DECOLORIZATION OF AZO DYES BY FREE LIVING DIAZOTROPHS

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Abstract: Biological oxidation of organic dyes is important for textile industry wastewater treatment. They are generally considered as xenobiotic compounds that are very recalcitrant against biodegradative processes. Nevertheless, during the last few years it has been demonstrated that several microorganisms are able, under certain environmental conditions, to transform azo dyes to non – colored products or even to completely mineralize them. The bacterial metabolism of azo dyes is initiated in most cases by reductive cleavage of azo bond, which results in the formation of usually colorless amines. The present study deals with the decolorization of some selected azo dyes (Congo Red, Erichrome Black T, Methyl Orange , Methyl Red and Trypan Blue) used in textile and dyeing industries by the selected two unidentified acclimatized diazotrophs (DA17 and DA26) isolated from sludge and soil, collected from nearby area of dye industry outlets. In the screen test, because the best results were showed against Methyl Orange, further decolorization procedures were maintained on this dye. The optimization process started with the addition of 1g/l yeast extract, where the decolorization ability of the two strains increased sharply. The effect of different conditional and chemical factors on the decolorization process of Methyl orange by two diazotroph isolates was studied. Factors that contributed to the difference were different pH, temperature, incubation period, inoculum size, carbon source and nitrogen source. Features of the decolorizing process related to biodegradation were also studied.

Keywords: Azo dyes, Bioremediation, Decolorization, Textile wastewater.

Introduction: Rapidity of industrialization and urbanization around the world has lead to the recognition and understanding of relationship between environmental pollution and public health. Whereas, the pollution triggered by the human activities become the top most challenge for modern civilization. Among the most concerned environmental pollutions that threatening our biodiversity, water pollution is a major one where effluents from dye based industries serve as principal source [9]. Textile dye wastewater has become one of the main reasons of severe pollution problems due to the greater demand for textile products and increase in production and application of synthetic dyes. Dyes are broadly classified into several types. Based on the chemical structure of chromophoric group synthetic dyes are classified as azo dyes, anthraquinone dyes etc. Among the used dyes, azo dyes are the most commonly utilized reagents owing to the presence of the azo group which confers to these chemicals a certain resistance to light, acids, bases and oxygen, the desired properties for clothes' makers [3],[8],[19]. Azo dyes, the largest chemical class of dyes with the greatest variety of colors, have been used extensively for textile, dyeing and paper painting. These dyes cannot be degraded and some are toxic to higher animals. Over $7x10^5$ metric tons of synthetic dyes are produced worldwide every year for dyeing and printing, and out of this, about 5% - 10% are discharged with wastewater [4]. The amount of dye lost depends on the class of dye applied; it varies from 2% loss with the use of basic dyes to about 50% loss in certain reactive sulfonated dyes [12],[20]. The presence of dyes in aqueous ecosystem diminishes photosynthesis [14] by impending light penetration into deeper layers thereby deteriorating water quality and lowering the gas solubility. Furthermore, the dyes and / or their degraded by –products may be toxic to flora and fauna [1].Azo dyes have long been recognized as a human urinary bladder carcinogen and tumorigenic in animals. Cyanogenic in fishes, reduction in seed germination and induce dwarfism in plants [17]. Azo dyes are considered as electron deficient xenobiotic components because they possess azo (N=N) and sulphonic ($-SO_3$) electron withdrawing groups, generating electron deficiency and making the component less susceptible to oxidative catabolism by bacteria [10],[13].The physiochemical methods are financially and often also methodologically demanding, time consuming and mostly not very effective. Green technologies to deal with this problem include adsorption of dyestuff on bacterial and fungal biomass or low cost non conventional adsorbents. Biological processes, such as biodegradation, bioaccumulation and biosorption, have received increasing interest due to their cost, effectiveness, ability to produce less sludge and environmental benignity[5],[6],[22],[26].

Some specialized **st**rains of aerobic bacteria have developed the ability to use azo dyes as sole source of carbon and nitrogen; others only reduce the azo group by special oxygen- tolerant azo reductase. However, these azo reductase have a narrow substrate range. The workers reported about bacterial strains which display good growth in aerobic or agitation culture, but color removal was obtained with a high efficiency in anoxic or anaerobic culture [7]. Azo linkages are easily reduced under anaerobic

conditions, yielding colorless aromatic amines which, with a few exceptions, are not mineralized anaerobically, but are readily degraded aerobically. Therefore, a combination of anaerobic and aerobic conditions is proposed for azo compounds' mineralization [15],[16],[23]. Bacterial decolorization under aerobic conditions usually results in adsorption of dye stuffs on bacteria rather than their oxidation. White rot fungi can degrade a wide variety of recalcitrant compounds by their extra cellular enzyme systems. However it is difficult to keep them in functional form in the activated sludge systems, because of their special nutritional and environmental requirements. More ever bacterial degradation is much faster than fungal degradation of textile dyestuffs [18].There is a very growing demand to revitalize the role of indigenous plant growth promoting Rhizobacteria for the bioremediation of azo dyes because they are generally considered as xenobiotic compounds as well as recalcitrant in nature that causes severe contamination of the rivers and ground water in those areas of the world with a high concentration of dyeing industries. Soil near to textile effluent outlet is readily get contaminated by the dyes and expected to harvest a variety of dye acclimatized plant growth promoting rhizobacteria for e.g. Heterotrophic Diazotrophs. These organisms have not been explored much for dye decolorization. Thus very little information is available on the ability to bioremediate these textile dyes by free living nitrogen fixing bacteria. The present study deals with revitalizing the role of dye acclimatized indigenous plant growth promoting free living diazotrophs for the bioremediation of azo dyes, isolated from soil near to textile and dyeing industries effluent outlets.

