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Biodecolorisation of Acid Orange-10 by *Pseudomonas sps* CMES-4.

Madhuri R. Basutkar[1], Pallavi P.Bapte, Deepak B.Methre, Channappa T. Shivannavar*

Corresponding authors address*
Dr. Channappa T. Shivannavar, Professor,
Dept of Microbiology, Gulbarga University, Kalaburagi-585106.

Abstract

Present work interest focused on the microbial biodecolorisation of dyes as a better alternative. Microorganisms which are as known nature's recyclers, convert toxic compounds to harmless products such as carbon dioxide and water. In this way a Acid orange-10(AO-10), which is widely used textile dye and samples were collected from different areas in textile industries, MIDC Solapur, Maharashtra. Out of fifteen strains, a bacterial strain CMES-4, tentatively identified as *Pseudomonas sps* CMES-4, which showed maximum decolorisation of Acid orange-10, by optimizing various abiotc and biotic parameters, hence the isolate has proven a better resource for the decolorisation of textile effluents.

Keywords: Biodecolorisation, *Pseudomonas sps*, textile effluents, Acid orange-10.

Introduction

Rapid industrialization has necessitated the manufacture and use of different chemicals in day to day life. The textile industry is one of them, which extensively use synthetic chemicals as dyes. Effluent discharged from the textile industries has variable characteristics in terms of pH, dissolved oxygen, organic, and inorganic chemical content, etc. Pollution problems due to textile industry effluent have increased in recent years, it can be estimated that approximately 75% of dye discharged by textile processing belong to the class of reactive (36%), acid (25%) & direct (15%) dyes. Approximately 50% of the applied dye is lost in effluents during textile dyeing processing (Pandey *et al*., 2007). Disposal of dyes into the environment causes serious damage and also they may be toxic to some aquatic organisms due to their breakdown products & significantly affect photosynthetic activity in aquatic life by reducing light penetration and phytoplankton form abnormal coloration (Duran and Esposito 2000; Mester and Tien 2000; Wu, *et al*., 2011). Recent studies have shown that dyes contribute to the mutagenic activity of ground and surface waters polluted by textile effluents (Sponza and Isik, 2005). Moreover; it is very difficult to treat textile industry effluents because of their high BOD, COD, heat, color, pH and the presence of metal ions (Gogate and Pandey, 2004). Therefore, treatment of industrial effluent having azo dyes is deemed necessary before its discharge into wastewater bodies. As the characteristics of dye wastewater are very variable, many different physical, chemical and biological treatment methods are in use for its treatment; which one is effective treatment depend upon the type of dye wastewater (McCurdy *et al*.,1992).

The presence of unnatural colors is aesthetically unpleasant and tends to be associated with contamination. Without adequate treatment these dyes will remain in the environment for an extended period of time. The various organisms which degrade dyes are fungi, bacteria and actinomycetes. The biological mode of treatment of dye bath effluents offers distinct advantages over the conventional modes of treatment. Most importantly, biological treatment of dye bath effluents is eco friendly. It causes mineralization of dyes to simpler inorganic compounds which are not lethal to life forms. The basic step in the decolorization and degradation of dyes is breakdown of chromophore bonds, leading to removal of color. So, for complete mineralization of dye the microbial population forming part of treatment system should be able to work efficiently. In this direction we selected of Acid orange-10 dye and Isolation of decolorising bacteria had been done.

Materials and Methods

Sources of samples

In this study we used, various environmental samples collected from following places, Soil sample from Textile Industrial areas MIDC Sholapur, Maharashtra, were collected. The effluents from discharging area and textile industrial effluent were also collected. The effluents at discharge point of textile industries and effluents from logging area were collected and deposable sites are the source of dye degrading microorganism.

Dye -The synthetic azo dye Acid Orange-10 used in this study were procured from colorise dyes Ahmadabad Gujarat.

The chemicals used in this work were of analytical grade and procured from HI-MEDIA.

Methods

The required media, reagent and solution were prepared by following standard method in the microbiological lab manual and concerned literature of microbial decolorisation of Acid orange-10.Mineral salt medium (Agar) used in this study with dye as a sole source of carbon.

