International Research Journal of Multidisciplinary Science & Technology
www.irjmrs.com ISSN : 2455-930X

Degradation and Decolourisation of Dye effluents by using Bacillus subtilis CBNR isolates

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Abstract - *Dyes are chemicals which binds to materials that imports colour due to presence of chromophore group. Azo dyes are the largest class of synthetic aromatic dyes. Azo dyes produce clear and strong colours, is a group of organic compounds. Through mineralisation, these dyes can be broken down into an aromatic amine, an arylamine to be carcinogenic and mutagenic. They reduce the efficiency of seed germination and plant growth, inhibits the elongation of shoot and roots. In the present study, to evaluates the ability of Bacillus subtilis to degrade and decolourise dye into non-toxic form. Bacillus subtilis could tolerate dye effluents at 10mg/ml. The maximum growth observed at 37°C. Maximum rate of decolourisation was observed (90%) when starch, glucose and peptone was supplemented in the medium, and within 72hr. Decolourisation was confirmed by Biomass measurement and UV – VIS spectrophotometer. Further, it is confirmed by Laccase enzyme assay. This work provides a general idea about microbial decolourisation (eco-friendly) of dyes with highlights the application of these processes for the treatment of dye containing waste water.*

Key Words: Irrigation, Dyes, Decolourisation etc...

1.INTRODUCTION

Dyes are generally called as ''chemicals'' which binds to one of the material then to imports colour due to presence of chromophore group. Industrially, the commonly used azo dyes are Acid dye, Basic dye, Direct dye, Disperse dye, Mordant dye, Reactive dye, and Solvent dyes. The Acid, Basic, Direct and Reactive dyes are ionic. (Sudhaet.al, 2014).

Azo dyes are one of the largest and most versatile classes of synthetic dye. Specifically reactive azo dye has complex aromatic structures. Mainly, those dyes are stable because very difficult to degrade. (UsmanAftabet.al, 2011). These are mainly used in the textile, rubber product, paper printing, colour photography, pharmaceuticals, cosmetics, foods, and other industries. (Syed et.al, 2009).

Biodegradation is defined as biologically catalysed reduction in complexity of hazardous organic contaminants in soil, sub-surface materials, and ground water system by using microbes. Bioremediation is a pollution control technology that uses natural biological species to catalyse the degradation or transformation of various toxic chemicals to harmful forms. This process is completed is called as ''mineralization''.(NezhaTahriJoutey et al, and Bhatnagar et al, 2013).

Many textile mills to release millions of litre (10 to 15%) of unwanted effluents in the form of waste water in rivers. This is toxic and harmful to environment. Employment of physicochemical methods for degradation of dyes such as adsorption, coagulation, flocculation, oxidation, filtration, nano-filtration, multiple effect evaporator, use of activated carbon and electrochemical methods. These are effective, but quite expensive. (Joshniet al, 2011).

Compared with physicochemical methods, biological process have more interest because of their cost effectiveness, lower sludge formation, environment friendly. (Gurulakshmi.,2008).These organic materials can be degrade both aerobic and anaerobically. Dyes are not readily degraded by using conventional aerobic treatment systems because create environmental problem. Under an anaerobic condition, dyes are degraded by the cleavage of azo bonds. (Syed et al, 2009).

During this process, microbes obtain carbon and energy through metabolism of organic contaminants. It includes many factors that is cell biomass concentration, contaminant, temperature, pH, moisture, supply of nutrient, carbon and energy source, soil structure,etc.Different waste water management technique handles several industries, but lack implementation due to high cost and low efficiency.

But using microbial technique includes Bacteria, Fungi, Yeast, Actinomycetes and Algae. These are degrading dyes easily. This is a cost effective methods for remove pollutant from environment. (Bhatnagar et al, 2013).Many bacteria involve azo dye degradation in an anaerobic condition that includes Bacteroides species, Eubacterium species, Closteridium species, Proteus vulgaris and Streptococcus faecalis. (Syed et.al, 2009).