Materials and Methods:

Sample collection: Samples were collected randomly in duplicate from various textile , dyeing and printing industries from units in and around Ulhasnagar. Samples were collected from different places such as drainage canal that carry textile effluent, soil samples polluted by such drainage effluent, and untreated sludge effluent. All the samples were collected in sterile glass screw cap tubes and preserved at 4°C in refrigerator and samples were tested within 24 Hrs. of collection.

Dyes: All selected dyes viz. Congo Red, Erichrome Black T, Methyl Orange, Methyl red and Trypan Blue and other chemicals used for study were of AR grade.

Acclimatization of Diazotrophs with dyes:

All these samples were homogenized on rotary shaker by taking 10 gm amount from each. To these homogenized samples, mixture of selected azo dyes in final concentration of 50 mg/l was added in increasing concentration viz.- 20%, 40%, 60%, 80% and 100% for 15 days[25]. After period of fifteen days, acclimatized sample was then added in 200 ml sterile Burk's Nitrogen free medium composition (g/l) $MgSO_4$ 0.2; $CaSO_4$ 0.08; $FeSO_4$ $7H_2O$ Trace; Na_2MoO_4 Trace; Glucose 10; K_2HPO_4 0.87; KH_2PO_4 0.68.pH-7.0±0.2) in 500 ml conical flask and incubated at Room temperature on shaker for one week. This enrichment process for Diazotrophs was repeated for thrice with 5% inoculum from the previous enrichment medium and at the end isolates were obtained on solidified Burk's Nitrogen free medium.

Enrichment and cultivation of the most efficient decolorizing Diazotrophs: The Mineral salt basic medium (Composition (g/l) Na_2HPO_4 $7H_2O$ 3.6; $(NH_4)_2SO_4$ 1.0; KH_2PO_4 1.0; $MgSO_4$ 1.0; $Fe(NH_4)_2$ citrate 0.01; $CaCl_2$ $2H_2O$ 0.1; pH-7.0 ± 0.2) was supplemented with 1 gm/lit yeast extract [1]. The enriched culture of Diazotrophs (10ml) was transferred into 500 ml flasks containing 200 ml mineral salts medium and dye (prepared using a mixture of 5 types of dyes). All dyes were mixed together to get stock solution of mixture of dyes 0.25 g/l (0.05 g/l of each dye). The stock solution was supplemented with a basic salt medium to get a final concentration of 0.05 g/l. The flasks were incubated at room temperature under static conditions for 3 days. This process of enrichment was repeated for thrice with same medium, dye concentration and incubation conditions. Every time these flasks were used to isolate the target microorganisms by streaking the mineral salts agar medium containing the same ingredients of the previous broth medium plus agar and 50 ppm of azo dyes mixtures. Separate colonies of the predominant types of microorganisms were purified by restreaking on the same medium. The purified isolates were examined microscopically to check for purity. Obtained pure cultures were maintained on the nutrient agar at 4°C (in refrigerator).

Screening to select the most potent decolorizing Diazotrophs : The primary screening was done to test the ability of the purified isolates to utilize dyes as the sole carbon source. Screening to test the ability of isolated organisms to utilize Congo Red(CR), Erichrome Black T (EBT), Methyl Orange(MO), Methyl red(MR) and Trypan Blue(TB) as the sole carbon source was carried out in mineral salt basic medium used in isolation. Here 0.1 g/l Yeast extract was replaced by 0.05 g/l of individual dye. Organisms were selected on the basis of their ability to grow and reduce pigmentation under these conditions. The secondary screening was performed to ensure the ability of selected isolates to decolorize individual dyes by using 100 ml of screening medium in triplicate sets with final concentration of dye 50 ppm in 250 ml flasks, with 5% of culture isolate with 0.1 OD at 600 nm against media control. The

uninoculated screening media supplemented with the azo dye was used as control under light exposure to compare abiotic color loss during the experiment. Dye decolorizing activity was determined under static conditions at room temperature [1].

The cells were harvested by centrifugation at 5000 rpm for 10 min. the supernatant was used to assay azo dye reduction by measuring residual absorption at the appropriate wavelength for each azo dye. An UV-Visible spectrophotometer was used for absorbance measurement.

Identification of the most potent dye decolorizing Diazotrophs: The two most potent diazotrophs (DA 17 and DA26) showing the highest decolorization potential during screening studies were selected to complete the study. They were identified on the basis of cell shape, cell arrangement, nutritional characteristics, physiological and biochemical characteristics .

Decolorization Assay: On the basis of obtained screening results, for further different parameters studies, the Methyl Orange azo dye and two selected diazotroph DA 17 and DA 26 as a potent dye decolorizers were used.

All samples were centrifuged at 10,000 rpm for 10 min. The supernatant was read at absorbance with maximum (λ max) values using spectrophotometer [1]. The efficiency of color removal was expressed as the percentage of the decolorized dye concentration to that of the initial one i.e., the difference between the initial dye concentration, Dye(i), and the residual dye concentration, Dye (r), of the sample:

$$\text{Decolorization (\%)} = \frac{\text{Dye(i)} - \text{Dye(r)}}{\text{Dye (r)}} \times 100$$

Bacterial growth was measured as a turbidity difference calculated by difference in OD at 600 nm using formula .Turbidity = $OD_{(\text{before centrifugation})} - OD_{(\text{after centrifugation})}$ Methyl Orange decolorization under different culture conditions by selected diazotrophs: The effect of various culture conditions such as pH, temperature, incubation period and inoculum size on decolorization of Methyl Orange by selected diazotrophs was examined.

