Journal of Advanced Scientific Research

Available online through http://www.sciensage.info

ISSN 0976-9595

Research Article

BIO-MINERALIZATION OF DISPERSE DYE AZO ORANGE -3 BY AN ALKALIPHILE *PSEUDOMONAS* DL 17

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ABSTRACT

Pseudomonas DL 17, isolated from hyper saline Lake Lonar (MS) Buldana, India was tested for complete mineralization of azo dye and other nitro-aromatics. After adapting for several days it was used for bio-degradation of Disperse orange 3 where it's experimental concentration was completely mineralized within 24 hrs. Solvent extracted metabolites were characterized as p-nitroaniline, p-phenylenediamine, acetanilide, catechol etc. On the basis of UV-Vis., FTIR, NMR, GCMS analysis, mechanistic pathway for biodegration was designed. CYP450 and other related enzymes involved in biodegradation were found hiked comparative to standard. Azo reductase, Nitro reductase, Tyrosinase, and SOD showed more activity than other enzymes under study.

Keywords: Bio-mineralization, *Pseudomonas* DL17, Azo Dye, Disperse Orange -3.

1. INTRODUCTION

Dyes are compounds widely used in textiles, printing, rubber, cosmetics, plastics, leather industries to color the products and results in generating an excess amount of colored waste water [1]. Azo dyes are the commercially largest group of dyes [2]. These are characterized by presence of typical one or more azo (-N=N-) bonds. Acid azo dyes are characterized by the presence of chromophoric azo groups where nitrogen atoms are linked to sp^2 hybridized carbon atoms of the aromatic ring. In addition, they also carry a sulfonic acid groups. The reduction of azo bond is purely a chemical reaction. They are extremely versatile colorants and constitute about 50% of dyes produced. Presence of dyes in water can cause sensitization reactions to human being or living animals such as nausea, hemorrhage, ulceration of skin and mucous membranes, dermatitis. Even it affects perforation of the nasal septum and causes severe irritation of the respiratory tract. Therefore, release of untreated dye from textile wastewater into various streams is unacceptable due to its negative impacts on ecosystem. Azo dyes are also toxic carcinogenic and mutagenic to humans [3]. The chemicals p-phenylenedimine and amino phenyl have been suggested as possible carcinogens or mutagens [4], may be metabolites of catabolic pathway of such dyes. Biodegradation of azo dyes were studied in anaerobic and aerobic condition using various microbes as neutrophiles, thermopiles, alkaliphiles [5] etc. There are very few reports adding the information of Azo dye under alkaline condition. Therefore our attempt was to add more information concerning bioremediation of such azo dyes using an Alkaliphile *Pseudomonas* DL17.

2. METHODOLOGY

All the chemicals were purchased from SRL India. The bacterial media reagents stains were purchased from Hi Media Mumbai.

2.1. Bacterial Isolation and Identification

Bacterial sediments were collected from Lonar Lake Buldana, Kamlja site. By dissolving 1 gm of sediment in 1 ml and after proper dilution 1:10 ml in distilled water a loop full sample was used to isolate the alkaliphiles on alkaline agar plate by spread plate or pour plate technique.

2.2. Media preparation for usual experiments

The strain *Pseudomonas* DL17 was isolated by method mentioned above and cultured on composition

containing yeast extract, peptone, NaCl-5g/L respectively and the micronutrients' in mg/L were KH_2PO_4 -160, Na_2HPO_4-280, $(NH_4)_2SO_4$ -100, $MgSO_4$ or MgO-0.2, $FeSO_4$ -0.04, $CaCO_3$ 0.25, $ZnSO_4$ - 0.09, $CuSO_4$ - 0.016, $CaSO_4$ - 0.018, Boric acid 0.07, and pH 9. The pH was adjusted by sodium bicarbonate (0.1 M or 1M) solution. The media was sterilized by autoclaving at 121°C for 15 pounds at 20 minutes. The solid media was prepared in same way by adding 2% agar. The same broth media of pH 9.00 was used for biotransformation study except the pH parameter changed. The experimental Azo Dye was added aseptically during experimental start up.

2.3. Biodegradation study

Six 250 ml conical flasks with 100 ml alkaline broth media were used for bio-degradation study. A 1% inoculum of 24 hr ($1X10^6$ bacteria) grown culture possessing 0.5 OD at 600 nm was added and allowed to grow for 24 hr in shaking incubator at 37°C, 110 rpm. At the end of 24 hrs, experimental concentration of Disperse Orange Azo dye was added in each flask keeping one as an abiotic control among them separately. The experimental flasks were monitored after six hrs. intervals. All experiments were carried in dark. To study the degradation capability of strain *Pseudomonas* DL17, the concentration (100-300 mg/L), were investigated. After adaptation of the strain, higher concentration was used for experimentation. Residual concentration was investigated by Eq.1

$$\{(A0-A)t/A0\} \times 100 \ldots\ldots 1$$

Where A_0 is initial absorbance and A_t is absorbance after time t.

2.4. Solvent extraction, purification and characterization of metabolites generated

The supernatant obtained after spinning the broth media with cells after certain time interval was used for solvent extraction. DCM or ethyl acetate was used for solvent extraction mostly to isolate the metabolites. Neutral silica gel was used for column chromatographic separation. Purified metabolites were characterized by ^{1}HNMR, FTIR and GCMS technique.