Specifically, Bacillus subtilis was gram-positive exposed to degrade variety of toxic organic materials. The bacterial cultures exhibit 90% decolourising ability at 50h. This activity was observed at pH 7.0 and incubates at room temperature. (Suad Ahmed., 2014).Fungi especially representative of white rot fungi should also degrade different classes of dyes. The white rod fungi were most efficient ligninolytic microorganisms used for decolourisation of industrial effluents.For example: Phanerocheatechrysoporium, Thelephora species,Gliocladiumvirense, Trichodermaharzianum, etc. (Lokendra Singh et al, 2010).

Various fungal treatments able to decolourise azo dyes using laccase enzymes it is a time consuming process. (Gurulakshmi., 2008).Laccase based decolourization treatments are potentially advantageous to bioremediation since the enzyme is produced in larger amounts. These copper containing enzymes are oxidative enzyme detected in plants and many fungi. The solid and aqueous state dye decolourisation assay carried out laccase enzyme identification. (sathiyamoorthi, et al, 2007).

2.MATERIALS AND METHODS

2.1.Source of inoculums

In the present study, the *Bacillus subtilis* organism was used against Dye effluents. The organism is isolates CBNR institute by using nutrient medium.

2.2.Sample collection

Two different dye effluent samples (black and sandal) were collected in sterilised bottles from erode textile mills areas. Some physicochemical parameters of dye effluents such as pH(6), color (Black and sandal) and smell were measured.

2.3.Degradation of Dye using medium

For dye degradation process Mineral salt medium was used that containing Peptone 0.5, yeast extract 0.25, glucose 0.5 or sucrose 1.0, K_2HPO_4 0.2, NaCl 0.25 and the medium (pH-7.0) was autoclaved at 121°C for 15 minutes.

After sterilisation, various concentration of (10ml, 20ml and 30ml) two Dye effluents were added and to inoculate the isolated *Bacillus subtilis*(100µl) organism and kept in inoculated shaker for 72 hours.

2.4.Effect of temperature and pH

Thebacterial cultures was inoculated in MSM broth containing dye effluent were incubated at different temperatures (27°C, 37°C and 47°C) and different pH (4, 5, 6 and 7). After 72 hours of incubation, the broth was study the growth of organism at dry weight and degradation at 450nm and 417 against a suitable blank.

2.5.Effect of carbon source on dye degradation

Degradation of dye was observed by the addition of different carbon sources like sucrose, starch, and maltose in MSM media. After 72hours of incubation, the broth was study the growth at dry weight and degradation at 450 and 417nm against a suitable blank.

2.6.Degradation study

Each day 2 ml of samples were withdrawn from the flasks andcentrifuged at 8,500 rpm for 10 minutes. Collect the supernatant and the degradation pattern was studied using a UV- Visible Nano drop spectrophotometer (ELICO SL-159) at 450 nm and 417nm...

Remaining pellet was used to determine the biomass measurement for identification of bacterial growth.

The optimum day, pH temperature and the effect of carbon source were measured by using this above said method.

Decolourization activity was calculated as

Decolourisation (%) = Control –Degradation / Control

2.7.Laccase enzyme assay

Laccase activity was assayed spectrophotometrically at 465nm as described byCBNR Institute standard procedure. Take 1ml of culture supernatant, 1ml Guaicoal and 3ml 5M phosphate buffer, mix and incubate this solution for 30minutes at room temperature.

2.8.Irrigation purpose

The treated water was used for green gram plant growth as follows; the treated water was centrifuged (5,000 rpm for 20 min) to remove such pellet. 5ml of treated water and added to the soil containing green gram seed. Each day an amount of 10mL of treated water, dye water and mixed dye waters were added to the plants and growth was measured at cm. Finally, the chlorophyll content was calculated using methanol and acetone extracts.

3.RESULTS AND DISCUSSION

In the present study, *Bacillus subtilis* was effectively degrades toxic dye enzymatically into non-toxic product form.

3.1.Sub-culturing of Bacillus subtilis

The *Bacillus subtilis* CBNR isolate was sub-cultured on a nutrient agar plate and incubated at 37°C for 24 hours. The grown culture was stored at 4°C for further studies.

