Figure 2. Effect of Temperature on decolourization performance of *Pseudomonas spp.*

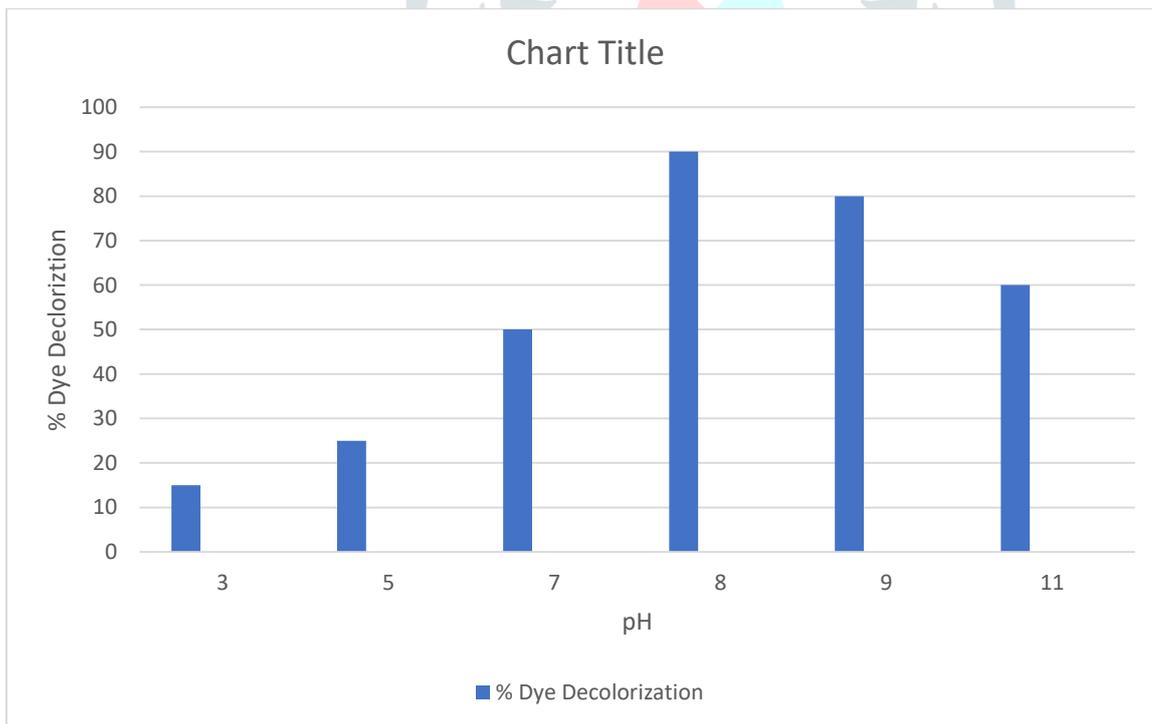


Figure 3. Effect of pH on decolourization performance of *Pseudomonas spp.*

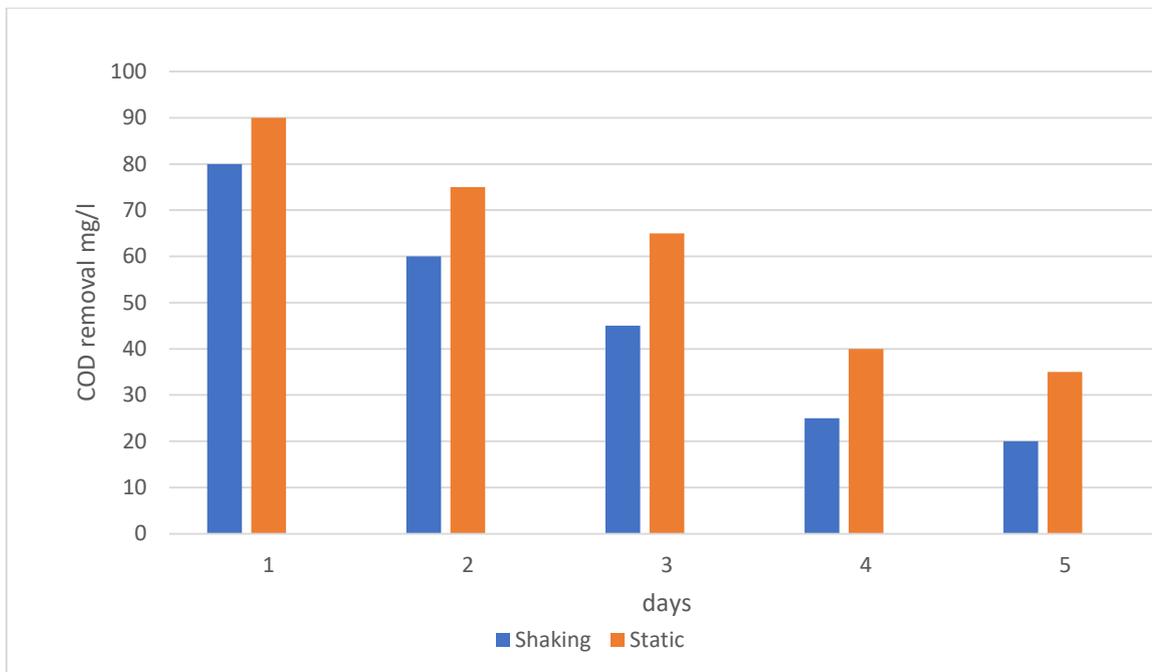


Figure 4. Effect of Static and Shaking condition on decolourization performance of *Pseudomonas spp*

