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SCREENING OF DYE DEGRADING BACTERIA FROM TEXTILE EFFLUENTS

T.Vinodhkumar[1], N.Thiripurasundari*[1], G.Ramanathan[2], G. Karthik[3]

[1, 3] Department Of Microbiology, Avscollege Of Arts And Science, Salem, Tamil Nadu, India

[2]department Of Microbiology, V.H.N.S.N. College,

Virudhunagar-626 001, India

ABSTRACT

The textile industry in India is one of the oldest industries. It provides direct employment to nearly thirty million people. Wastewater from textile industries are a complex mixture of many polluting substances such as organochlorine based pesticides, heavy metals, pigments and dyes. This study deals about the biodegradation of Reactive yellow 81, which is a commercially important dye, with a wide range of applications across the textile and leather industries. Decolourisation was assessed as color disappearance during bacterial growth. Textile effluent samples were collected from different dyeing units near Salem. Seventeen isolates were isolated. Using plate assay method the decolourisation activity of bacteria was detected. Among the seventeen isolates seven organisms showed the decolourisation activity and they are Bacillus sp 1, Staphylococcus, Kurthia, Legionella, Pseudomonas, Sulfidobacillus, Bacillus Sp.

Key words Textile Industry, Dye, Degradation, Effluent

INTRODUCTION

Environmental pollution has been defined in various ways. It is considered as the release of unwanted substances to the environment by man in quantities that damage either the health or the resources itself. Water pollution involves the release of small amounts of substances directly through point sources or indirectly through nonpoint sources. Industrial effluents from various industries like textile, dye stuffs, paper and pulp, distillery, Olive oil mill and metal industries are the major contributor to water pollution.

A dye can generally be described as a colored substance that has an affinity to the substrate to which it is being applied. The dye is generally applied in an aqueous solution, and may require a mordant to improve the fastness of the dye on the fiber.

Dyes are widely used in the textile, rubber product, paper, printing, color photography, pharmaceuticals, cosmetics and many other industries. Waste water from textile industries creates a great pollution problem due to the dye content. Industrialization is vital to a nation's economy because it serve as a vehicle for development. However, there are associated problems resulting from the introduction of industrial waste products into the environment. Many of these products are problematic because of persistence (low biodegradability) and / or toxicity.

India's dye industry produces every type of dyes and pigments. Production of dyestuff and pigments in India is close to 80,000 tones. India is the second largest exporter of dye stuffs after china. The textile industry accounts for the largest consumption of dye stuffs (nearly 80%).

The Indian textile industries now predominantly use synthetic organic dyes like direct dyes, processing dyes, reactive dyes etc. The large variety of dyes and chemicals used in an attempt to make more attractive popular shades of fabrics for a competitive market render them very complex. During the last decade, environmental issues associated with dye stuff production and application have grown significantly and are indisputably among the major driving forces affecting the textile dye industry today.

Management of water pollution is at present one of the major challenges for environmentalists. More than 10,000 different textile dyes with an estimated annual production of $7x10^5$ metric tones are commercially available worldwide (Mc Mullan et al., 2001). 2% of these dyes are directly discharged as aqueous effluents and 10% are subsequently lost during textile coloration process (Pearce et al., 2003). Color is one of the most obvious indicators of water pollution, and discharge of highly colored synthetic dye effluents can be damaging to the receiving water bodies (Nigam et al., 1996).

Reactive Dyes

Reactive dye is a class of highly colored organic substances, primarily utilized for tinting textiles that attach themselves to their substrates by a chemical reaction that forms a covalent bond between the molecule of dye and that of the fiber. In this reactive dye cold brand dyes, hot brand, vinylsulphone based, highly exhausted and printing dyes are present. "Cold" reactive dyes such as procion Mx, Cibacron F, and Drimarenek, are very easy to use because they can be applied at room temperature. Reactive dyes are by far the best choice for dyeing cotton and other cellulose fibers at home or in the art studio.

Impact of Textile Effluents in Environment

The textile industries produce effluents that contain several types of chemicals such as dispersants, leveling agents, acids, alkalis, carriers and various dyes (Cooper, 1995). The release of colored compounds into water bodies is undesirable not only because of their impact on photosynthesis of aquatic plants but also due to the carcinogenic nature of many of these dyes and their breakdown products (Weisburger, 2002). This alters the P^H, increases the biological oxygen demand (BOD) and chemical oxygen demand (COD) and gives the rivers intense colorations (Ajayi and osibanjo, 1980). The use of these water resources is limited and the ecosystem is affected.

Treatment of Dye effluent

The textile industry in India is one of the oldest industries. It provides direct employment to nearly thirty million people. Wastewater from textile industries are a complex mixture of many polluting substances such as organochlorine based pesticides, heavy metals, pigments and dyes.

The wastewater characteristics from a dye house are highly variable from day to day, depending on the type of dye, the type of fabric and the concentration of the agents added. Treatment of such wastewaters is therefore, essential but difficult. The removal of dyes from the textile waste effluent has been carried out by physical, chemical and biological methods such as flocculation, membrane filtration, electrochemical techniques, ozonation, coagulation, adsorption and fungal discoloration. (Fu et al., 2001). In recent years a number of studies have focussed on some microorganisms which are able to biodegrade and biosorb dyes in waste waters.