Effect of PH: Two milliliter of cell suspension (0.1 OD at 600nm) was used to inoculate 100ml Mineral salt basic medium, supplemented with 0.1 g/l Methyl Orange dye and 1 g/l Yeast extract. The medium was adjusted to pH of 4,6,7,8 and 10 using (1 N) Hydrochloric acid and (1 N) Sodium Hydroxide. Flasks were incubated for 5 days at Room temperature (30˚C).

Effect of Incubation temperature: The experiment was carried out with 100 ml of Mineral salt basic medium supplemented with 0.1 g/l of Methyl Orange and 1 g/l Yeast extract. The medium was adjusted to pH 7.0 and each flask was inoculated with a predetermined equal cell density for the two strains. Flasks were divided to be incubated at different temperature: 0˚C, 10˚C, RT (30˚C), 37˚C and 55˚C.

Effect of Incubation period: Decolorization of MO dye by both the diazotrophs isolates was recorded at different intervals of time from 15 Hrs. to 120 Hrs. under predetermined optimum pH (pH-7) and optimum temperature (30˚C) conditions.

Effect of Inoculum size: Different inoculation sizes 1-5 milliliters of the two bacterial diazotroph isolates DA 17 and DA 26 ranging from OD 0.1- 0.5 at 600 nm wavelength were used. All other optimal culture conditions were taken into consideration. At the end of incubation period the Methyl orange dye decolorization was determined as previously determined.

Effect of Carbon source: Different carbon sources were introduced into the Methyl orange mineral salt media at an 1% concentration. The carbon sources were represented by Mannitol, Glucose, Lactose, Sucrose, Maltose and Xylose. In all cases, other previously mentioned optimal conditions were taken into consideration.

Effect of Nitrogen source: Different Nitrogen sources were introduced into the Methyl orange mineral salt media at an 1% concentration. The nitrogen sources were represented by Potassium nitrate, Sodium nitrite, Ammonium chloride, Ammonium citrate, Ammonium sulphate, Ammonium oxalate, Ammonium molybdate , Casein hydrolysate and Peptone. All other optimal factors were carried out as previously mentioned.

Decolorization and COD removal under static and shaker conditions: This experiment was carried out in order to investigate the Methyl orange decolorization and COD removal under different incubation conditions by Diazotroph isolates DA17 and DA26. This was carried out by incubating the flasks under static and shaker conditions, containing the Mineral salts medium with Methyl orange dye in addition to the optimal sources of carbon and nitrogen resulting from the previous optimization. At the end of incubation period, Methyl Orange decolorization was assayed as previously mentioned and COD was measured according to a standard procedure [2].

Effect of dye concentration: The various concentrations of dye (100, 200, 300, 400 and 500 mg/l) were added into Mineral salt basic medium in order to examine the effect of initial dye decolorization in static conditions. Percent decolorization and biomass at different time intervals were measured.

Spectrophotometric analysis of dye decolorization: Decolorization of Methyl Orange dye under predetermined optimal conditions was

studied for both the isolates for 120 Hrs. at different wavelengths ranging from 425-650 nm in order to observe the progress of biodecolorization.

Fed batch decolorization of dye**:** The fed batch decolorization of Methyl orange was studied by adding 100mg/l of dye into the 24 Hrs. grown culture of diazotrophs, after decolorization 100 mg/l dye added into the decolorized broth without supplement of additional nutrient. Dye was added continuously until culture does not lose decolorization ability [24]. The dye concentration was determined by monitoring the absorbance of dye and the percent decolorization was determined by procedure reported earlier.

Results and Discussion: Diazotrophs with efficient decolonization potential for textile dyes particularly azo dyes were isolated from dye polluted soil collected from effluent channel of a local textile and dyeing industries. it was expected that sites near to textile and dyeing industries pollutes with dyes harbor several microorganisms which are capable to coexist with higher toxic levels of pollution. These microorganisms adapt to the new polluted environment thus they can play an important role in clearance of this environment through their growth and function. Pure strains were isolated from the microbial consortium to find out the most promising strains with the higher degradative activity. The final aim was to assure that they do not produce toxic metabolites and are safe if used in bioremediation process.Total 32 diazotroph strains were isolated from a dye polluted soil and dye effluent waste water. Out of which two potent dye decolorizing strains of diazotroph were selected based on their performance in primary screening and secondary screening and the Methyl Orange dye was selected for further studies based on its suitability for biodegradation by the isolated diazotrophs.

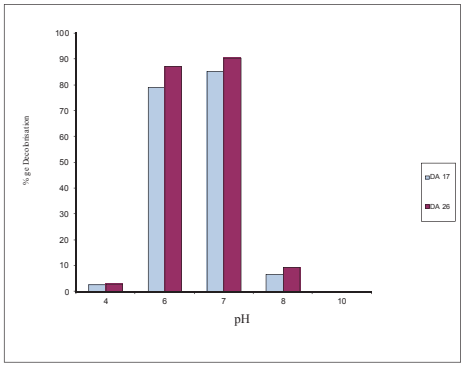


Fig.-1 – Effect of pH on MO dye decolorization by isolates DA17 and DA26.

pH value affects not only on the decolorization capability, but also on the color stability . The effect of different initial pH values (4-10) on decolorization of Methyl Orange by two selected diazotrophs DA 17 and DA26 was recorded after 48 Hrs. as presented graphically in Fig.-1. The optimal pH for decolorization by both strains was pH 7 and the decolorization percentage was decreased when solution pH made more acidic / alkaline. This seems to indicate that neutral pH values would be more favorable for the decolorization process of Methyl Orange by diazotroph isolates DA17 and DA26. The medium pH is also an important factor with regard to decolorization. The pH has a major effect on the efficiency of dye decolorization and many authors had reported that the optimal pH for color removal is often between 6.0 and 10.0. Many authors had reported the rate of color removal is higher at the optimum pH and tends to decrease rapidly at strongly acid or strongly alkaline pH [21]. It is thought that the effects of pH may be related to the transport of dye molecules across the cell membrane, which is considered as the rate limiting step in the decolorization.