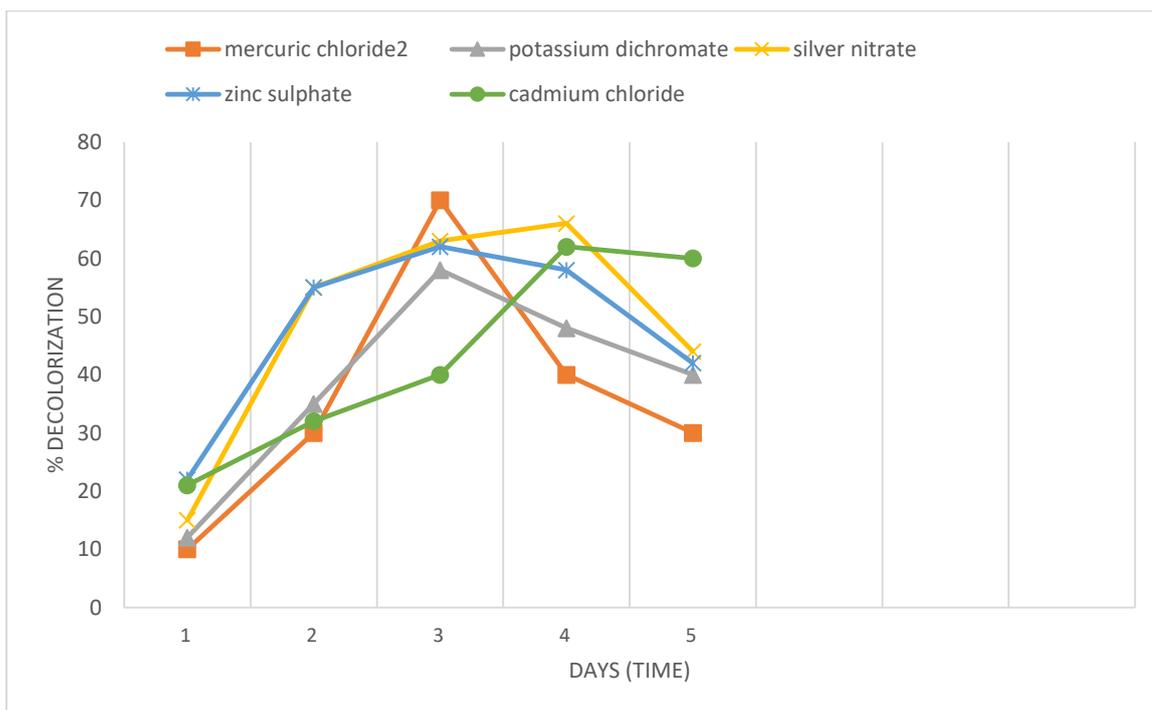


Figure 5. Effect of Heavy metals on rate of uptake of dye during decolourization by *Pseudomonas spp*

