Analytical Study On Microbial Degradation of Textile Dyes

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Abstract

A bacterial strain ETL-1949, identified as Aeromonas spp. was isolated from sediment from a textile wastewater treatment plant in Ankleshwar, Gujarat, India. The strain could decolorize an azo dye, Orange 16 under the static condition after the shaking culture. In order to increase cell concentration by the shaking culture, the optimum culture condition was investigated for following the decolorization incubation. In present research two plausible bacterial isolates were selected to decolorize Orange 16 dye. At static conditions, successful decolorization was achieved and decolorization percentage varied from 81% to 97, while at pH 7 and temperature 37 °C maximum decolorization is observed.

Keywords: Textile dyes, Orange 16, Biodegradation, Decolorization.

1. INTRODUCTION

One of the major problems that humans are facing is the restoration of the contaminated environment. Textile dyes contribute as the most important environment-polluting agents. Several classes of such contaminants have been synthesized, and still new products are being synthesized now and then. The textile industry is a large water consumer and produces large volumes of contaminated water. One of such examples is the Ankleshwar Industrial Estate, Ankleshwar, Gujarat, India, which is a seriously industrialized area and produces millions of liters of improperly treated effluents that are released directly without giving proper treatment. In this study efficiency of a bacterial species Aeromonas spp. ETL-1979 was evaluated for decolorization, and degradation of textile azo dye Orange 16. A bacterial strain, ETL-1949 isolated from sediment of a textile industrial wastewater treatment plant, showed strong ability to remove the color of Orange16 under static condition. The effect of several culture conditions such as temperature, initial pH, and nutrient concentration of the medium were investigated.

Dyes are an important source in various industries such as textile, leather, paint, food, cosmetic and paper industries. There are approximately twenty-five types of dye groups available based on their chemical structure of chromophore. More than thousand dyes have been classified as textile dyes which are used to color variety of fabrics. Dye intermediates are precursors of dyes. They can be obtained from raw constituents, such as naphthalene and benzene, with an aid of various chemical reactions. Present review intends to expand biodegradation scope of dyes. It includes types of dyes, dye intermediates and impact of dyes. It also narrates types of dye degradation techniques and through light on factors affecting biodegradation of dyes. Direct Black 38 is majorly used azo dye; hence microbial degradation pathway for Direct Black 38 has been discussed. It also provides an overview about role of genetically modified organisms (GMOs) in dye(s) biodegradation.

The microorganisms are being employed for bioremediation of wastes of many industries, be it heavy metals, antibiotic degradation etc., this microbial degradation has been used for dye degradation as well. A dye is a colored substance which absorbs a particular wavelength and gives color corresponding to that wavelength. There are two types of dyes- natural dyes (obtained from leaves, wood, and bark) and synthetic dyes. About 50% of dyes were lost in effluent after dyeing process. The chemical or physicochemical treatment methods are inefficient, expensive, have limited applicability, and cannot be applied to a large scale effluent treatment process.

2. LITERATURE REVIEW

T. Marimuthu (2013) Various synthetic dyes from the textile sector have been discharged and environmental safety has been threatened. Azo dyes are mostly utilised in the textile, paper, food, leather, cosmetics, and pharmaceutical sectors since they are used in large numbers. Due to its colour rapidity, stability and degradation resistance, existing

effluent treatment methods cannot fully remove different colours from the wastewater. Under specific environmental circumstances, bacterial decoloration and dyes degradation. The treatment technique is cost-effective, environmentally benign and may be used for many similar teeth. The primary emphasis of this study is on the various processes of bacterial colouring and finding a solution for decolored dyes and colouring effluents.

M.Sudha (2014) A colouration is used to provide colour to things that are part of human existence. As acids are widely employed in the textiles, leather, pharmaceutical and cosmetics sectors, they constitute a risk for all living forms, they represent significant synthetic dyestuffs. The industrial wastewater treatment physical-chemical technique does not successfully remove the colours. Azodys have lately become increasingly attractive to microbial degradation and colorization due of their environmental friendliness and cheapness. Aerobic as well as anaerobic metabolism may decolorize the dyes. Furthermore, the effectiveness of the decoloration microbial enzymes was examined in biotransforming hazardous azodyes. This paper offers a basic concept of microbial decoloration and azody degradation with different physicochemical parameters, and stresses the use of these processes for azodytic wastewater treatment.