3.2.Effect of optimum day on degradation

The optimum day was calculated for the degradation as follows, after inoculation of organism in medium to take the reading for degradation found or not, day by day, these are follows, finally we conclude that third day is optimum for both effluents.

Table 1: Calculate the degradation by *Bacillus* at optimum day

Days	Dye		Dye	
	E1	E2	E1	E2
	Biomass in grams		% of degradation	
1	0.14	0.26	14.64	18.26
2	0.26	0.42	58.46	64.33
3	**0.52**	**0.057**	**83.88**	**79.4**
4	0.50	0.042	62.04	64.30
5	0.46	0.040	43.14	52.84
6	0.44	0.038	40.03	48.80
7	0.047	0.041	38.9	38.9
8	0.036	0.030	12.2	18.4

3.3.Effect of pH on degradation

The optimum pH was calculated for the degradation as follows, after inoculation of organism in medium to note the pH day by day and the degradation was done at pH 7.0 and to get maximum decolourisation.

Table 2: Evaluate the optimum pH on degradation

pH	Effluent 1	Effluent 2
4	43.14%	52.84%
5	58.46%	64.30%
6	62.04%	64.33%
7	**83.88%**	**79.4%**

3.4.Effect of temperature on degradation

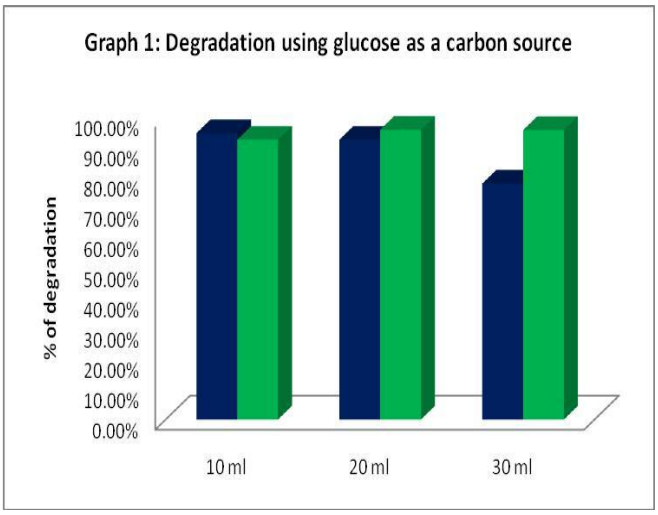
The optimum temperature was calculated for dye degradation analysis as follows, after inoculation of an organism in medium to note the optimum temperature and the degradation was done at temperature 37°C and to get maximum decolourisation.

Table 3: To optimize the optimum temperature during degradation by *Bacillus*

Temperature	Effluent 1	Effluent 2
27°C	62.04%	64.30%
37°C	**83.88%**	**79.4%**
47°C	40.03%	48.80

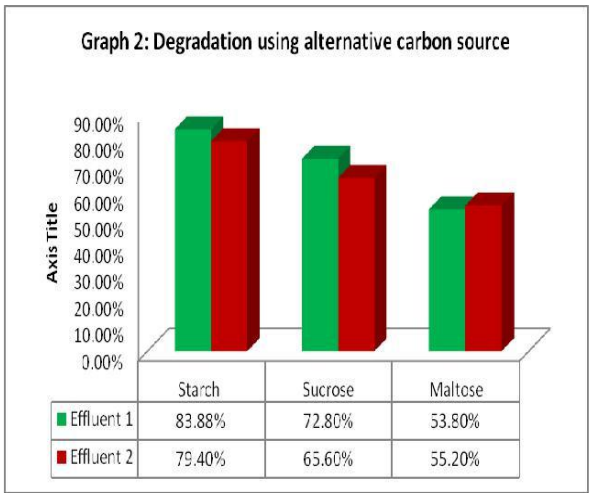
3.5.Effect of carbon source as glucose on degradation:

The different concentration of effluent 1 and 2 was added to mineral salt medium containing glucose as a carbon source with pH 7.0 and temperature 37°C.After 72 hours of incubation biomass and level of degradation was measured in UV- visible spectrophotometer.For effluent 1 the biomass was obtained as 0.04 g, 0.02 g and 0.01 g and its degradation level is 94.65%, 92.5% and 78% respectively at 10 ml, 20 ml and 30 ml. Similarly for effluent 2, biomass was obtained as 0.026 g, 0.022 g and 0.029 g and its degradation level was 92.5%, 95.8% and 95.7% respectively.