2.5. Bio-catalytic Study

Cell mass was harvested after 24 hours induction with Azo Dye Disperse Orange 3 by Du-Pont Sorvall RC-5B centrifuge spinning at 10000 x g for 13 min at 4°C. The cell mass was washed with phosphate buffer at pH 8.0 twice and physiological saline. Cell disruption was carried by Sonicator Ultra O Sonic (Mumbai) in Tris buffer pH 7.50. The resulting homogenate was centrifuged in cold condition at 15000 x g for 20 min. Protein determination and enzyme activities were checked by Standard methods.

2.6. Construction of bio mineralization path-way

After producing the data regarding UV-Vis, FTIR, NMR, GCMS etc., the mechanism of bio-mineralization was designed.

3. RESULTS

3.1. Bio-degradation of Disperse Azo-Orange-3 by *Pseudomonas* DL17

The **Fig.1** clearly shows the absorbance of original dye at 443 nm get totally decreased within 24 hrs which indicated that almost all the experimental dye had underwent complete biodegradation.

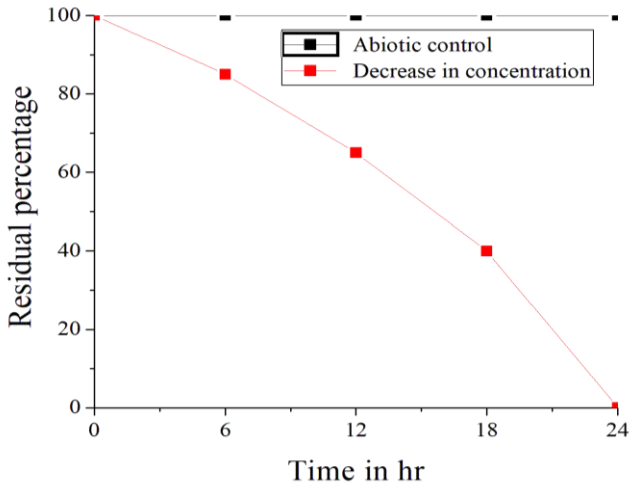

Fig. 1: Residual percentage of Disperse Azo-Orange- 3 during mineralization

3.2. UV- Vis spectra of column purified meta-bolites

In the fig.2 clear appearance of band at 350 nm was assigned for p- nitroaniline (band 1). The second might be showing catechol 275 nm as an additive spectrum. This situation was observed within 6 to 12 hr. Although spectrum of p-phenylenediamine does not appeared in this case which might be due to least concentration or faster reactions like deamination, hydroxylation or acetanilide hydroxylation type reactions immediately have gone through. Although it had not observed in 12hr metabolite. In fig. 3 it exists as spectrum number

2. As the Azo reductase works by Ping pong mechanism even at 6 hr also p-phenylenediamine might have formed. The spectrum 1 in this fig.2 was judged as p-nitro-aniline. These are column purified metabolites further used for characterization by FTIR NMR and GCMS. These metabolites were appeared in 3:1 chloroform methanol phase.

The spectrum number was 3 identified as p-phenylenediamine. GCMS and other techniques clearly showed that it is also vanishing from the media. One another metabolite formed and traced as acetanilide as shown **in Fig.4.**

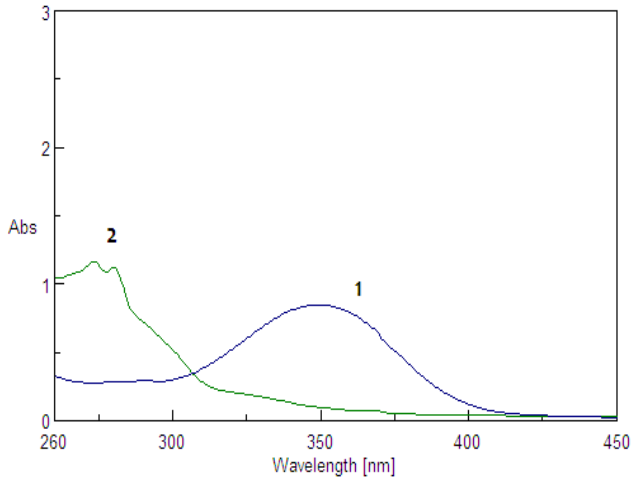

Fig. 2: Spectral data of major metabolite observed after bio-catalysis by Pseudomonas DL17 at 6 hr

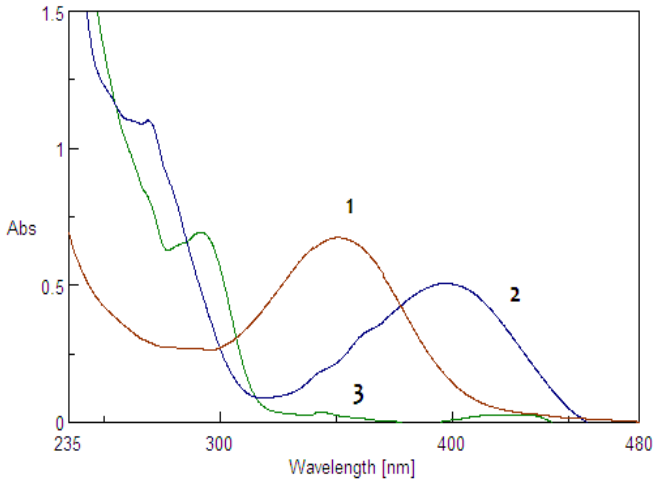

Fig. 3: Spectral data of major metabolite observed after bio-catalysis by Pseudomonas DL17 12 hr

3.3. Metabolite observed in Alkaline broth at 18hr. with other mrtabolites detected previously

From the fig. 4 it has been observed that metabolite was confirmed as acetanilide with UV-Vis maxima at 240 nm which was also supported by FTIR, NMR and GCMS analysis. Bio-catalytic action of *Pseudomonas* DL17 was also monitored by standard quantitative method has shown in fig. 6.