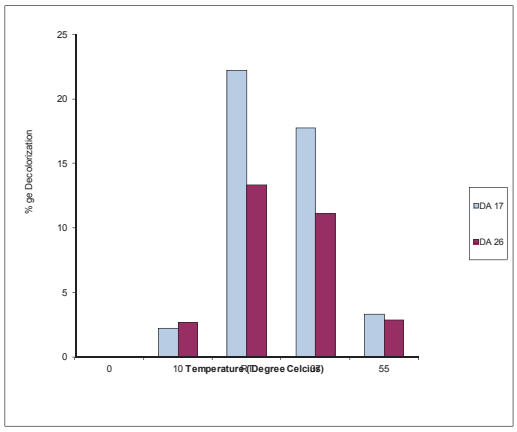
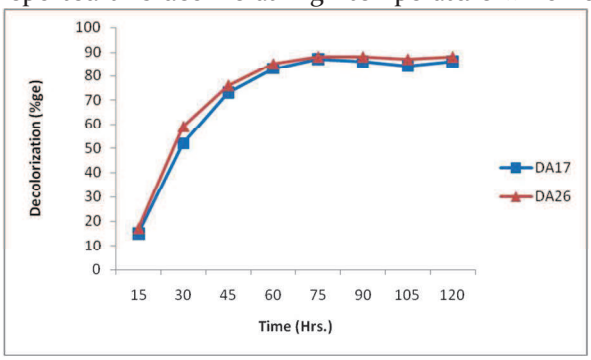


Fig.-2- Effect of temperature on MO dye decolorization by isolates DA17 and DA26.

The experiments were performed at different temperatures ranging from 0˚C – 55˚C (Fig.-2).The decolorization increased as the temperature increased as evidenced in Fig.2. But the color removal ability of both the strains decreased sharply at a temperature of 37˚C and further increases in temperature resulted only in marginal reductions in decolorization activity of the two isolates. The optimal incubation temperature for the decolorization process by the two isolates was Room temperature (30˚C). Decolorization percentage decreased as temperature dropped below Room temperature or increased over this particular value. Therefore the optimum temperature for both the isolates was determined as room temperature (30˚C). The decolorization rate of azo dyes increases up to the optimal temperature and afterwards there was a marginal reduction in the decolorization activity. In

accordance to our results, many workers also had reported this decline at high temperature which can



be attributed to the loss of cell viability or the denaturation of an azo reductase enzyme [21].

Fig.3.Effect of Incubation period on decolorization process of MO dye by Diazotroph isolates DA17 and DA26. The results recorded for decolorization of MO dye by isolates DA17 and DA26 in Fig.-3, showed that there were slight changes in color removal after first 15 Hrs. of incubation, but after 30 Hrs. of incubation decolorization was increased with maximum at 75 Hrs. of incubation period for isolate DA17 (87%) and isolate DA26 (88%) and after wards decolorization ability remain stagnant for both the isolates. The difference between the results recorded at 75 Hrs. and 120 Hrs. was only 1.2% of dye color removal.

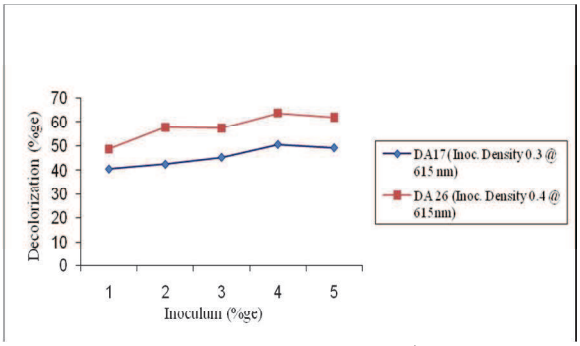


Fig.-4 – Effect of inoculum %ge (@ predetermined density) on MO dye decolorization by isolates DA17 and DA26.

Results presented graphically in Fig.-4 indicated that there was increase in decolorization up to 4 ml of 0.3 OD @615nm in case of DA 17 and 4 ml of 0.4 OD at 615nm in case of isolate DA 26. Below this concentration of inoculum the decolorization process was decreased due to less available degradative enzymes compare to substrate. While above 4 ml concentration of culture the stagnancy in decolorization could be due to limitation of substrate concentration or cell aggregation formation which make unavailable the reacting sites.

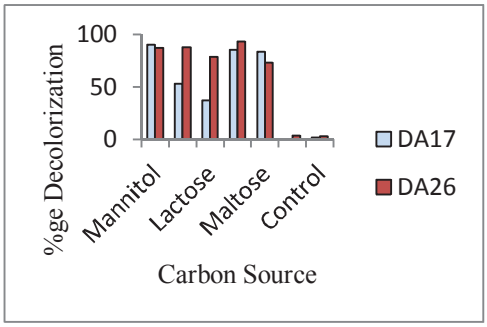


Fig.-5- Effect of different carbon source on decolorization of MO dye by isolates DA17&DA26.