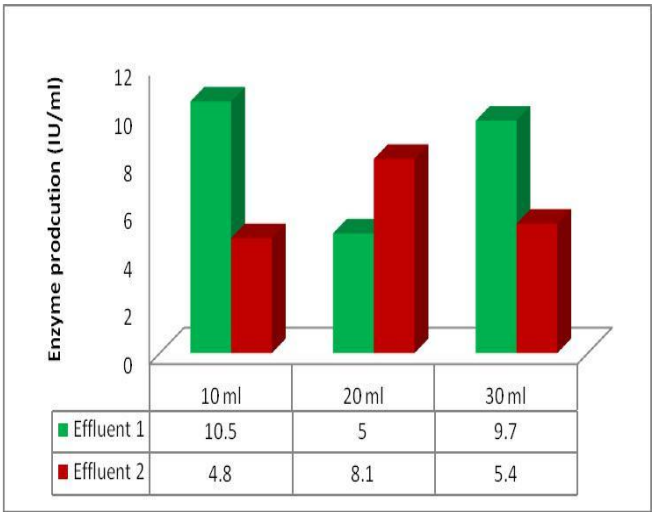
3.6.Effect of carbon source as starch, sucrose and maltoseon degradation:

The 10 ml of effluent 1 and 2 was added to mineral salt medium containing starch, sucrose and maltose as a carbon source with pH 7.0 and temperature 37°C.After 72 hours of incubation biomass and level of degradation was measured in UV- visible spectrophotometer. For effluent 1 the biomass was obtained as 0.05 g, 0.03 g and 0.02 g and its degradation level is 83.88%, 72.8% and 53.8% respectively. Similarly for effluent 2, biomass was obtained as 0.05 g, 0.04 g and 0.03 g and its degradation level was 79.4%, 65.6% and 55.2% respectively.



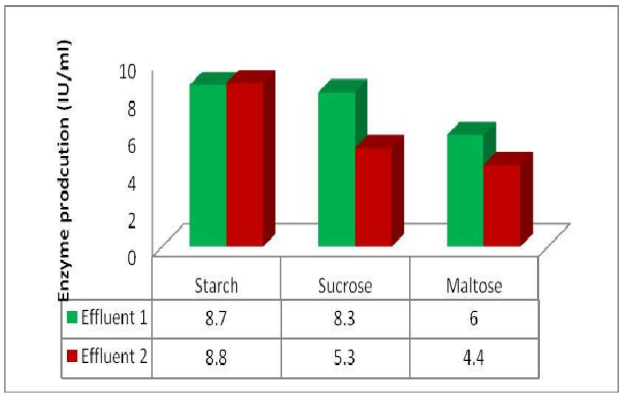
3.7.Effect of laccase on degradation:

Degradation of effluent 1 and 2 with glucose as a carbon source was measured by the activity of laccase using enzyme assay. Production of laccase enzyme in degraded effluents was measured in spectrophotometer and enzyme units were calculated. For effluent 1, 10.5 IU/ml, 5 IU/ml, 9.7 IU/ml in 10 ml, 20 ml and 30 ml respectively. Similarly for effluent 2, 4.8 IU/ml, 8.1 IU/ml and 5.4 IU/ml in 10 ml, 20 ml and 30 ml of effluents respectively.



Graph 3: production of laccase enzyme by glucose as a major carbon source

Degradation of effluent 1 and 2 with starch, sucrose and maltose as a carbon source was measured by the activity of laccase using enzyme assay. Production of laccase enzyme in degraded effluents was measured in spectrophotometer and enzyme units were calculated. For effluent 1, 8.7 IU/ml, 8.3 IU/ml, 6.0 IU/ml in 10 ml, 20 ml and 30 ml respectively. Similarly for effluent 2, 8.8 IU/ml, 5.3 IU/ml and 4.4 IU/ml in 10 ml, 20 ml and 30 ml of effluents respectively.