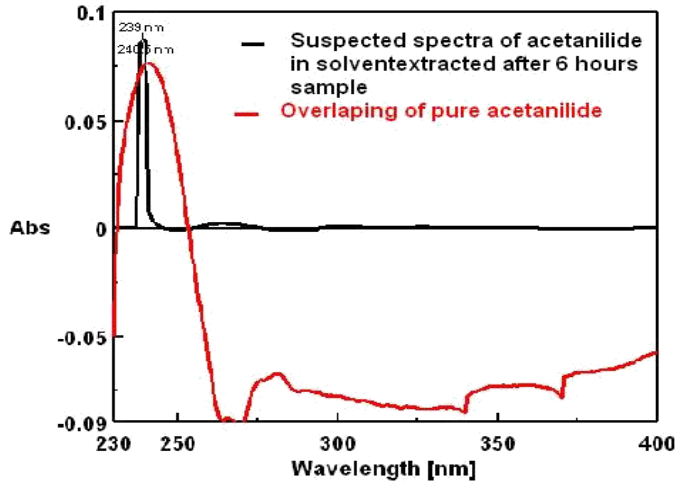

Fig. 4: Spectral data of purified metabolite compared with standard Acetanilide

3.4. Biocatalyic monitoring of CYP450

CYP450 are the super family of enzymes containing Fe as a cofactor which functions as monoxygenase or dioxygenase involved in biotransformation of recalcitrant's. Its spectrum changed from 418 nm to 450 nm on stimulation of Disperse dye Azo Orange -3. It seems from this fig. 5 that *Pseudomonas* DL17 is actively involved in bio-degradation process.

The Fig.5 a shows spectrum of CYP 450 without stimulation by Azo Orange 3 while Fig.5 b after induction of the cell by experimental Azo Orange 3.

3.5. Enzymatic activities in cell Extract

Azo reductase is showing most hikes than nitroreductase. The fig. 6 made it clear that azo bond had been preferably cleaved by experimental strain of bacteria to that of nitro group reduction. From the fig. 6, it has been observed that hike in the enzyme involved in biotransformation comparative to control may bring a good opportunity to isolation of various enzymes at commercial level.

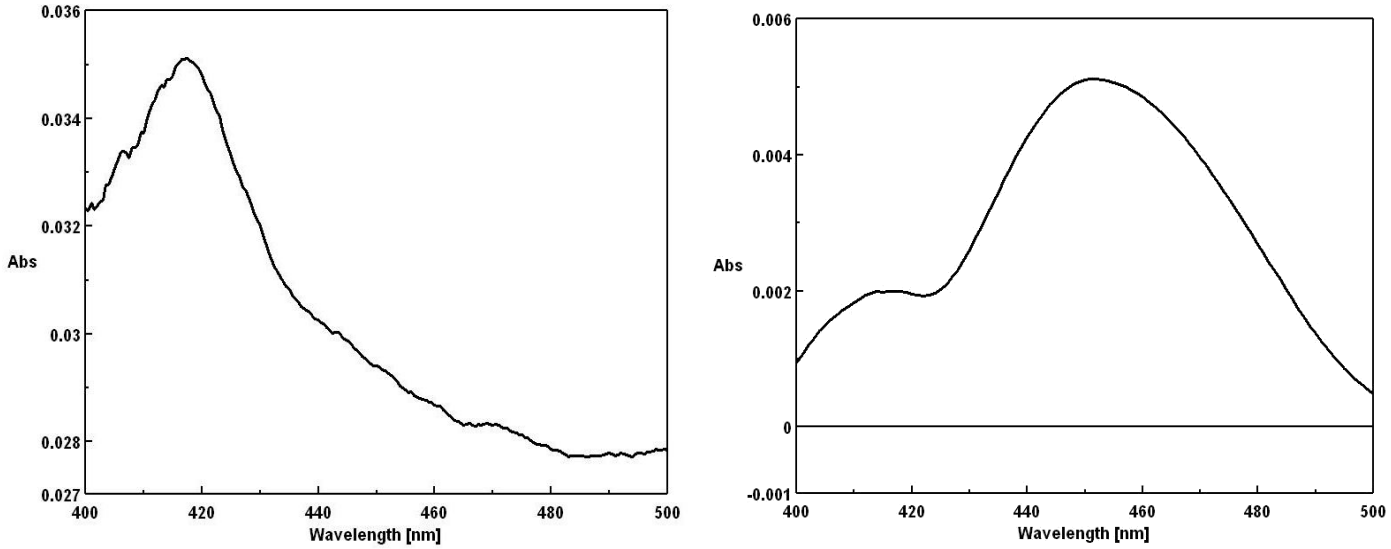

Fig. 5: CYP450 at normal level a) and Dye induced CYP450 b)