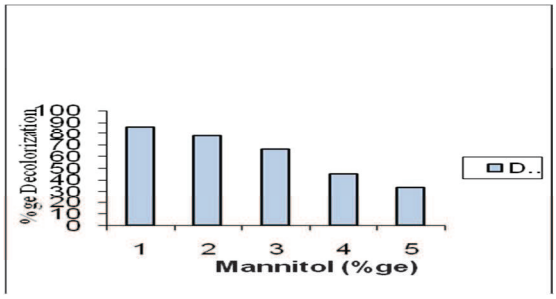


Fig.-6- Effect of Mannitol conc. on MO dye decolorization by isolate DA17.

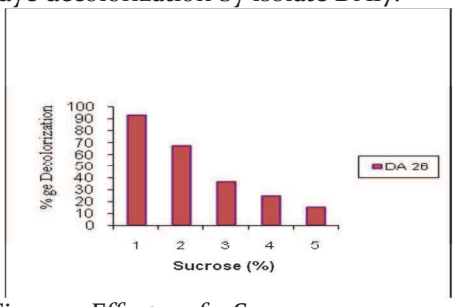
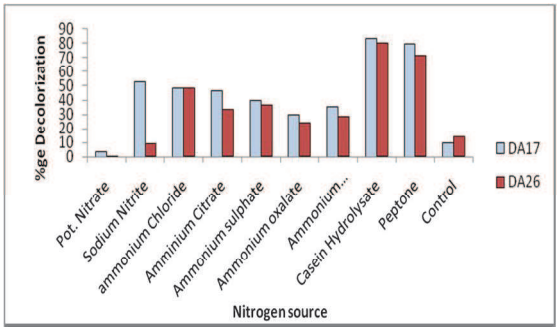


Fig.-7- Effect of Sucrose conc. on MO dye decolorization by isolate DA26.

During decolorization of azo dyes by reduction of azo bonds, it was reported that reducing equivalents from various carbon sources are transferred to the dye. Textile industrial effluents are deficient in carbon content and biodegradation without any extra carbon source is very difficult to achieve. Therefore, different co substrates such as Glucose, Sucrose, Maltose, Mannitol and Lactose (1%) were supplemented in the medium and dye decolorization for all were studied. Results graphically in Fig.– 5 to Fig.-7 revealed that the decolorization of Methyl Orange was increased with 1% concentration of Mannitol in case of isolate DA 17 whereas in case of isolate DA 26, decolorization was more with 1% of Sucrose. After this concentration of carbon source, the decolorization was decreased could be due to excessive acid production in the medium which effect the growth of isolates. In contrast to our findings, some authors had reported that addition of carbon sources seemed to be less

effective in promoting decolorization probably due to the preference of the cells in assimilating the extra carbon sources over using the dye compound as the carbon source [21].

Fig.-8- Effect of Nitrogen source on decolorization of MO dye by isolates DA17 &DA26.



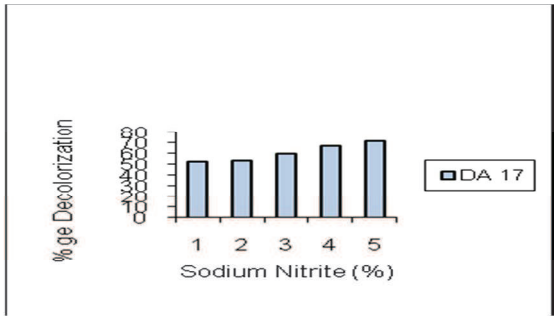


Fig.- 9 – Effect of $NaNO_2$

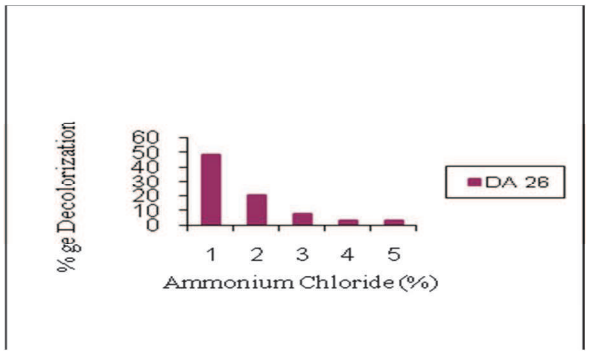


Fig.- 10 – Effect of NH_4Cl concentration on dye decolorization concentration on dye decolorization by isolate DA17.by isolate DA26.

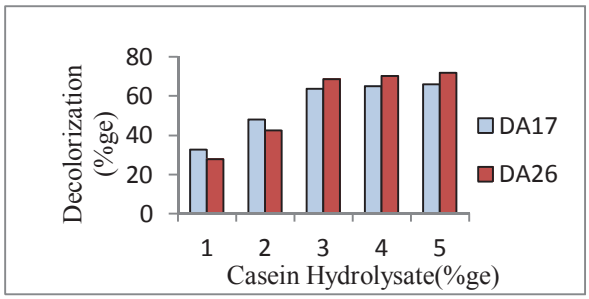


Fig.-11- Effect of Casein hydrolysate conc. on MO decolorization by Diazotroph isolates.

A number of organic and inorganic sources of nitrogen were used in this experiment. Results presented graphically in Fig.-8 to Fig.-11, indicate that, among Organic nitrogen sources, up to 3% Casein hydrolysate showed the enhanced decolorization for both the isolates whereas in case of inorganic nitrogen source, increase in concentration of Sodium nitrite showed the best support for decolorization of methyl orange by isolate DA17 whereas in case of isolate DA 26 , 1% Ammonium chloride showed the promising results. The lowest dye color removal in the case of Potassium nitrate was attained by both the isolates. In accordance to our findings, many authors also had reported that addition of the organic nitrogen sources such as Peptone, Casein hydrolysate, Beef extract, Urea, Yeast extract and so on can regenerate NADH, which acts as an electron donor for the reduction of azo dyes by microorganisms and thus effective decolorization was observed [21]. To make the process economically feasible and practically applicable, some investigators have used agricultural waste as an additional supplement for effective decolorization.