Graph 4: production of laccase enzyme byalternative carbon source

From the above calculated enzyme units *Bacillus subtilis* and laccase enzyme was effectively degrade and decolourise the dye effluent and it would be useful in the agriculture field.

3.8.Recycling of treated effluent for green gram:

At last, both treated effluents were used for cultivation of green gram as in-vitro. The growth of plant has some variations compared with normal tap water that has amount of chlorophyll present in

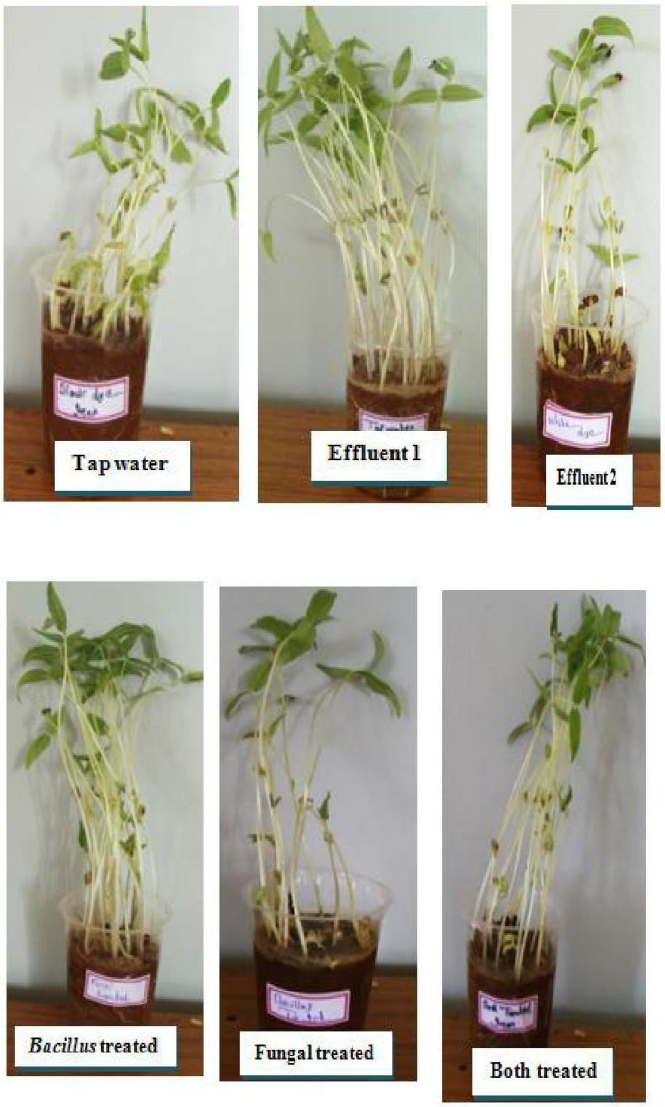


Figure 1: Cultivated green gram using tap water, effluents and treated water.

3.9.Effect of degraded water on green gram

Green gram leafs were analysed by find out the chlorophyll content using degraded water. Methanol and acetone solvents were used to determine the chlorophyll content and it was measured in spectrophotometer reads at 663 nm and 645 nm.

Table 4: Measurement of chlorophyll a

Categories	Methanol extract	Acetone extract
Tap water	9.45 g	9.01 g
Effluent 1	2.81 g	1.72 g
Effluent 2	3.62 g	2.03 g
***Bacillus* treated**	2.92 g	3.44 g
Fungi treated	1.88 g	5.96 g
Both treated	1.14 g	7.04 g

Table 5: Measurement of chlorophyll b

Categories	Methanol extract	Acetone extract
Tap water	4.95 g	29.72 g
Effluent 1	1.67 g	3.40 g
Effluent 2	4.93 g	19.02 g
***Bacillus* treated**	2.69 g	18.95 g
Fungi treated	2.15 g	4.16 g
Both treated	1.26 g	13.18 g

Our work is compared with other researchers as follows; textile dyes are chemicals with complex aromatic structures. A great number of dyes and other chemicals are used in textile wet processes. There are more than 105 commercially available dyes with over 1×10^6 tons of dye stuff produced annually world-wide. Among these available dyes, azo dyes constitute about 70% in the world that represents 70% of total dye produced per year. Thus making the largest and the most important group of synthetic colorant released into an environment.