Preparation of Mineral salt medium (MSM) for the study

Mineral salt medium (liquid & salt) was prepared according to Brilon *et al*.,(1981) used.

Mineral salt medium (MSM) with composition (grams per litre distilled water): $Na_2HPO_4.2H_2O$ (12gm), KH_2PO_4 (2gm), NH_4NO_3 (0.50gm), $MgCl_2.6H_2O$ (0.10gm), $Ca(NO_3).4H_2O$ (50.00mg), $FeCl_2.4H_2O$ (7.50mg), Agar agar (18gm/l for solution medium), Distilled water (1000ml), pH 7.

Isolation of bacteria from sample by inoculation

The soil samples were collected from different sites of dye industry in sterile containers and brought to laboratory within 24hrs to isolate potent dye decolorizing bacteria. The isolation of bacterial strains were carried out by serially diluting the soil samples in 100ml saline water in conical flask respectively and placed in orbital shaker for 1hour at 120rpm After 20min supernatant solution is collected and transferred into MSM containing flasks 50mg/L of Acid orange-10 dye is added and mixed well and 20ml of supernatant solution of sample and in one conical flask control is taken that is media without addition sample. Daily the color change noticed by visually and more 70% decolorized flask selected for the further experiments. Decolorized flask supernatant of 10ml transferred to another freshly prepared 100ml of MSM with 50mg/L acid orange 10. Once the complete decolorization seen and flask was subjected to the UV-VIS Spectrophotometer for the optical density analysis. The O.D was taken at 500nm. Compared with the control.

Screening of sample

Mineral salt agar plate with 50mg concentration of acid orange containing plates had been prepared on which a loop full of culture taken from decolorized bath and streaked on it , kept for incubation. The colonies showing zones are picked and streaked on MS plate after 24hr of incubation colonies were inoculated in nutrient bath and checked for further decolorization activity.

Dye Decolorization Assay

In order to examine the effect of initial dye concentration on decolorization under static condition 100 mg/l of Acid orange10 was added to the sterile nutrient broth inoculated with different soil samples and incubated at 37°C under static condition is added to mineral salt medium. The % decolorization was measured. Out of 5 sample sample1 & sample4 shown the fastest decolorization within 24hrs of duration.

Dye decolorization assay was measured in the terms of percentage decolorization using UV-Spectrophotometer. The percentage decolorization was calculated from the following equation

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

The bacterial strain giving maximum decolorization values was selected and used for further decolorization experiments.

Characterization of isolated dye decolorizing bacterial strain

The isolated potent dyes decolorizing bacterial strain were characterized on the basis of their cultural, morphological, staining, biochemical, and physiological properties. Based on these properties the most potent bacterial strain have been identified tentatively up to the genus level.

They were

- ✓ Culture media used
- ➢ MS Liquid medium with dye
- ➢ Nutrient agar and nutrient broth
- ✓ Morphological characters; Shape, size, color, arrangement of cell, motility, and spore formation was checked
- ✓ Biochemical test: Indole, citrate, Methyl red, Voges proskauer, Gelatin, Triple sugar Iron, Urease test.

Optimization of maximum decolorization of Acid orange-10 by isolate CMES-4

Various environmental conditions were standardized by optimum decolorisation of isolated bacterial strain by varying particular parameter and other parameter constant. Following parameters were standardized, Static and Shaking (Aeration), pH, Temperature, Inoculums size, Salt concentration, Dye concentration, study of effect of carbon and nitrogen sources, optimization of yeast extract, declorisation in different dyes.

Results and Discussion

The various biotic and abiotic factors were optimised for the maximum decolorisation by *Pseudomonas sps* CMES-4.

Optimization of Static and shaking condition

Optimization of static and shaking condition for decolorization of Acid orange-10 by bacterial isolate CMES-4 has showed after incubation of 24hours, highest decolorization in static condition of 83.03% than the shaking condition that is 29.88% observed similar with our results (Mallikarjun, *et al.*, 2014) Showed static condition only favorable for the degradation also (S. Satheesh Babu, *et al* 2013) found that decolorisation rate decreased in shaking condition.