Shanooba Palamthodi (2011) Textile waste water is a very varied blend of various pollutants, from inorganic chemicals and compounds to polymers and organic molecules. Proper technology for full degradation of dyes should be used to guarantee the safety of effluents. Traditionally, physical and chemical techniques are used for treating textile waste water. But there are numerous shortcomings in both the physical and chemical approaches. Biodegradation is an environmentally beneficial process which may have little or no secondary risk. During this study, the textile industry effluent has been degraded in situ. The deterioration of the blue and green colours of two distinct colours. The isolated bacterium that exhibited the capacity to destroy the colour, was studied by different biochemical methods and identified as Paenibacillus azoreducers. The decoloration test and the measurement of COD and BOD levels verified dye degradation. A rolling sheet reactor was developed to efficiently treat the wastewater of a textile industry.

Harshad Lade (2015) The release to the environment of textile azo dyes is a health problem, while the employment of microbes has shown the best way of cleaning up. In the current research, for the breakdown and detoxification of structurally diverse azo dyes a Bacterial Consortium comprising of Providencia rettgeri strain HSL1 and Pseudomonas sp. SUK1 is being explored. The consortium has experienced a 98-99% decolourization of the selected azo dyes in the 12-30 hours at a concentration of 100mg L-1, at 30 ± 0.2°C under the microaerophilic, sequential and microaeropilic/ aerobilic processes, i.e. the Reactive Black 5 (RB 5), Reactive Orange 16 (RO 16), the Disperse Red 78 (DR 78) and the Direct Red 81 (DR 81). The formation of aromatic amines resulted in coloured effects under micro-aerophilic conditions such as the RB 5 processes (0,26 mM), RO 16 processes (0,18 mM), DR 78 processes (0,20 mM) and DR 81 processes (0,23 mM) and sequential aerobic processes (RB 5 processes (0,08 mM), RO 16 processes (0,06 mM), DR78 (0.07 mM) and DR 81 (0,09 mM). A distinction, the synthesis of amines does not exhibit sequential microaerophilic / aerobic process. In the course of successive microaerophilic/aerobic processes indicating efficient methods in the mineralization of the dyes, 62-72 percent reductions were found in the total organismic carbone content of all dyes decolored broths. Significant induction of azoreductase and NADH-DCIP decreases in a sequential microaerophilic/aerobium process (97 and 229 percent for RB 5, 55 and 160 percent for RO 16 and 63 percent, and 196 percent for DR 78, 108 and 258 percent) suggested that their critical implications were in the initial collapse of azo bonds, whereas a slight increase in laccases and ve was observed. The acute toxicity test using Daphnia magna showed, in successive microaerophilic / aerobiic processes, also the non-toxic character of dye-degraded metabolites. Since all textile azo teeth are fully detoxified in successive microaerophilic/aerobic processes, additional efforts should be made to apply such processes for broad-scale wastewater treatment technologies.

3. MATERIALS AND METHODS

Measurement of dye and cell growth

Orange 16 was obtained from local textile industry of Ankleshwar, Gujarat, India. Concentration of Orange 16 in the medium was determined by measuring the absorbance at 492 nm corresponding γ max of this dye. Bacterial growth of the cultures was measured as OD at 600.

Microorganism and Culture condition

Bacterial strain ETL-1949 used in this work was isolated from sediment of the wastewater treatment facilities of a textile industry in Ankleshwar, Gujarat, India. The medium used for screening of bacteria with 0.02 g/l Orange 16 decolorizing potential contained 5 g/l glycerol, 4 g/l yeast extract, 0.5 g/l ammonium sulfate, 0.2 g/l MgSO4 ·7H2 O, 0.5 g/l K2 HP O4 , and 1.5 g/l K2 HPO4 . The initial pH was adjusted to 8.0. The strain was cultivated in a reciprocal

shaker at 30°C. The culture conditions including the medium composition were varied to determine the optimum condition for the bacterial growth. Batch culture experiments under the aerobic condition were carried out in 500 ml conical flask, containing 100 ml medium inoculated with a loopful of I-day grown-cells from the slant. Flasks were shaken at 125 rpm in a reciprocal shaker. Aliquots collected at intervals were used for measurement of the cell growth. For decolorization experiments, the bacterial cells under optimal growth condition were transferred to 100 ml of Erlen-Meyer flask. Orange 16 was added to give 0.15-2 mg/l and further incubated at 30° C under the static condition.