The industrially manufactured azo dye used for textile finishing process to generate waste water stream. It is difficult to treat by conventional waste water treatment methods. For this reason to use such physicochemical methods, it has some advantages and disadvantages. But the biological methods are preferable because of economical and low possibilities of production of products likes sludge formation. (UsmanAftab*et al,* 2011).

But, it have also some risk such as screening of that specific micro-organism, is a one of the critical step in this remediation system. These kind of work is performed by numerous workers, is discussed as follows,

In 2013, Sri Devi Neelam and J. Chandra SekhraRao isolates bacteria from effluent water contaminated soil sample, it decolourise Remazol Red at 92% within 72 hours. Some other bacterial strains were also isolated namely IBs (92.63% - best), MTCC 2588 strains (69.77%) and more number of Soil *micro flora* isolated but incapable of degradation's.

Kamlesh Shah (2014) use azo dye for degradation process. *Bacillus subtilis* producing enzyme has been used for microbial degradation and gained more attention because of eco-friendly inexpensive in nature. This process gives a general idea about decolourising the dye by aerobic and nonaerobic. Gurulakshmi*et al,* demonstrate Bacillus subtilis will degrade leather Acid dye 113 at 90% level, the medium containing pH 7, starch and peptone. These are monitored by TLC and UV-Visible spectrophotometer at 560nm. She finally confirms the degradation by COD and POD analysis.

In 2011, Tripathi use *Pseudomonas putida, Bacillus cereus, Staphylococcus aureus, Pseudomonas flourescens, Bacillus subtilis* and *Alcaligen species* for degrade Acid orange 10. *Pseudomonas putida* potent decolouriser at pH10 but it has a good activity even in alkaline region at temperature 370C. At lost, he is conclude this organism have a good potential removal of dyes. Sheth and Dave (2009) reported that *Pseudomonas aeroginosa* exhibited 91% decolourization of Reactive Red BS with in 5.5h at a pH range from 5 TO 10.5 and temperature ranging from 30 to 40^0C.This is occurs under static condition in the presence of glucose, peptone or yeast extract.

Kalyani et al, (2008) reported that *Pseudomonas sp. SUK1* decolourized Red BL 1 99% within 1hr under static condition at pH range from 6.5 to 7.0 and 30^0C. Similarly Manikandan et al use mixed culture for bio bleaching of dye, isolated from sludge and dye effluent contaminated soil and also use immobilised packed bed as a bio bleaching agent. Among these respective genera, *Pseudomonas putida, Bacillus subtilis* decolourise 80-90%.

From the above references, several workers degrade dye effluents by using many more microbes like bacteria, fungi and actinomycetes, and get better results. But such peoples don't implement certain things. But we use single *Bacillus* species to effectively degrade dye effluents then treated effluent was used to cultivate plant. This might reduce more water lose and improve treaded water usage.

4.CONCLUSION

Microbial activities are very important for the renewal for our environment and maintenance of global carbon cycle. These activities are included in the term biodegradation. The present study confirms the ability of bacterial culture *Bacillus subtilis* and white rod fungi, its enzyme to degrade the dye effluents with decolourising efficiency of 90%, thus suggesting its application to use in many textile industries. Presence of such co-substrate like glucose/ starch and peptone is essential for attaining maximum decolourisation if dye effluents. Such approaches are ultimately successful in bioremediation of pollutants may make a difference in our ability to reduce wastes, eliminate industrial pollution and enjoy a more sustainable future.

ACKNOWLEDGEMENT

We wish to thank our Institution of Centre for Bioscience and Nano science Research, Coimbatore for providing the necessary facilities for this study.

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