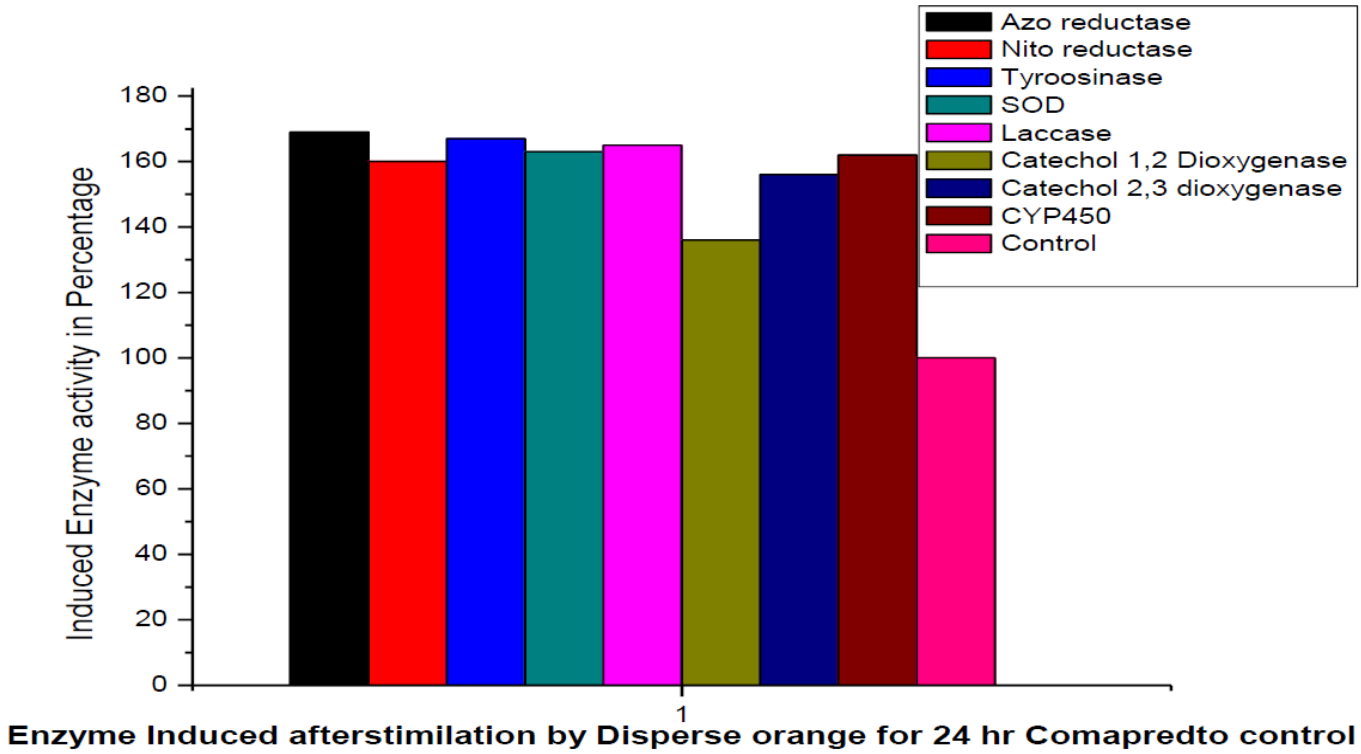

Fig. 6: Enzyme induction Effect in Pseudomonas DL17

3.6. Reducing concentration of metabolite p-phenylenediamine

As earlier it was confirmed that p-phenylenediamine was a metabolite formed in broth. Its FTIR (fig.7) was taken to review the concentration in the alkaline broth media for Azo dye mineralization parallel to reduction in absorbance at 443 nm as an optical density to calculate the residual concentration from 12 hrs. Fig.7 shows reducing concentration of p-phenylenediamine which might be due to the toxicity of p-phenylene-diamine formed. It might have transformed into acetanilide, acetaminophen or hydroxyl amine by enzymes monoxygenase dioxygenases, hydroxylases etc. involved in biotransformation.

