ISSN 2278-7763

MICROBIAL DECOLOURISATION OF AZO DYES - A COMPARITIVE ANALYSIS

Mabel Joshaline.C[*], Subathra.M[*], Shyamala.M[*], S.Padmavathy[**]

***- Department of Microbiology, Sourashtra College, Madurai.625004, Tamil Nadu, India.**- Department of Microbiology, Thiagarajar College, Madurai.625009, Tamil Nadu, India. Email : jesmabmary2512@gmail.com**

Abstract

Textile industries are vital components of improving the economy of a country. Neverthless they contribute a major propotion to the category of environmental pollutants. Coloured dyes were collected from select outlets of Madurai city and the native actinomycete was isolated . The dye decolorisation capability of the native isolate was tested against a standard MTCC strain of ***Phanerochate chrysosporium*.** The declourisation percentage of ***Streptomyces sp*** decreased as the amount of dye effluent added to the media increased. Red dye reported 55% for 1ml of the dye effluent and green dye reported 60%. This was reduced to 30% when 25ml of dye effluent was added. The standard MTCC strain reported 79% and 42% respectively for the same amount . The same trend was observed for the protein content also.

Key words: Textile dye, Effluent, Azo dye, ***Streptomyces sp***, Decolorisation percentage.

Introduction

Textile dye industries use a huge volume of water of processing and discharge them as effluents. They consume substantial volumes of water and chemicals for wet processing of textiles. These chemicals are used for derizing, scouring, beaching, dyeing, printing and finishing. They range from inorganic compounds and elements to polymers and organic products. (1,2). The only thing in common is their ability to absorb light in the visible region. Colour is the first contaminant to be recognized in waste water and has to be remarked before discharging into water bodies or on land. The presence of very small amount of dye in water, even less than 1 ppm is highly visible and affects the aesthetic merit, water transparency and gas solubility in lake's rivers and other water bodies (3).

Thus the textile industry, effluents are continuously being discharged directly into surrounding agricultural lands without neutralization. Uncontrolled discharge of untreated effluents from these industries on to the land may have profound influences on physic chemical and biological properties and their by affect land fertility (4). Azo-dye constitutes the largest of dyes used commercially in the textile industries. (5) Toxicity of azo-dye, especially Benzedrine based dyes is well known particularly they are mutagenic, carcinogenic and allergic in nature. Azo-dye have chromophoric a group as N=N which is the color forming component if dye stuff. A very large number of dyes belonging to the azo class can be found in direct, acid, basic mordant, disperse and reactive dyes.

Decolourisation techniques

To depollute the dye waste water, the physic – chemical methods, absorption, chemical precipitation, flocculation, photolysis, chemical oxidation and reduction, electro chemical treatment and ion – pair extraction Have proved to be costly and less effective (6).

Biological decolourisation

The treatment of textile waste water by purely biological process may be possible even without the inclusion of other carbon sources, (eg) municipal waste water. This has been the subject of intensive research in recent years. Many biological systems are available. Aerobic activated

ISSN 2278-7763

sludge (7). Aerobic – ananerobic packed bed reactors (8). Aerobic anaerobic sequential batch (or) continuous – flow reactors (9). Anaerobic batch reactors (10).

Decolourisation capability of microbes

Bacteria

Numerous bacteria capable of dye decolourisation have been reported. Efforts to isolate bacterial cultures capable of degrading azo dye started in the 1970s with reports on ***Bacillus subtilis***. (11) similar reports were also made on *Bacillus cerus* ***Pseudomonas*** and ***Aeromonas (***6,12) described a bacterial consortium capable of mineralizing the sulfonated azo dye mordant yellow. *Pseudomonas luteola* grown well in media containing low glucose concentration (0.125) and without N-source showed 95% colour removal within 5-6 days, under static incubation process (12).