Identification of bacterial strain ETL-1949

The taxonomical studies were carried out according to Bargey's manual.The 16s rRNA gene in this strain was amplified by 30 cycle of PCR using genomic DNA from strain ETL-1949 as the template and two specific primers, 20F (5'-GATTTTGATCCTGGCTCA-3') and 1500R (5'-GTTACCTTGTTACGACTT-3'). Each cycle was carried out at 95°C for 30 sec, at 55°C for 20 sec, and at 72°C for 90 sec. An amplified DNA fragment was sequenced by the dideoxynucleotide chain termination method using Big Dye terminator Cycle Sequencing Kit (Perkin-Elmer, Foster City, California) and a DNA sequencer (model 310, Perkin-Elmer). Homology of the DNA sequence was searched by using the BLASTn program at DDBJ.

Effect of physicochemical degradation

Physico-Chemical degradation is a combination of chemical and physical techniques. Physico-chemical treatment is the process in which physical changes are constantly present, while chemical changes in the process at different phases may or may not take place. In this process chemicals such as Lime, Ferric chloride (FeCl3), Ferrous sulphate (FeSO4·7H2O) and Alum ((Al2SO4)3·18H2O) are widely used to alter physical state of dye molecules. Treatments such as flocculation, wet oxidation, membrane separations, adsorption and precipitation are examples of physico-chemical treatment. The disadvantages of this methods are high chemical requirement, high maintenance, costly and large amount of sludge is generated which requires safe dumping.

Microbial degradation

For degradation of various dyes different microbes can be used, they have different mechanisms and pathways for degradation of dyes.

Dyes are useful class of dyes with highest diversity of colors. Under anaerobic condition and with help of azoreductase, microorganisms degrade azo dyes and as end product they form colorless aromatic amines. Benzidine is generally used in construction of direct azo dyes and has been reported as potential carcinogen. Direct dyes are inexpensive and used to dye fibers, leathers or papers without any pre-treatment. Among benzidine based azo dyes most generally used dye is Direct Black 38. Degradation of Direct Black 38 dye can be achieved using Enterococcus gallinarum. Direct Black 38 has three azo bonds in its structure which are the active sites for azoreductase. Direct Black 38 through metabolic reactions is converted to benzidine which upon deamination results in 4-amion phenyl. It has been reported that dyes which have benzidine as a base is highly carcinogenic as compared to the dyes without Benzidine. This is due to existence of pollutant(s) like 4-amino biphenyl and 2–4, diaminoazobenzene, which have been reported as carcinogens

Effect of pH on Decolorization

Decolorization of Orange16 which is thought to be recalcitrant, was observed using UV-Vis Spectrophotometer at different pH values.

Decolorization experiment

Suspension of isolate were prepared by inoculating an individual bacterial colony (obtained on agar plates) in nutrient broth, under shaker at 200 rpm for 24 h. 1 ml of this suspension culture was added to a fresh medium containing dye which is to be decolorized. Then at the intervals of 24, 48, 72, 96, upto 408h, culture was withdrawn and centrifuged at 10,000 rpm for 15-20 minutes. The λmax (maximum wavelength) of the dye Orange16 is 470 nm. Decolorization percentage was calculated by the formula suggested by Saratale et al.

$$\text{Decolorization (\%)} = \frac{\text{(Initial absorbance – Observed absorbance)} \times 100}{\text{Initial absorbance}}$$

Comparative analysis of dyes before and after incubation in presence of bacteria was carried out by following the protocol of Asad et al. and Kurade et al.