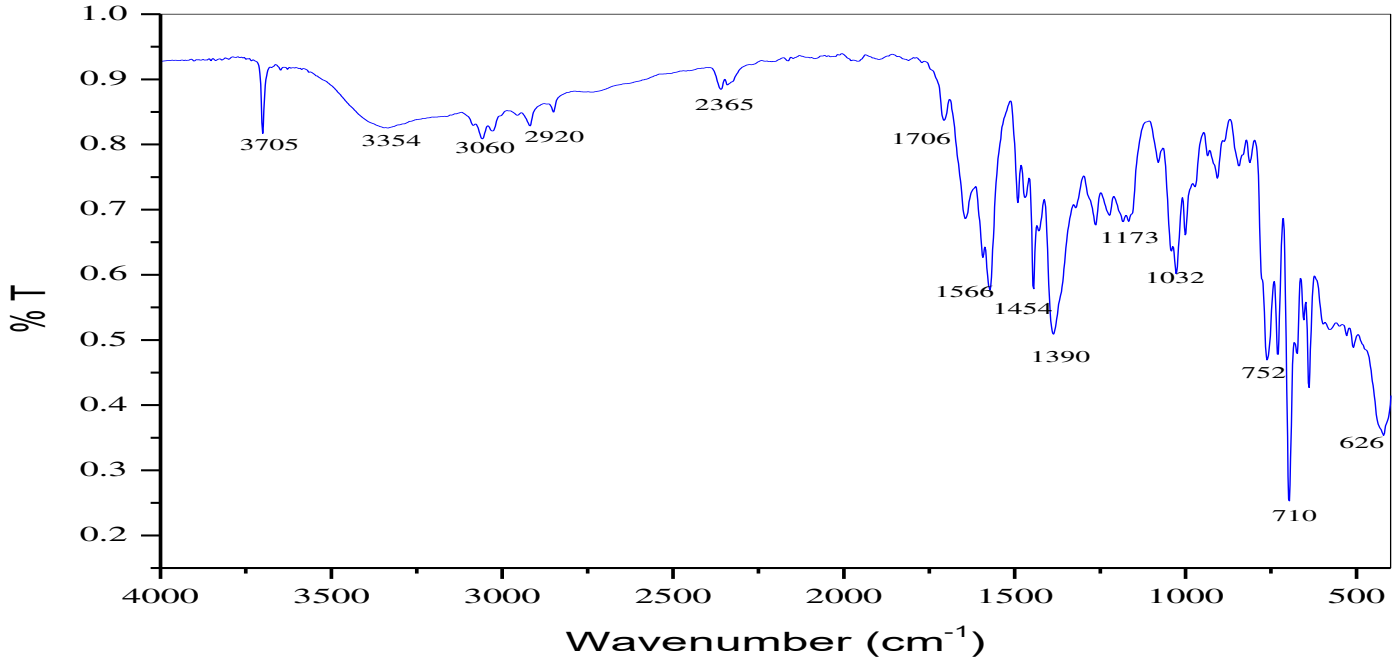

Fig. 7: FTIR showing disappearing concentration of metabolite Diphenylamine from broth

3.7. Mechanism of Azo dye Disperse Orange 3 bio-mineralization

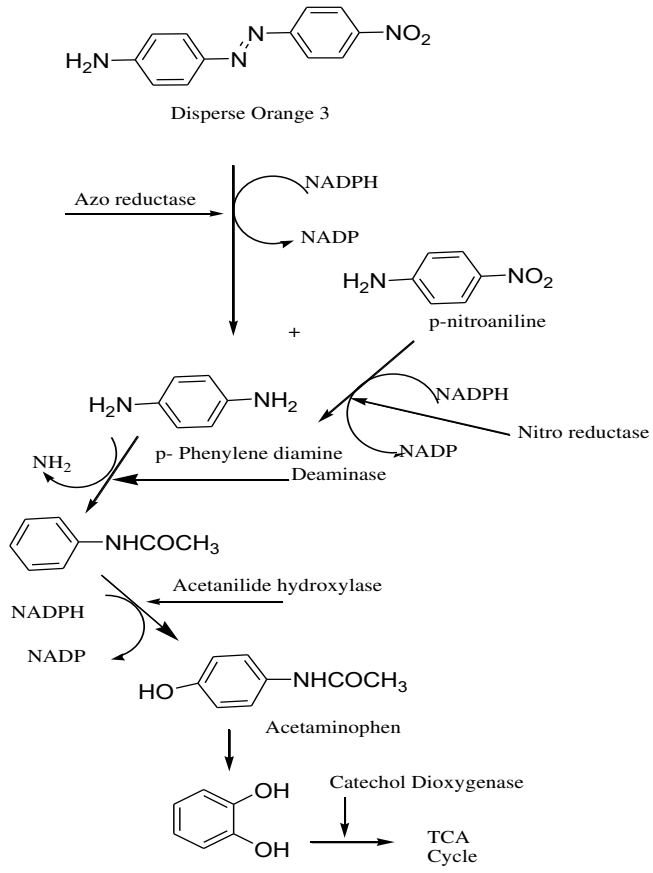

Fig. 8: Mineralization pathway of Azo orange by *Pseudomonas* DL17

4. DISCUSSION

Thus Azo dyes are not readily degraded by microorganisms. The industrial effluents containing chemicals of synthetic origin are seldom found toxic in nature [6]. It was found that under oxygen deficient conditions, redox mediators of abiotic or biotic origin catalyze the transfer of redox equivalent from donor to azo bond results in reduction of the dye and formation of aromatic amines which has been recognized as more carcinogenic than their parent compound by bio-activation [7]. Several physicochemical methods such as adsorption, coagulation, and flocculation are most commonly used worldwide for textile effluent treatment [8]. Silver nano particles also been employed for degradation of Disperse Orange 3 azo dye [9]. Few of the bacterial strains were found resistant to these types of dyes and nitro-aromatics which carry the biodegradation. Moderately alkaliphilic, halotolerant microbes were used for such bio-remediation purpose earlier [10]. Here an attempt was made for detoxification of study considering such dyes by using alkaliphiles isolated from Lonar Crater Lake (MS) Buldana District, India.

Several other studies performed by scientists are as: Laccase biocatalysis was studied by Albert and Gelderblom using ABTS [11-13]. Nitro reductase activity was analyzed by Caballero [14-15]. Catechol 1,2 dioxygenase activity was studied by Guzik Urszula and Gren Izabela [16].

Catechol 2, 3 dioxygenase was monitored by Std method Nozaki and others [17]. CYP450 was studied by standard method of omura and Sato [18]. SOD activities were performed as per Mishra and Fredioch [19]. Azo reductase activity was monitored by Yan [20] et al. Tyrosinase activity was monitored by C. K. Sung and S. H. Cho [21].

On the basis of Initial absorbance A_0 and decreasing absorbance A_t the residual concentration in percentage was calculated and average values used for plotting the graph after repeating it thrice. Fig.1 clearly indicates complete mineralization of the dye. Azo dyes possess toxicity and lethal effects like genotoxicity, mutagenicity, and carcinogenicity to plants and animals [22]. Disperse dyes are the most common allergens among textile dyes. It is not known whether the purified dyes, impurities in the commercial dyes, or metabolites are the actual sensitizers. Disperse Yellow 3 observed allergic with their potential metabolites after azo reduction on contact. Azo dyes contribute to mutagenic activity of ground and surface waters polluted by textile effluents after percolation. Furthermore, their discharge into surface water obstructs light penetration and oxygen transfer into the water bodies with living organisms and threatens the aquatic life affecting environment negatively [23].