Fungi

Bio decolourisation of lignin containing pulp and paper waste water as measured by the decrease in colour absorption using two basidiomycete fungi ***Phanerochaete chrysosporium*** and ***Tinctoporia*** sp was reported as early as 1980 Since then ***Phanerochaete chrysosporium*** in particular has been the subject of intensive research related to the degradation of a wide rqance of recalcitrant xenobiotic compounds including azo dyes. Later several works were done ***Phanerochate chrysosporium*** its capability and mechanism of decolourisation (13). A strain of Trichoderma species was also shown to decolorize lignin containing plant effluent (14). Myrothecium verrucaria was shown to have a very strong binding affinity to some azo dye which were recoverable by extraction with methanol (16).

Actinomycete

Nutritionally the ***Streptomyces*** species are quite versatile. Growth factor requirements are rare and a widely variety of carbon sources, sugars, alcohols, organic acids, amino acids and some aromatic compounds can be utilized. Most isolates produce extra cellular hydrolytic enzymes that permit utilization of polysaccharides (Starch, cellulose, hemicelluloses) proteins, fats hydrocarbons, lignin, tannin or even rubber. ***Streptomyces*** spp can often be obtained by spreading a soil dilutioj on an agar medium containing polymers such as casein and starch. A single isolate may different carbon sources. (12) compared the efficiency of a soil ***Actinomycete*** culture, ***Streptomyces chromofcesus*** to that of ***P.chrysoporium*** and reported that fungi performed better than the ***Actinomycetes***

Materials and methods.

Collection of soil sample

Dye amended soil samples were collected from the 4cm of the dye industry effluent soil. The soil was store in polythene bags at 4^{o}C.

Characterization of samples:

Native isolates were examined for colony morphology, pigmentation, cell shape and gram reaction as per the standard procedures they include Gelatin hydrolysis, starch hydrolysis, Nitrate reduction test, catalase test.

Methodology

Phanerochaete chrysoporium MTTC NO. 787 was obtained from IMTECH, Chandigarh. It was sub-cultured in malt agar (2%) and used throughout the experiment. Spore

ISSN 2278-7763

inoculums was prepared by washing the spore of the fungus from 14 days old slants into 10ml of sterile distilled water. Liquid cultures were maintained by inoculating 100µl of spore suspension in 10ml of basal medium (2% Malt extract broth)

Dye sample collection

The dye effluent samples were collected from a colour yarn, near sellur at Madurai city and these samples were used for experiments.

Sample inoculation of dye effluent

100ml conical flasks containing the sterilized minimal synthetic media at a concentration of 49ml, 45ml,40ml,25ml,10ml and 5ml to this add a dye effluent sample was added with a sterilization glass pipette making up the volume to 50ml. This was inoculated with the isolated strains of ***Streptomyces*** spp (Broth culture containing beads) and ***Phanerochate chrysosporium*** MTCC No 787 separately. The sets were incubated.

Estimation of protein

Lowry's method

Reference observation were made on 7,14 and 21 days. The protein content was also estimate before and after incubation by Lowery's et al., method.

Decolorisation Percentage (4)

Decolourising activity expressed in terms of percent decolourisation were determined by calculation the decrease in absorbance for red and green dye at 600nm and 40nm respectively. Decolourisation activity was calculated according to the following formula

$$D = 100 \times \frac{A_{ini} - A_{fin}}{A_{ini}}$$

Where,

D = Decolourisation

A_{ini} = Initial Absorbance

A_{fin} = Final Absorbance

The samples were withdrawn at intervals, centrifuged at 10,000rpm for 5 min and the supernatant was taken separately and its absorbance was made at 600nm and 540nm for red and green dye effluent respectively.

Experimental Results

Investigations were carried out to find out the decolourization capacity of the Streptomyces spp which was isolated from dye amended soil. Two dyes (Red and Green colour) were selected, their effluent was inoculated with *Streptomyces Spp*. The decolourisarion capacity of the *Streptomyces spp* was compared with *Phanerochaete chrysosporium* MTCC No 787

Identification of isolate

Streptomyces spp was isolated from dye amended soil and was characterized based on its shape, colour, margin and elevation of the colony. Simple and differential staining was done to confirm its shape and Gram's reaction. Biochemical test were performed for the isolate and its was found that, it was catalase positive and capable of nitrate reduction and utilize gelatin and starch.