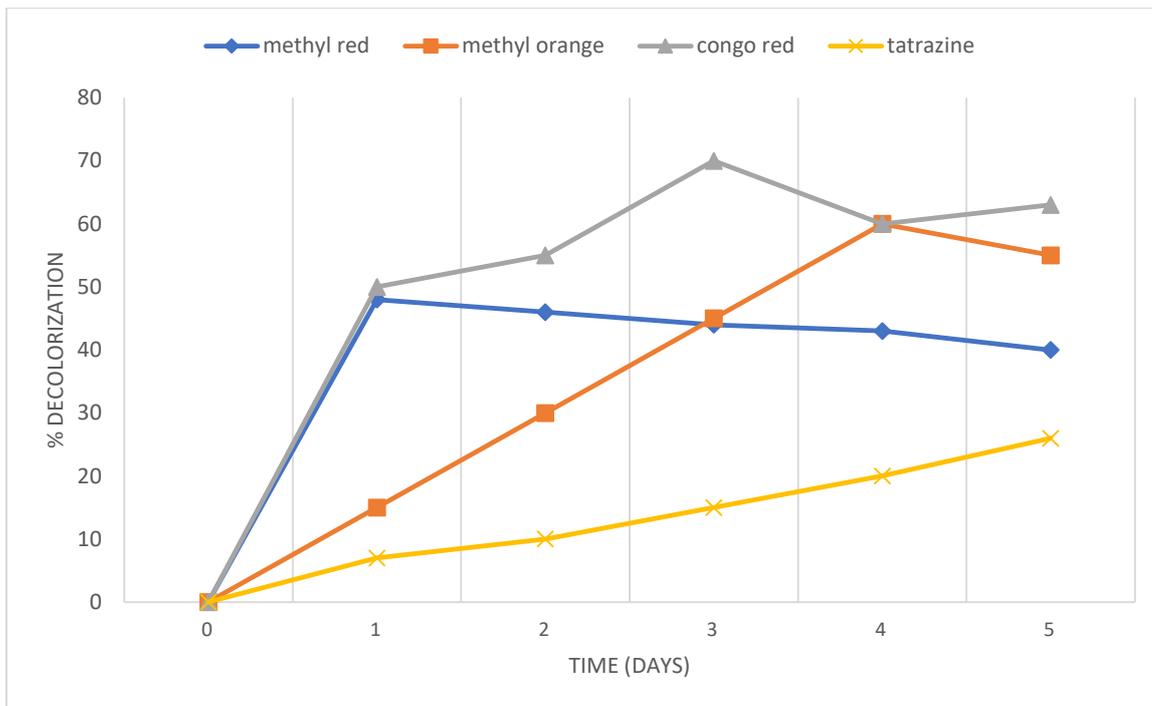


Figure 6. Decolourization of different azo dyes by *Pseudomonas spp*

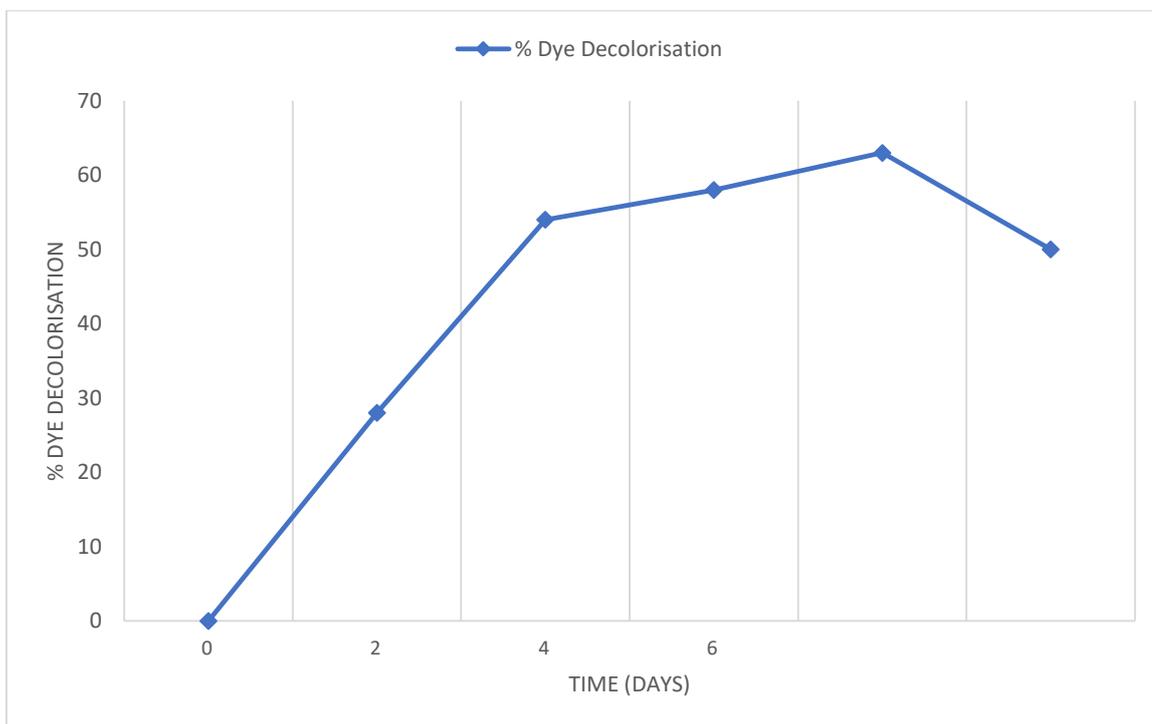


Figure 7. Decolourization of azo dye in consortium by *Pseudomonas spp*

4. CONCLUSION

In this study, a decolourizing bacterial strain, *Pseudomonas spp.*, ETL-M was isolated from dye contaminated soil. *Pseudomonas spp.* ETL-M showed decolourizing activity through a degradation mechanism rather than adsorption, and it could tolerate high concentrations (up to 500 mg⁻¹) of Methyl Orange. With high degradative and decolourizing activity against various reactive dyes commonly used in

the textile industries, it is proposed that *Pseudomonas spp.* ETL-M has a practical application potential in the biotransformation of various dye effluents. The effects of oxygen, pH, Temperatures, and dye concentration on the decolourization of methyl orange 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 the products from the degradation of Methyl orange dye by *Pseudomonas spp.* ETL-M 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, decolourize azo dyes at high concentration gives it an advantage for treatment of textile industry waste waters. However, potential of the strain needs to be demonstrated for its application in treatment of real dye bearing waste waters using appropriate bioreactors.

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