The structure assessment of anthropogenic chemicals could enhance our understanding that how the new metabolites get generated by enzymatic pathway and what way the detoxification or bio activation of recalcitrant takes place [24]. *Shewanella xiamenensis* G5-03 isolated from contaminated landfill soil reported as efficiently decolorized five textile azo dyes under static conditions. One of them, Reactive Red 239 (RR239), was completely decolorized [25] at a pH range of 7 to 11 (at 35°C) within 3-6 hr. The most common chemicals used in permanent hair colors are phenylene-diamine, 3-aminophenol, resorcinol, toluene-2, 5-diamine sulphate, sodium sulfite, oleic acid, sodium hydroxide, ammonium hydroxide, propylene glycol and isopropyl alcohol are few components of such type of dyes [26].

Synthetic dyes are widely used in textile, paper, food, cosmetics and pharmaceutical industries with the textile industry as the largest consumer. Several physico-chemical methods have been employed for treatment of textile wastewater but these methods have many limitations due to high cost, low efficiency and problems like secondary pollution. As an alternative to physico-chemical methods, biological methods comprise bacteria, fungi, yeast, algae and plants, enzymes which have been received increasing interest due to their cost effectiveness and eco-friendly nature [27]. Decolorization of azo dyes by biological processes may take place either by bio-sorption or biodegradation. A variety of oxidoreductases might be involved in the degradation of such dyes. Different forms of azoreductases and nitroreductases are produced by several anaerobic bacteria and can be evaluated by various gel assays having azoreductase and nitroreductase activities on the gel. Microbes could decolorize the dyes by both aerobic and anaerobic metabolism. Further, the efficacy of microbial decolorizing enzymes on biotransformation of toxic azo dyes has been discussed here [28].

Alkaliphiles typically grow well at pH 9.0. Obligate alkaliphiles grows up to pH values as high as pH 12 -13. Interest in extreme alkaliphiles arise because they are sources of useful, stable enzymes, their cells can tolerate high salinity, high pH and also found resistant to recalcitrant's [29]. Extracellular reduction' is the electron transfer process from the inside of microbial cells to the outside and finally to reducing extracellular substrates on the cell surface also reported by some researchers and implemented for bioremediation process [30]. Reduction does not mean complete mineralization. It could just be a starting process of mineralization or break down of the azo bond by ping pong mechanism. Sometimes other groups like nitro groups are also present in it might get reduced which shows decolorization in case of active bacterial culture or in case of enzymatic suspensions. Sulphonic acid along with azo group(s) (-N=N-) generates electron deficiency which confers recalcitrant nature to dye molecules. Benzene rings being thermodynamically stable increase their persistence in the environment by providing resistance to xenobiotics compounds [31].

The azo reductase isolated from *Rhodobacter sphaeroides* AS1.1737 was shown to be a flavodoxin having nitroreductase and FMN reductase activities. Both NADH and NADPH could serve as electron donors of azo reductase but NADPH was observed the preferred one. Externally added FMN was also reduced by azo reductase by ping-pong mechanism and was a competitive inhibitor of NADPH in case of methyl red and Nitrofurazol [32]. Several anaerobic bacteria from the human intestinal tract were known to reduce azo dyes and nitro polycyclic aromatic hydrocarbons to the corresponding aromatic amines are having azo reductase and nitroreductase activities [33]. The biological activity of microbes for nitro-substituted compounds is derived

from reductive metabolism of the nitro moiety catalyzed by variety of nitroreductases. Resistance of bacteria to nitro-substituted compounds is believed to result primarily from mutations in genes encoding oxygen-insensitive nitroreductases [34].

One of the major problems that humans are facing is the restoration of the contaminated environment. The textile industry is a large water consumer and produces large volumes of contaminated water. Seriously industrialized area produces millions of liters of improperly treated effluents that are released directly without giving proper treatment severely damages to ecosystem [35].

The experimental bacterial culture was tested for p-nitro aniline bio-tranformation separately using similar alkaline broth media and supernatant was tested for formation of the metabolite by usual methods employed for its characterization confirmed *Pseudomonas DL17* bacterial strain is also having nitro reductase activity. Earlier study referred Disperse orange 3 (DO-3) dye degradation resulted in formation of 4-nitroaniline [36]. As nitro aniline toxicity leads to bladder cancer while 4-ethoxyacetanilide (phenacetin), is a major component of the analgesics consumed in excessively large quantities turned to development of renal papillary necrosis and renal, pelvic and bladder cancer. Thus the experimental strain *Pseudomonas* DL17 could be much useful in maintaining environmental safety if used for bio-remediation at polluted sites.