ISSN 2278-7763

Comparision between Streptomyces spp and Pharnerochaete chrysosporium MTCC No 787.

Decolourisation percentage

Green colour effluent

The decolourisation percentage was calculated a detailed in the methodology and was tabulated. For the isolated **Streptomyces spp** the decolorisation was calculated after 7 days and for the standard strain of ***Phanerochaete chrysoporium*** MTCC No 787, it was calculated after 7,12 and 21 days. It was found that the decolorisation percentage ranged from 8 to 60 for Streptomyces spp. Lower the concentration of the dye higher the decolorisation. For a standard strain of ***Phanerochate chrysoporium*** MTCC No 787 the decolorisation percentage ranged 12 to 85 on the whole. But for the whole 21 days the decolorisation percentage was maximum after 21 days. It was very low after 7 days. Both the organism exhibited slow decolorisation when the concentration of the dye effluent increased in the media.

Estimation of protein

Streptomyces spp

The amount of protein was estimated both before and after incubation which in turn relate to the extent of decolorisation. Green dye was better when compared with red dye. Thus was shown by the increase in the protein content.

S.No	Dye effluent concentration (per 50ml media)	Decolonization percentage	
		Red dye	Green dye
1.	1ml	55	60
2.	5ml	49	52
3.	10ml	41	48
4.	25ml	30	30
5.	40ml	18	13
6.	45ml	3	8

Phaerochate chrsosporium MTCC No 787

The amount of protein was estimated both before and after incubation. The increase in protein content was more for green dye effluent than red dye effluent. Generally the increase in protein content was more for the fungi than the actinomycete.

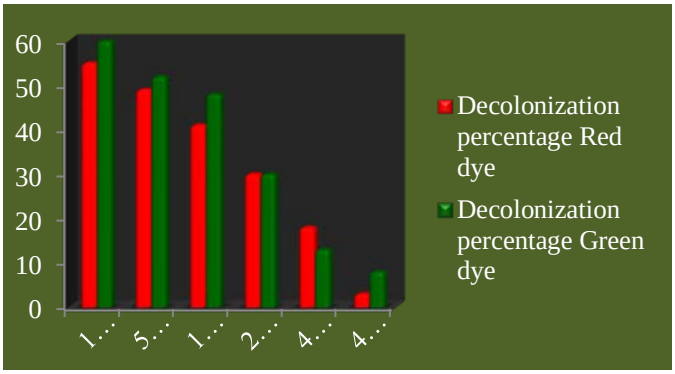

Table: 1. Decoloriosation percentage of *Streptomyces sp for* Red and Green Dye

ISSN 2278-7763

S.No	Dye effluent concentration (per 50ml media)	Decolonization percentage					
		Red dye			Green dye		
		7 days	14 days	21 days	7 days	14 days	21 days
1.	1ml	55	61	79	60	65	85
2.	5ml	49	53	68	53	56	72
3.	10ml	40	42	55	47	52	63
4.	25ml	21	30	42	33	42	54
5.	40ml	16	22	31	18	25	33
6.	45ml	10	13	24	12	15	30