Optimization of pH

Decolorisation studies were carried out at different pH levels ranging from 4 to 12 at an interval of 1. The pH of medium was adjusted either by 0.1N HCL or NaoH. Optimization of pH for decolorisation of Acid orange-10 by bacterial isolate CMES-4,After incubation for 24hours, effect of pH was compared for the dye decolorisation. CMES-4 Strain showed the decolorizing activity at pH 6 (77.67%), pH 7 (79.06%), pH 8, (84.49%), pH 9 (83.44%) respectively. From this data it is cleared that the isolate CMES-4 has capacity to decolorize in wide range of pH. From results isolate *Pseudomonas sps* CMES-4 ,has shown maximum decolorization of Acid orange10 at pH-8 with 84.49% in alkaline medium. This isolate had capacity of decolorization in wide range of pH6-9.Earlier few reports have been reported on the use of *Pseudomonas sps* for the decolourization of various dyes (Shah *et al*.,2013) used the *Pseudomonas sps MPS-2* for degradation of Reactive red showed decolorization between pH 6.5-8.5.The isolate *Pseudomonas putida MS-7* showed maximum decolorization of Remazol Black B was achieved at pH 7.0 with 93.23% in 48hrs, the optimum pH of the growth of *Pseudomonas putida* was neutral (S.Kannan *et al*,. 2013).

Optimization of Temperature

To study temperature effect on isolate, selected temperature range from 20-50 °C with interval at 5 °C and incubated for 24 hours and the effect of temperature was compared for the dye decolorizing strain CMES-4, it revealed the highest decolorizing activity at 35°C (85.04%), and decreased at 40°C (83.86%), 45°C (80.25%), 50°C (77.74%) respectively. Similarly isolate *Pseudomonas Putida* in decolorization of Remazol Black B has shown decolorization at 35°C with 94.25% (S.Kannan *et al*., 2013). Sartale *et al*., (2011) and Bhatt Nikhil *et al.*, *(*2012) reported that 37°C temperature gave maximum decolorization by bacterial consortium.

The bacterial strain *Pseudomonas* species S2 revealed the highest decolorization of Red m5B at 37°C with 90.08% after incubation of 96hrs Lone *et al*.,(2015).

Optimization of Inoculums size

Results of optimization of inoculums size for effective decolorization of Acid orange-10 by bacterial isolate CMES-4 was , decolorization percentage is recorded with increased inoculums size from 1ml/L to 20m/L. At 10ml of inoculums size, the highest percentage of decolorization was observed that is 84%, while lowest of 56.07% in 1ml was observed. 10ml inoculums size is suitable for further decolorization. Inoculum concentration varies from species to species in the report of Kumar and Bhatt, (2011) on decolorization of Red 3BN by *B. cereus* optimum inoculum size was 8% and *B. megaterium* inoculums concentration was 10%.

Optimization of Salt concentration

Optimization of salt concentration for decolorization of Acid orange-10 by bacterial isolate CMES-4 was selected (1-6%/L) showed decolorization percentage was highest in 1% of salt 82.75% and in 6% of salt concentration it showed 67% of decolorisation, it reveals isolates CMES-4 had salt tolerance capacity, similar with our results (Mallikarjun *et al.*, 2014)reported paracoccus sps shown decolorisation up to 6% salt concentration.

Optimization of Dye concentration

Decolorization of Acid orange-10 with Percent decolorization values obtained at 24hr of incubation were found to be 87.41%, 84%, 82.05%, 73.15%, 43.46%, 37.83% respectively over a dye concentration of 100mg, 200mg, 400mg, 600mg, 800mg, 1000mg/L respectively.

Highest decolorization of 87.41% of Acid orange-10 by bacterial strain was observed at a dye concentration of 100mg/L. In other reports ;(Moosvi *et al*., 2007) the complete decolorization of initially added 1500mg/L of reactive violet 5 within 42 h by a bacterial consortium SB4. Further high concentration of reactive azo dye inhibits nucleic acid synthesis in microbial cell growth. Jain *et al*., (2012). Tariq Ahmad Lone *et al.*, (2015) after incubation of 48hrs dye degrading Pseudomonas species has shown decolorization in 300mg/L in degradation of Red m5B.