Ligninolytic enzymes, including Laccase, Manganese peroxidase and Lignin peroxidase have attracted much attention in the degradation of such contaminants [37]. Laccase is an enzyme which belongs to the oxidoreductase class (EC 1.10.3.2) [38]. Laccases are recognized for their wide spread applications including ethanol production, food processing, dye bleaching, paper and pulp processing, as well as production of value added chemicals from lignin, bioremediation, effluent detoxification and bio-polymer modification [39] etc. Phenolic compounds are toxic in nature and these compounds are able to cause environmental hazards as well as health hazards which include cardiac arrhythmias, renal diseases, skin cancer and even death. Laccases and tyrosinases catalyze the oxidative transformation of a large number of phenolic and non-phenolic aromatic compounds *viz.*, phenol, o- and p-cresol, catechin, gallic acid, phenylenediamine and benzenediols to their corresponding quinines. Qunones and semiqunone formation may generate free oxygen radicals. This might have induced the SOD at higher level than normal or standard level in experimental strain *Pseudomonas DL 17*. As the experimental setup involved a part of bio-catalytic study, fig.6 showed increase in activity of SOD, Tyrosinase, Laccase comparative to standard means there might be formation of little semibezoquinone from catechol in the pathway. Fig. 8 shows that the experimental strain seems to going through catechol pathway. The semi bezoquinone can attack the bacterial genome and to prevent the damage of bacterial DNA from the free radical generated or avoid the effect of semi bezoquinone or phenolic compound like acetaminophen generated in excess. As the CYP450 and other related enzymes showing their hikes comparative to standard suggested that the experimental strain is very much active against the Azo dye under alkaline condition.

Complete mineralization of azo dyes generally involves a two-step aerobic and anaerobic treatment for degradation. Oxygen insensitive azo reductases are attracting our sight in present days. Molecular studies have revealed that the first reductive process can be carried out by two classes of enzymes involving flavin-dependent and flavin-free azo reductases under anaerobic or low oxygen conditions. The second step that is carried out by oxidative enzymes primarily having broad specificity of Peroxidases, Laccases and Tyrosinases [40]. Few of the examples concern with flavodoxin based azo reductases were also shown nitro reductase activity [41]. A nitroreductase with distinct properties had shown the activation of the prodrug 5-aziridinyl-2,4-dinitrobenzamide isolated from *Bacillus amyloliquefaciens*.

The experimental strain *Pseudomonas* DL 17 isolated from Lonar Lake Buldana (MS) India showed higher degradation potential depicted in fig. 1 at pH 9.00 and at temperature 37°C. It has found that the experimental concentration was completely removed by this strain as per another study compared to azo dye tolerant strain of *Acinetobacter* sp.SRL8 had used up to 300 mg/L^{-1} and it had a shown broad decolorizing spectrum [43]. It is well known that mostly the bacterial strains showed positive effect on biodegradation after bio-augmentation. In this experiment, the broth media was used with rich nutrient and at the ending time of nutrients the azo Dye was added to media aseptically. After specific time of interval, supernatant was collected to observe optical density to calculate residual concentration as shown in fig. 1 and used for solvent extraction, purification to characterize the metabolites formed by FTIR, NMR and GCMS. As p-phenylenediamine, p-nitro aniline,

acetanilide, acetaminophen, catechol etc. were found appeared in broth media, on the basis of metabolites generated and the bio- catalytic study the mechanism of degradation of this recalcitrant dye was designed. It was tried to monitor the FTIR spectra of p- phenylinedimine which is one of the principal metabolite generated. Its existence from 12 to 24 hr was recorded by FTIR and found that its concentration also diminishing as per increasing time (Fig. 7). It explains that in later phase of experiment, the concentration of Azo dye was exhausting from the broth media used for experimental study.

The enzymatic study or bio-catalytic expression suggests that stimulation of the recalcitrant changed the CYP450 absorption in crude extract as shown in fig. 5a and fig. 5b. while fig. 6 shows percent induction of the enzyme involved in biotransformation. CYP450, Azo reductase, Nitroreductase, Laccase, Tyrosinae, SOD, Cat 1, 2 dioxygenase as well as Cat 2, 3 dioxygenase found hiked comparative to control indicates that this experimental bacterial strain is having higher potential of bioremediation. Oblgate alkaliphiles and adaptation of microbes to higher pH showed higher Cytochrome-C content, may cause to produce even CYP450 and may involve more actively in bio-remediation [44].

Although several physical, biological and chemical methods like adsorption, coagulation, flocculation, membrane filtration, ozonization, electrochemical, radiolysis, are employed for removal of toxicants. Few oxidation processes have been known to decolorize the textile effluent including use of nanao particles [45- 46]. Bio-remediation techniques involving use of bacterial, algal, fungal methods are found cost effective. Among these bacterial bioremediation is more preferential due to high growth rate and no fear of clogging the tubes of bio-reactors by fungal fibers. CYP50 are heme protein family of enzyme with an imidazole ligand such asmyoglobin and hemoglobin as well as an inactive form of P450 are characterized by Soret maxima at 420 nm [47] activated after the experimental azo dye induction for 24 hrs shown in fig. 5.

5. CONCLUSION

The strain *Pseudomonas DL17* is an alkaliphile having higher degradation potential of recalcitrant azo orange. It can be used at polluted site for decolorization of the effluent as well as making the site clean.

6. ACKNOWLEDGEMENT

Authors are thankful to Prof. K R Gawai for gifting the strain and allowing most of the work in his laboratory, Department of Chemistry, Bio-chemistry Division, Savitribai Phule Pune University, Pune. Authors are also thankful to UGC, New Delhi for granting the Teacher Fellowship for this Research work.