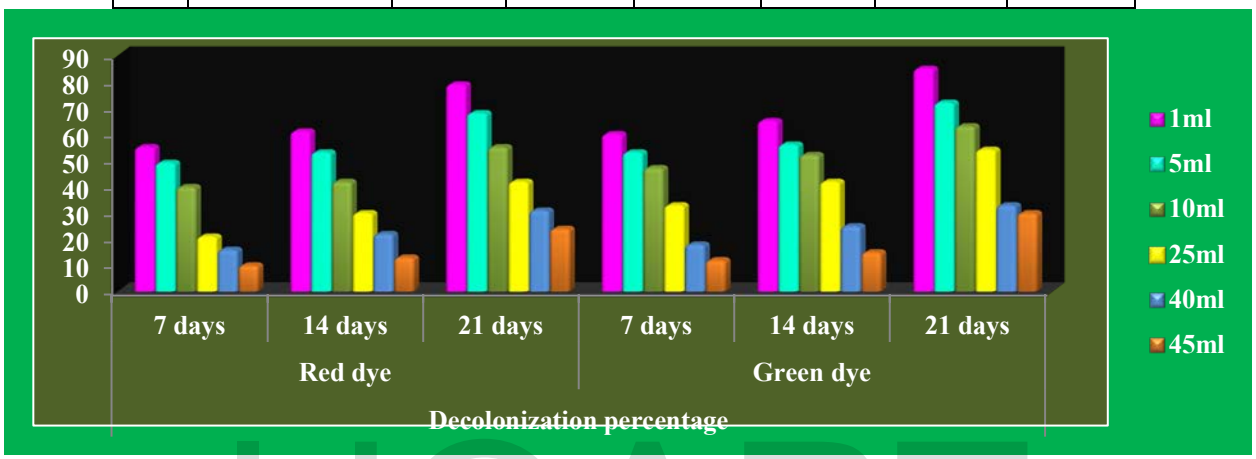

Table: 2. Decoloriosation percentage of *Phanerochaete chrysosporium sp for* Red and Green Dye

S.No	Dye	Period	Protein (mg)	
			Streptomyces sp	*Phanerochaete chrysosporium*
1.	Red dye	Before incubation	2.5	4.3
		After incubation	3.2	5.8
2.	Green Dye	Before incubation	2.5	4.3
		After incubation	4.1	7.2

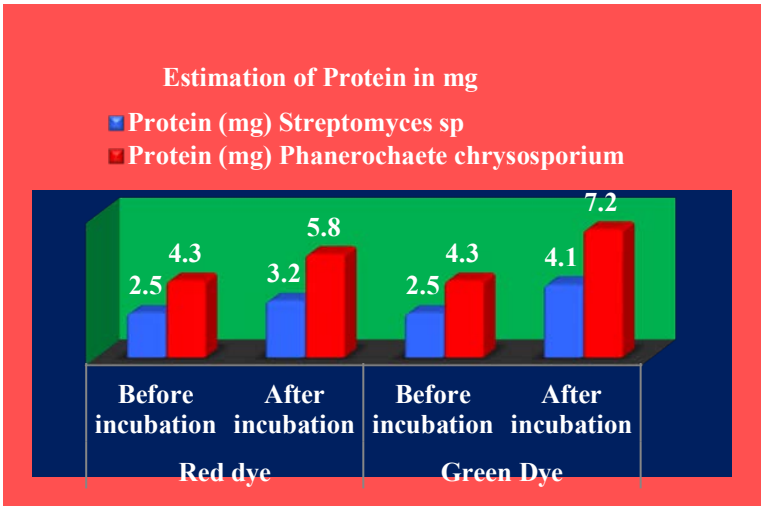

Table: 3. Estimation of Protein

Discussion

An attempt was made in this study of decolourise the textile industry effluent (Green and red) using Streptomyces spp. It was isolated from dye amended soil. It was identified and characterized based on its non spreading nature on solid media and its Gram reaction and

ISSN 2278-7763

biochemical test. A standard strain capable of decolourisation of textile dye effluent was got from IMTECH, Chandigrah. It was tried along with the isolated Streptomyces spp.

Certain group of microorganisms is capable of degrading these dyes enzymatically. On the basis of dye components they produce the enzyme and utilize the substrate. Thus this study also focuses on the capacity of degrading one aromatic compound are able to decolorize other similar compounds. Our isolates *Streptomyces spp* is capable of degrading the dye industry effluent. This is found by the change in colour of the minimal media change in colour of the beads and an increase in the protein content of the organism after the treatment. Similar reports were made by (17). The decolorisation percentage was around 60 which was very low than fungi and bacteria. This is may be due to the slow growth.(18) Microbial transformations have long been beneficial to mankind. More recently the environmental implications and biological application are well studied in the degradation of dye industry effluent. Therefore the isolate was compared with a standard dye decolorizing strain Phanerochaete chrysosporium. The decolourisation percentage was high when compared with the actinomycetes. Among the 2 days selected for the study green was better decolouisied than red. The percentage difference was more 1. (11) This indicated than greed dye was preferred by the organism than red dye (4). The protein content also increased significantly after incubation indication its better growth. Thus green dye was better decolorized because of its increased the protein content. Thus the experimental results infer that the actinomycetes isolated from dye amended soil are able to decolorize by the dye industry effluent. When compared with a standard strain Phanerochaete chrysoporium MTCC No 787 it's less efficient.