4. RESULTS

Decolorization of Orange 16

Decolorization studies were carried out by noting down the absorbance value for the samples after inoculation with the bacterium. Media used was nutrient broth at pH = 7 and temperature used was 30 °C at static condition. Orange16 was decolorized to light pink color, color variation was seen after 48 hr of incubation.

Effect of pH on decolorization of Orange 16

The effect pH on the decolorization of Orange16 dye was studied over decolorization percentage. Decolorization and degradation was achieved at a wide range of pH from 3 to 10. All experiments were done in triplicates along with the uninoculated control. Decolorization in both the isolates follows polynomial trends when plotted against pH (Fig. 1). Decolorization increased above pH 5 and decreased below pH 5 in most of the cases. It was found that pH (3-6) was the most unacceptable pH for decolorization of dyes. Maximum decolorization was observed at pH 7 and temperature 37 °C by W2 isolate.

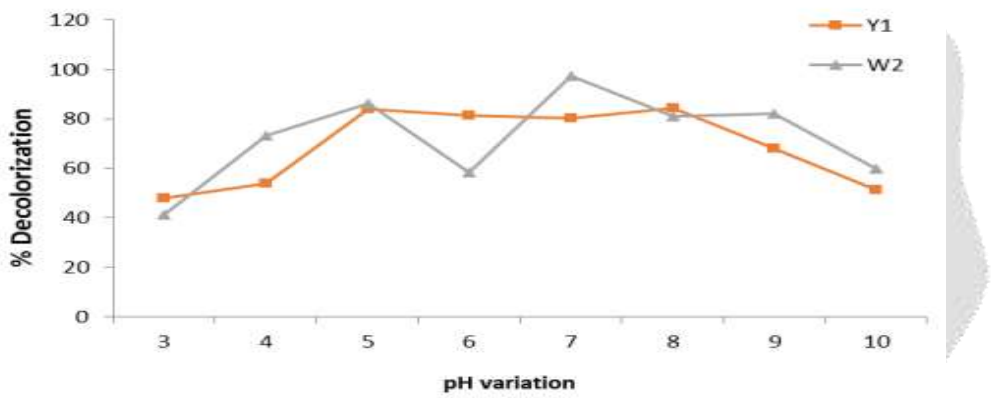

Figure 1: Effect of Ph on Decolorization By Bacterial Isolates (Y1 And W2)

Spectrophotometric Analysis

Decolorized medium was taken and centrifuged at 5000 rpm for 20 minutes and supernatant was used to measure OD. We obtained maximum decolorization of Orange16 dye by W2 isolate at pH 7 and temperature 37 °C (Fig. 2). Marked decrease in the absorbance of degraded samples was observed at λmax (470 nm) of the dye. This can be an outcome of cleavage of azo bond. Change in absorbance readings for the dye indicates a conspicuous change in the structure of the dye.

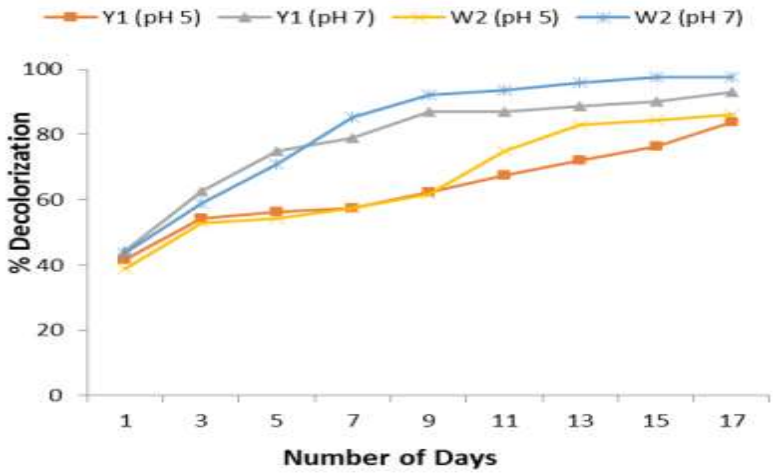

Figure 2: Decolorization of Orange 16 by bacterial isolates (Y1 and W2) at pH (5 and 7) at 37 °C temperature