Conflict of interest

None declared

7. REFERENCES

1. Agarwal K. *Indian Journal of Fibre & Textile Research,* 2009; **34:**384-399.
2. Andrzej Paszczynski, Maria B. Pasti-grigsby. *Applied and Environmental Microbiology*, 1992; **11:**3598-3604.
3. Amjad Hussein, Miklas Scholz, *et al.*, *Environmental Science and Pollution Research,* 2018; **25:**6870-6889
4. Abdel Naser Zaid, Rowa' AL- Ramahi, *et al.*, *International Journal of Pharmacy and Pharmaceutical Sciences,* 2013; **5:**485-488.
5. Awoke Guadiea,d, Samson Tizazua, Meseretu Meleseb. *Biotechnology Reports,* 2017; **15:**92-100.
6. Forgacs E, Cserhati, *et al*, *Environment international*, 2004; **30(7):**953-971.
7. Hong Y, Xu M, Guo J, et al, *Applied and environmental microbiology*, 2007; **73(1):**64-72.
8. Harshad L, Sanjay G, *Journal of Chemistry,* 2015; Article ID 894109.
9. Anuradha, Manoj G et al, *Indian Journal of Chemical Technology,* 2015; **22:**167-170.
10. Radhika B, Aruna K. *Journal of Global Biosciences,* 2019; **8(7):**6324-6367.
11. Alberts JF, Gelderblom WCA, Botha A, Vanzyl WH. *Int J Food Microbiol.,* 2009; **135:**47-52.
12. Woro Triarsi Sulistyaningdyah, Jun Ogawa, *Microbiology Letters,* 2004; **230:**209-214.
13. Lu L, Zhao M, Zhang BB, et al, *Appl. Microbiol. Biotechnol,* 2006; **74:**1232-1237.
14. Caballero A, Lazaro JJ, Ramos JL, Esteve-Nunez A, *Environ Microbiol,* 2005; **7(22):**1211-1219.
15. Caballero A, Ramos JL. *Environ Microbiol,* 2006; **8:**1306-1310.
16. Guzik Urszula, Gren Izabela, Wojcieszyska Danuta, abuek Sylwia, *Brazilian Journal of Microbiology,* 2009; **40:**285-291.
17. Nozaki M, *Methods of Enzymol,* 1970; **17A:**522-525.
18. Omura T, Sato TR. *J. of Biol. Chem*, 1964; **239:**2370-2378.
19. Misra HP. Fridivich I. *J. Biol.Chem*, 1972; **247:**3170-3175.
20. Yan B, Zhou J, Wang J, Du C, Hou H, Song Z, Bao Y. *Microbiol Lett.,* 2004; **236:**129-136.

21. Sung CK, Cho SH. *Journal of Biochemistry Molecular Biology,* 1992; **25:**564-572.
22. Sudha M, Saranya A, *et al.*, *Int. J. Curr. Microbiol. App. Sci,* 2014;**3(2):**670-690.
23. Laura Malinauskiene, university Sweden, thesis, 2012, Occupational and Environmental Dermatology Unit,
24. Chetan CO, Milind SP *et al.*, *Bioresource Technology* 2013; **130:**360-365.
25. Aline Silva Cossolin, Hélen Cristina Oliveira dos Reis, *An Interdisciplinary Journal of Applied Science* doi:10.4136/1980-993X.
26. Chua-Gocheco A, Bozzo P, Einarson A. *Can Fam Physician,* 2008; **54(10):**1386-1388.
27. Pradeep KS, Ram LS, *Int. J. Appl. Sci. Biotechnol.*, 2017; **5(2):**108-126.
28. Sudha M, Saranya A, *et al.*, *Int. J. Curr. Microbiol. App. Sci,* 2014; **3(2):**670-690.
29. Laura Preiss, David B. Hicks *et al.*, *Frontiers in Bioengineering and Biotechnology,* 2015; **3:**75.
30. Lovley DR. *Geobiology,* 2008; **6:**225-231.
31. Díaz E. *International Microbiology,* 2004; **7(3):**173-180.
32. Guangfei Liu, Jiti Zhou, *et al.*, *Appl Microbiol Biotechnol,* 2007; **76:**1271-1279.
33. Fatemeh Rafii, Carl E. Cerniglia, *Environmental Health Perspectives,* 1995; **103:**17-19.
34. Whiteway J, Koziarz P, *et al.*, *Journal of Bacteriology,* 1998; **180:**5529-5539.
35. Maulin PS, Patel KA et *al.*, *Journal of Bioremediation & Biodegradation,* 2013, **6:**511-517.
36. Valéria PB, Marilda DA, *J. Braz. Chem. Soc.*, 2013; **24(5):**830-836.
37. Siqi Liu, Xiaolin Xu. *Romanian Biotechnological Letters,* 2016; **21(3):**235-240.
38. Giardina P, Faraco V. *Cell Mol. Life Sci.*, 2010; **67(3):**369-385.
39. Shahid M, Azeem K et al., *Crit Rev Biotechnol,* 2015; **15:**1-13.
40. Guangfei Liu, Jiti Zhou, et al., *Appl Microbiol Biotechnol,* 2007; **76:**1271-1279.
41. Gill MA, Thomas V, *Microbiology* 2002; **148:**297-306.
42. Zhiqiang C, Wenjie Z et al. *Water Environment Research,* 2015; **87(6):**24-37.
43. Matsuno T, Yumoto I. *BioMed Research International* 2015, Article ID 847945.
44. Mei Huang, Kun Zhao, *Engineering,* 2011; **18:**358-362.
45. Gupta VK, Khamparia S, *Global J. Environ. Sci. Manage*, 2015; **1(1):**71-94.
46. Wells AV, Li P, Champion PM, Martinis SA, Sligar SG. *Biochemistry,* 1992; **31:**4384-4393.
47. Abhinav L, Ilia G. Denisov *et al.*, *Arch Biochem Biophys,* 2011; **504(1):**26-35.