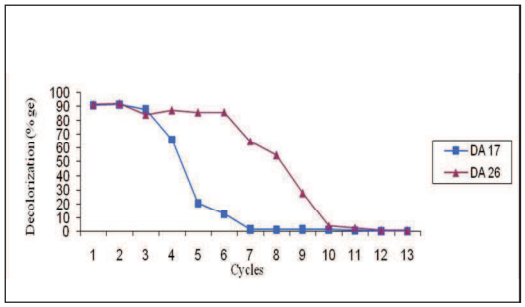
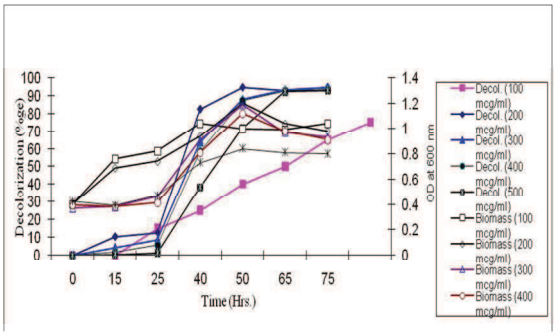


Fig.- 12 – Fed batch MO dye decolorization by isolates DA17 and DA26.

The intention of fed batch study (Fig.- 12) was to check the ability of isolates for the repeated decolorization of Methyl Orange dye. The isolate DA 17 decolorized the repeated addition of Methyl Orange dye up to seven cycles with variable decolorization rate (92 – 10%), whereas the isolate DA 26 showed decolorization for repeated addition of Methyl Orange dye up to ten cycles with variable decolorization rate (93-7%).

Fig.- 13 – Effect of MO dye concentration on growth



and dye decolorization by isolate DA17.

It is clearly shown in graphical presentation (Fig.-13 & Fig.-14) that with increase in concentration of Methyl Orange from 100 – 500 mg/lit, growth of bacteria get affected with increase in lag period and same effect can be seen in decolorization ability of Methyl Orange dye with lesser extent and increase lag period in the case of both the isolates. This could be due to the toxic effect of dye at higher concentration for the isolates. Similar results were reported by other workers [21] that with increasing the dye concentration gradually decolorization rate decreases, probably due to the toxic effect of dyes with regard to the individual bacteria and/or inadequate biomass concentration (or improper cell to dye ratio) as well as blockage of active sites of azoreductase.

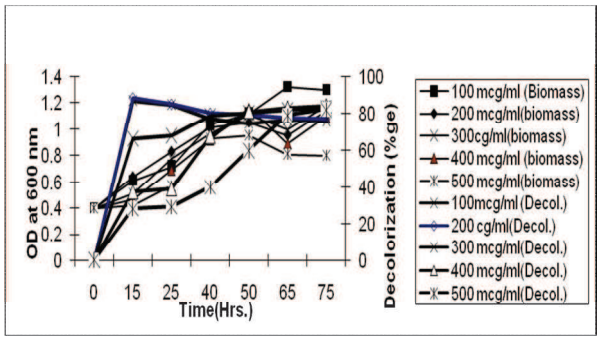


Fig. – 14 – Effect of MO dye concentration on growth and dye decolorization by isolate DA26.

It is clearly shown in graphical presentation (Fig.-13 & Fig.-14) that with increase in concentration of Methyl Orange from 100 – 500 mg/lit, growth of bacteria get affected with increase in lag period and same effect can be seen in decolorization ability of Methyl Orange dye with lesser extent and increase lag period in the case of both the isolates. This could be due to the toxic effect of dye at higher concentration for the isolates. Similar results were reported by other workers [21] that with increasing the dye concentration gradually decolorization rate decreases, probably due to the toxic effect of dyes with regard to the individual bacteria and/or inadequate biomass concentration (or improper cell to dye ratio) as well as blockage of active sites of azoreductase.

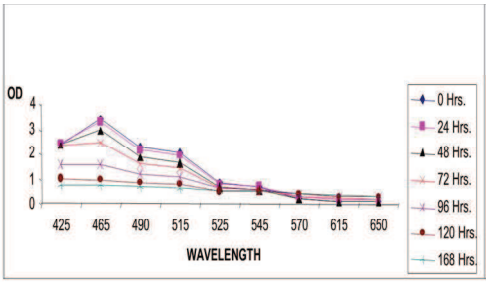


Fig.- 15- Variation of spectra of Methyl Orange azo dye solution after decolorizing cultivation with isolate DA17.

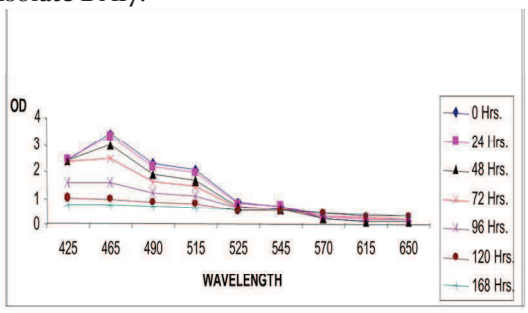


Fig.- 16 – Variation of spectra of Methyl orange azo dye solution after decolorizing cultivation with isolate DA26.