Optimization of additional nutrients for maximum decolorization of isolate CMES-4:

Optimization of additional carbon source:

Optimization of carbon source for effective decolorisation of Acid orange-10 was different . Decolorization percentage was observed in carbon source that is glucose, lactose, starch, highest percentage was recorded for glucose 82.05%, lactose 80.45%, starch 69.95%. Among the three carbon sources tested, glucose proved to be very effective in the decolorization of Acid orange10 than lactose and starch.Similarly in our result Arulazhagan (2016) have reported that cellulose, glucose and NH_4OH and Na_2NO_3 were suitable source of carbon and nitrogen for the growth of *B. subtilis*.

Optimization of nitrogen source

Optimization of nitrogen source for effective decolorisation percentage was observed in nitrogen source i.e, peptone, beef extract, yeast extract, ammonium nitrate, sodiumnitrate. Highest percentage of decolorization was recorded for yeast extract 83.93%, beef extract 82.75%, and peptone 79.06% was recorded. Among the nitrogen source tested yeast extract proved to be very effective than beef extract and peptone in the decolorization of Acid orange-10. based on our results and other reports nitrogen sources like Beef extract, peptone, yeast extract in the synthetic azo dye decolorizing medium activate the co-enzyme required for the metabolic pathway of azo reductase and serve as a key component for enhanced azo dye decolorization (Lade *et al.*, 2015; Chang *et al.*, 2001).

Optimization of yeast extract:

The result of optimization of concentration of yeast extract for effective decolorization of Acid orange10. The percentage decolorization values at 0.5,1, 1.5, 2 mg/L concentration of yeast extract was found to be 75.66, 84.84, 85.04, 84.49 % respectively. Highest percentage of decolorization was observed at 1mg/L of yeast extract concentration. From the results it is clear that the decolorization of Acid orange10 enhanced by the addition of yeast extract as nitrogen and energy source, hence we used yeast extract in further decolorizing experiment. Many reports showed that yeast extract concentration between 0.1 to 1% ideal for the growth and decolorization of azo dyes by *Bacillus* sp. (Dawkar *et al.*, 2009). *Paracoccus* sp. by Bheemaraddi *et al.* (2014); Hu (1998) reported that decolorisation efficiency of *Psedomonas luteola* was directly related to the concentration of yeast extract.

Optimization of degradation in different dyes

The result of optimization of decolorisation of Acid orange-10 in different dyes. The highest decolorisation was observed in Reactive orange- 84 at 500nm 85.80%, Reactive blue-59 at 530nm 85.33%, Reactive orange-16 at 500nm 84.63%, Reactive violet-1 at 540nm 82.54%, Reactive red-11 at 540nm 78.76%, Reactive crystal violet at 540nm 60.50%, Reactive blue-4 at 530nm 49.15%, Methylene blue at 540nm 47.53%, Reactive yellow -84 at 540nm 39.88% within 24 hours of incubation.

Acid orange10 decolorisation study

Isolate-CMES-4 at various incubation time beginning with 2-hr of incubation time to 24 hrs. The percentage decolorization values were calculated by taking O.D of culture supernatant of decolorizing culture at different incubation period and results Maximum of 85.18% dye decolorisation was achieved by the isolate- CMES-4 at an incubation time of 24 hours it was , 84.49% at 24hr and 85.5% at 48hr. So we observed this organism can decolorize up to 85% of acid orange-10 within 24 hours.

Conclusion

Among synthetic dyes reactive dyes are major pollutant present in textile industry effluent because of its low fixation capacity to fiber. Many reactive dyes and their intermediate products are toxic, mutagenic and potentially carcinogenic in nature, leading to health hazards to human and environment. Biological method have found effective for dye degradation and is considered most cost-effective and eco-friendly.

Hence, by comparing these aspects, the present was carried out to isolate and characterize the azo reactive dye degrading bacteria from various dye polluted areas.

We have used different parameters to standardize the condition for maximum decolorization of Acid orange-10 by selected bacterial strain. Isolated bacteria *Pseudomonas sps* CMES-4 species has shown good results in dye decolorisation under static condition with pH -7, tempreture-35^{0C}, 10ml/L Inoculum concentration with 100mg/L acid orange -10 dye.

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