5. DISCUSSION

It has been proclaimed repeatedly that bacteria dwell in textile effluents to utilize its ingredients for their survival. Similarly, the textile dye effluent examined in this work was found to accommodate an assorted turf of microbes. These bacteria are native inhabitants of the textile effluent as the latter serves as their supply of essential nutrients. This finding is in compliance with certain previous studies. Industrial dye effluent and sludge generated by effluent treatment plant is loaded with dye degrading and decolorizing microbial populace. Two plausible isolates were selected on the basis of ability to decolorize the dye and were inoculated in the presence of Scarlett RR dyes. At static conditions, successful decolorization was achieved and decolorization percentage varied from 81% to 97%. Maximum decolorization was observed at pH 7 and temperature 37 °C. But, one thing was observed that prolonged incubation time is required for complete degradation. Spectrophotometric analysis done after 5 days did not yielded the complete degradation, so with the prolonged incubation, newly formed metabolites were also degraded. Similar results of decolorization studies have been reported earlier by Ito et al.

We can recommend further research to develop a customized alternative treatment for textile dye degradation. This can resolve leading problems touching contamination of the water bodies because of textile effluent discharge. Bacteria are helpful in many other ways and it is found bacteria isolated from heavy metal soils have potential of plant growth promoting traits. Various zinc solubilizing bacteria have been found to promote growth and nutrition of rice plants

6. CONCLUSION

Current investigation has confirmed the decolorization of Azo dye Orange 16 by the isolated Aeromonas spp. under in vitro conditions. Thus the study has confirmed the potential of Aeromonas spp. ETL-1979 in the decolorization of the dye indicating their possible application for treatment of textile effluents. By shifting the culture condition from the aerobic growth to the static condition, which is an oxygen limiting or anaerobic condition, Orange16 was efficiently decolorized.

7. REFERENCES

1. Maulin P Shah, Kavita A Patel, Sunu S Nair, Darji AM (2013) Microbial degradation of Textile Dye (Remazol Black B) by Bacillus spp. ETL-2012. J Bioremed Biodeg 4:1-5.
2. Shanooba Palamthodi, Microbial degradation of textile industrial effluents, African Journal of Biotechnology Vol. 10(59), pp. 12657-12661, 3 October, 2011
3. Lade, Harshad et al. "Biodegradation and detoxification of textile azo dyes by bacterial consortium under sequential microaerophilic/aerobic processes." *EXCLI journal* vol. 14 158-74. 29 Jan. 2015, doi:10.17179/excli2014-642
4. M.Sudha, Microbial degradation of Azo Dyes: A review, Int.J.Curr.Microbiol.App.Sci (2014) 3(2): 670-690
5. Vilaseca M, Gutie MC, Grimau VL, Mesas ML, Crespi M (2010). Biological Treatment of a Textile Effluent After Electrochemical Oxidation of Reactive Dyes. Water Environ. Res. 82:176-181.

6. Morias JL, Zamora PP (2005). Use of advanced oxidation process to improve the biodegradability of mature landfill leachate. J. Hazard. Mater. 123: 181-186.
7. Andleeb S, Atiq N, Ali MI, Hussnain RR, Shafique M, Ahmad B, Ghumro PB, Hussain M, Hameed A, Ahmad S (2010). Biological treatment of textile effluent in stirred tank bioreactor. Int. J. Agric. Biol. 12: 256- 260.
8. Fang, H., Wenrong, H., Yuezhong, L. 2004. Biodegradation mechanisms and kinetics of azo dye 4BS by a microbial consortium. Chemosphere, Vol. 57, No. 4, pp. 293- 301, ISSN 0045-6535.
9. Chekir-Ghedira,L. 2010. Acid Violet 7 and its biodegradation products induce chromosome aberrations, lipid peroxidation, and cholinesterase inhibition in mouse bone marrow. Environ.Sci.Pollut.Res.Int.177: 1371- 1378.
10. Abedin.M.A.R.,2008. Decolorization and Biodegradation of crystal Violet and Malachite Green by Fusarium solani Martius Saccardo, A Comparative study on Biosorption of Dyes by the Dead Fungal Biomass, Am-Euras.J.Bot.,12:17- 31.