Decolorization 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 colored because of adsorbed dyes, whereas those retaining their original colors are accompanied by the occurrence of biodegradation. Both the Fig.-15 and 16, display the change of visible spectra of Methyl Orange; using the supernatant fluid of the culture after every 24 Hrs. up to 168 Hrs. decolorizing cultivation with Diazotroph isolates DA17 & DA26. The absorbance peak at 465 nm completely disappeared after cultivation for decolorization. In addition, as the azo dye was reduced, the broth returned to its original color. These results support the presumption that color removal by Diazotrophs may be largely attributed to biodegradation. It is reported by many workers that under anaerobic conditions, reductive enzyme activities are higher; however a small amount of oxygen is also required for the oxidative enzymes which are involved in the degradation of azo dyes. Some studies had reported that during bacterial degradation of azo dyes both oxidative and reductive enzymes play a role. Many workers suggested that for efficient color removal, aeration and agitation which increases the concentration of oxygen in solution should be avoided. Whereas some authors reported that the aerobic condition is required for the complete mineralization of the azo dye molecules [21].Thus for the most effective effluent treatment an anaerobic process with subsequent aerobic treatment can be used to decolorize waste waters containing dyes and improve their biodegradability.

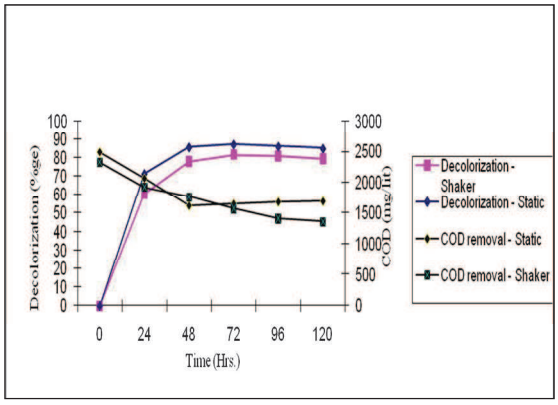


Fig. – 17 – Methyl Orange Dye decolorization and COD removal by isolate DA17 under static and shaker conditions.

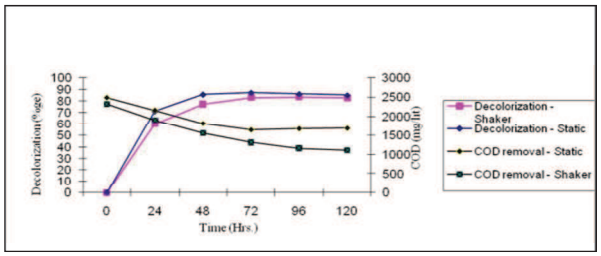


Fig.-18 – Methyl orange dye decolorization (%ge) and COD removal by isolate DA26 under static and shaker conditions.

Experiments on decolorization using Methyl Orange under laboratory conditions were performed. Two strains of diazotrophs DA 17 and DA26, were incubated under static and shaking conditions. The results obtained from treatment of Methyl Orange for both the isolates are shown in Fig.17 and Fig.18. In case of isolate DA 17 (Fig.-17), Under static conditions within 120 Hrs., decolorization of Methyl Orange was 80% with COD removal of 28.50%, while under shaker conditions, dye decolorization was 77.2% with COD removal of 50% were observed. The Fig.-18 shows the results for Decolorization of Methyl Orange by isolate DA26 with 85% dye decolorization and 31.85% COD removal under static conditions. Whereas under shaker conditions, Dye decolorization (80%) was near about same to static but COD removal was increased to 49.47%. Anaerobic conditions are usually referred to as favorable to the reduction step, but it has also shown that isolated strains of diazotrophs can reduce azo dyes in the presence of oxygen. However, the ultimate purpose of complete mineralization of dye molecules cannot usually be reached under anaerobic conditions.

Conclusion: Accumulation of dyestuff and dye wastewater creates not only environmental pollution, but also medical and aesthetic problems. As regulations are becoming even more stringent, there is an urgent need for technically feasible and cost effective treatment methods. Microbial and enzymatic decolorization and degradation of azo dyes have significant potential to address this problem due to their environmentally friendly inexpensive nature and also because they do not produce large quantities of sludge. In addition bacteria have many other advantages such as a fast growth rate and high hydraulic retention time and they could be efficient in treating high strength organic wastewaters. In this study, lab scale studies with isolated free living diazotrophs have been conducted on decolorization of Methyl Orange solution using pure bacterial culture; however there is still a need to generate relative performance data on industrial effluent. Examination of the mechanism of the decolorization process by isolated diazotrophs indicated that it proceeded primarily by biological degradation associated with a minor portion of the dye adsorbing onto the cell surface. To disclose the possible mechanisms of dye decolorization, increased understanding of the biochemical basis (enzymatic studies) and more detailed characterization of the intermediates and metabolites produced during biodegradation using various analytical techniques is to be carried out. Further to ensure the safety of the decolorized wastewater, studies are in progress, on the toxicity of the MO dye and its degraded products. Based on the successful laboratory results, efforts should then be made to scale up and apply bacterial decolorization techniques in real industrial effluent. Moreover, as the knowledge base and funding in this area of research increases, it is hoped that free living diazotroph treatments will become the predominant solution to the problem of colored waste water in the textile coloration industry.

References:

1. Alalewi Amar, Cuiling Jiang , " Bacterial influence on textile wastewater decolorization", Journal of Environmental Protection, Vol.3, 2012, pp 889-901.
2. APHA , "Standard methods for the examination of water and wastewater", 18th Edition, American public health association Washington D.C.1992.
3. Ashjaran Ali, Yazdanshenas Mohammad Esmail, Rashidi Abosaeed, Khajavi Ramin and Rezaee Abbas , " Biosorption thermodynamics and kinetic of direct dye from aqueous solutions on bacterial cellulose", African Journal of Microbiology Research, Vol.6 (6),2012, pp1270-1278.
4. Bayoumi R.A., Musa S.M., Bahobil A./S., Louboudy S.S. and El-Sakawey T.A.
5. "Biodecolorization and Biodegradation of azo dyes by some bacterial isolates" J.Appl.