References:

1. Society of Dyers and colorists. 1976. Colour index 3rd edition. Society of dyes and colorisets Yorkshire U.K.
2. Zollinger. H.1987. Colour chemistry- synthesis properties and application of organic dyes and pigments VCH publishers newyork.92-100.
3. Poots. V.J., G.Mekay and J.J.heak 1976. The removal of acid dye from effluent using natural absorbents. Water Res.10.1067-1076.
4. Nikhath. K., D. Seshikala and M.A. Singara Charya. 2000. Decolorisation of textile dyes by fungi. I. J. Microbiol 40(9): 191-197.
5. Eaton.D., H.M.Chang and T.K.Kirk.1980.Fungal Decolorisation of Kraft bleach plant effluent. Tappi.J.,63: 103-109.
6. Yatome., CT. Ogawa K. Itoh, A.Sugiyama and E. Idaka, 1987. Degradation of azo dyes by cell free extract from Pseudomonas stutzeri. Soc. Dyers and colorists 106: 280-282.
7. Shaul G.M., G.M., T J.m Holds worth, C.R.Dempsey and K.A .Dosstal.1991. Fate of water soluble azo dyes in the activated sludge process chemospere. 22: 107-119.
8. Schliephake.K., G.T. Lonergan.C.L.Jones and D.E. Manimwaring 1993. Decolorisation of pigment plant effluent by Pycnoporus cinnabarinus in a packed bed reactor. Biotechnol Lrtt, 15:1185-1188.
9. Ganesh.R.,G.D.Boardman and D.Michel sen. D. 1994. Fate of azo dyes in sludge. Water.Res.28:1367-1376.

ISSN 2278-7763

10. Carliell.C.M., S.I. Barclay, N.Buckley,E.Senior 1995. Microbial decolorisation of reactive of a reactive azo dye under anaerobic conditions. *Water S of 21:61-69.*
11. Horitsu.H.,M. Takada, E.Idaka,M.Tomayeda and T.Ogawa 1997. Degradation of P-aminoazo benzene by Bacillus subtilis. Eur.J.Appl.Microbiol.$:217-224.
12. Haug.W.,A.Schmitt, B.Nortemann, D.C.Hempel,A.Stolz and H.J.Knackmurs 1991. Mineralization of the sulphonated azo dye mordant yellow 3by a 6- amino naphthalion 2-sulphonate – degrading bacterium consortium .Appl.Environ.Microbiol.57:3144-3149.
13. Cripps.C., J.A. Bumpus and S.D. Aust.1990. Biodegradtion of azo and heterocyclic dyes by Phanerochaete chrysosporium *.Appl. Environ Microbio.S6:1114-1118.*
14. Prasad.D.Y and T.K.Joyee.1991. Colour removal from kraft bleach plant effluent by Trichoderma sp Tappi. J. 165-169.
15. Cripps.C., J.A. Bumpus and S.D. Aust.1990. Biodegradtion of azo and heterocyclic dyes by Phanerochaete chrysosporium *.Appl. Environ Microbio.S6:1114-1118.*
16. Brahimi-Horn M.C., K.K.Him,S.L.Liancy and D Mou, 1992. Binding of textile azo dyes by *Myrothecium verrucaria.J.Ind Microbiol.10:31-36.*
17. Paszczynski, A., M.B. Pasti-Grigsby, S.Goszczyanski.R.L.Crawford and D.L. Crawford 1992. Minerisation of sulfonated azo dyes and sulfanilic acid by *Phanerochaete chrysosporium and Streptomyces spp.*
18. Hayasse F.2000. isolation and charcterisation of Aeromonas spp. B-5 capable of decolorizing various dyes. Journal of bioscience and bioengineering 90(5) 570-573