Environ.Biol.Sci., 1(1), 2010,1-25.

6. Bibi R., Arshad M., and Asghar H.N., " Optimization of factors for accelerated biodegradation of reactive black-5-azo dye", International Journal of agricultural Biology, 14, 2012, 353- 359.
8. Celik leyla, Ozturk Ayten and Abdullah Meysun I., " Biodegradation of reactive red 195 Azo dye by the bacterium *Rhodopseudomonas palustris* 51ATA", African Journal of Microbiology Research ,Vol.6 (1), 2012, pp 120-126.
9. Chen K.C., Huang W.T., WU J.Y. and Houng J.Y. , "Microbial decolorization of azo dyes by *Proteus mirabilis",* Journal of Industrial Microbiology and Biotechnology, Vol. 23, 1999, 686-690.
10. Gharbani P., Tabatabaii S.M., Metrizad A. ," Removal of Congo Red from textile waste water by Ozonation", Int. J. Environ. Sci. Tech., 5(4), 2008, 495-500.
11. Hassan M.M., Alam M.Z. and Anwar M.N. , " Biodegradation of textile azo dyes by bacteria isolated from dyeing industry effluent", International Research Journal of Biological Sciences, 2(8), 2013, 27-31.
12. Jagwani J.S., Lakshmi B., Sharma M.C., " Isolation and identification of a bacterial consortium for biodegradation of a textile dye reactive orange M2R and study of phytotoxicity of the dehraded product on *Phaseolus mungo* and *Triticum aestivum",* Global Research Analysis, 2(8),2013,
13. Jalandani- Buan Aileen C., Anna Lynn A. Decena-Soliven, Ernelea P. CaO, Virgina l.Barraquio and Wilfredo L. Barraquio , " Congo red decolorizing activity under Microcosm and decolorization of other dyes by Congo red decolorizing bacteria", Philippine journal of Science, Vol.138 (2), 2009,
14. Jalandani- Buan Aileen C., Anna Lynn A. Decena-Soliven, Ernelea P. CaO, Virgina L.Barraquio and Wilfredo L. Barraquio , " Characterization and identification of Congo red decolorizing bacteria from monocultures and consortia," Philippine journal of Science, Vol.139 (10), 2010, 71-78.
15. Mahbub K.R., Mohammad A., Ahmed M.M. and Begum Salma , "Decolorization of synthetic dyes using bacteria isolated from textile industry effluent", Asian Journal of Biotechnology, 4(3), 2012, 129-136.
16. Mazumder Debabrata ," Process evaluation and treatability study of wastewater in a textile dyeing industry," International Journal of Energy and Environment, Vol.2, Issue 6, 2011, pp 1053-1066.
17. Mona E. M. Mabrouk and Hoda H.Yusef ," Decolorization of Fast Red by *Bacillus subtilis* HM, Journal of Applied Sciences Research, 4(3), 2008, 262-269.
18. Moutaouakkil A., Zeroual Y., Dzayri F.Z., Talbi M., Lee K., Blaghen M. , "Bacterial decolorization of the azo dye Methyl Red by *Enterobacter agglomerans*." Annals of Microbiology, 53, 2003,
19. Perumal K., Malleswari R. Baby, Catherin A. and Sambanda Moorthy T.A. , "Decolorization of Congo Red dye by bacterial consortium isolated from dye contaminated soil, Paramakudi, Tamil Nadu.", J. Microbial. Biotech. Res., 2(3), 2012, 475
20. Pourbabaee, Ali Ahmad, Matekzadeh, Fereydon, Sarbolouki, Nabi Mohammad, and Ali Mohajeri, " Decolorization of Methyl Orange (As a Model Azo dye) by the newly discovered *Bacillus* sp.," Iran .J. Chem. Eng. Vol.24, No.3.,2005.
21. Rajendran R., Karthik Sundaram S., and Uma maheshwari K., " Aerobic biodegradation of mixture of azo dye containing textile effluent using adapted microbial strains." Journal of Environmental Science and Technology. 4(6), 2011,568-578.
22. Rajesh Sawhney, Arun Kumar, " Congo red (Azo dye) decolorization by local isolate VT-II inhabiting dye effluent exposed soil," International Journal of Environmental Sciences, Vol .1. No.6, 2011, pp.1261-1266.
23. Sratale R.G., Saratale G.D., Chang J.S., Govindwar S.P. " Bacterial decolorization and degradation of azo dyes: A review.", Journal of the Taiwan Institute of chemical Engineers 42, 2011, 138-157.
24. Selvam K., Shanmuga Priya. M , " Biological treatment of Azo dyes and textile industry effluent by newly isolated white rot fungi *Schizophyllum commune* and *Lenzites eximia*," International Journal of Environmental Sciences, Vol.2 No.4,
25. Shah M.P., Patel K.A., Nair S.S., darji A.M., " Microbial decolorization of methyl orange dye by *Pseudomonas* spp." OA Biotechnology. 2(1).10,
26. Telke Amar, Kalyani Dayanand, Jadhav Jyoti and Govindwar Sanjay , " Kinetics and Mechanism of Reactive Red 141 degradation by a bacterial isolate *Rhizobium radiobacter* MTCC 8161," Acta. Chim. Slov, 55, 2008, 320-329.
27. Thorat P.R., Sayyad M., " Microbial decolorization and degradation of crystal violet by aerobic bacteria." The Bioscan. 5(4). 2010, 591-594.
28. Vidali M., " Bioremediation .An Overview.", Pure Appl. Chem., Vol.73 (7), 2001, pp.1163-1172.

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