Role of Microbes:

A wide variety of microorganisms, capable of decolorizing a wide range of dyes include some bacteria fungi and algae (Fu et al., 2001). The use of microorganisms for the removal of synthetic dyes from industrial effluents offers considerable advantages. The process is relatively inexpensive, it is simple method and the running costs are low. The end products of complete mineralization are not toxic (Zheng et al, Forgacs et al and Park et al., 2004).

Biodegradation processes may be anaerobic, aerobic or involve a combination of the two (Forgacs et al.,2004). However, it has been observed a number of cases that the efficiency of aerobic treatment was inferior to that of anaerobic decolorization process. Although anaerobic reduction of azo dyes is generally more satisfactory than aerobic degradation, the intermediate products (carcinogenic aromatic amines) have to be degraded by an aerobic process.

Bacteria involve in biodegradation of dyes are Bacillus firmus, Bacillus laterosporus, Legionella, Chryseobacterium, Flavobacterium, Pseudomonas Enterobacter sp, Serratia, sp Yersinia sp, Erwinia sp, and Bacillus subtilis. Some of the fungus such as Bjerkandera sp, Schizophyllum commune, Penicillium oxalicum, Rhizopus arrhizus, Phanerochaete chrysosporium, Fusarium moniliforme. A.terreus, A.niger, Mucor racemosus, Cladosporium cladosporioides, Trichoderma viride, A.ochraceus, Thermotolerant yeast, Kluyveromyces marxianus are involved in dye degradation. Algae such as

Nostoc muscourm, Scenedesmus bijugatus, Cyanobacteria, and diatom Nitzschia perminuta are also take part in dye degradation. Biological degradation of dye effluent is evaluated as a good treatment method.

This study is aimed to asses the potential of bacteria isolated from textile effluent in Salem district to degrade the dye reactive yellow81 (yellow HE4G) is chosen for this degradation study, as it is one of the frequently used dye in dyeing industry in Salem.

MATERIALS AND METHODS

Collection of samples

10 Samples from dye house effluent were collected from a dyeing unit in Salem, Tamil Nadu, India. They were stored in refrigerator at $4^{0}C$ and used without any pretreatment.

Dye

The dye used for the degradation study is Reactive yellow 81 (yellow HE4G). It is a bi functional monochlorotriazine dye of the azo class. The molecular formula of this dye is C_{52} H_{34} Cl_2 N_{180} $20S_6$. 6Na.

Structure of Reactive yellow 81 (HE4G)

Analysis of Physiochemical Parameters:-

Physiochemical parameters of the collected textile dye effluent such as P^H, color and odour were measured using standard methods.

Isolation of bacteria from Textile Effluent

The textile effluent is serially diluted and spread plated in nutrient agar. An each type of isolated colonies were subculture for their enrichment and stored in agar slant.

Identification of the Isolates

A pure colony of the unknown isolates identified presumptively on the basis of the following tests Gram staining Method, Spore staining, Motility Test, Catalase Test, Oxidase Test, Nitrate Test, Gelatin hydrolysis.

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Inoculum Preparation

The 24 hours old culture of the bacteria was used to investigate their ability to decolorize the reactive yellow 81.

Plate Assay Method

Plate assay was performed for the detection of decolorizing activity of bacteria. The nutrient agar and reactive yellow 81 dyes were autoclaved at 121^0C for 15 minutes. The isolated organisms were plated on nutrient agar, plates containing reactive yellow 81 (500mg /l). The plates were wrapped with parafilm and were incubated at 37^0C for 7 days. The plates were observed for clearance of the surrounding the colonies.

Medium used for Decolourization Test

The experiments were carried out in 100 ml flasks containing 50 ml of the tested reactive yellow 81 dye solution in the concentration of 500 mg/L, trace of yeast extract and sucrose. The P^H was adjusted to 7 ± 0.2 using diluted Na_2CO_3 and HCL solutions.

Then two sets of dye solution were prepared for each isolate inoculated with 1ml of inoculum of each bacterial isolate. The flaks were incubated at 37^0C for 12 days one set of the dye solution maintained in stationary condition and another one in shaking incubated to identify the effectiveness of dye degradation.

At 24 hours interval the samples were analyzed for degradation. 5 ml of the test dye solution was centrifuged at 5000 rpm for 15minutes and the optical density of the supernatant was measured by spectrophotometry at λ max (570 nm) of the reactive yellow 81.

Analytical Methods:

Absorbance of the supernatant withdrawn at 24 hrs intervals were measured at the maximum absorption wavelength (λ max - 570 nm) for reactive yellow 81 in the visible region on Spectrophotometer. The percentage of decolourisation was calculated.

RESULTS

Analysis of Physiochemical Parameters

10 different textile dye effluent samples were collected from a dyeing unit in Salem. P^H of the all textile effluent was 7.5. As the dye effluent was collected from different sites, it was having different color (Green, Orange, Pink, Red, Blue) based on the dye which was used for dyeing in the dyeing unit. There was no odor in the effluent samples.

Isolation of Bacteria from Textile Effluent

Well isolated colonies were obtained by spread plate technique. Mixed populations of organisms were separated by this technique and discrete colonies were isolated. A total of 7 different isolates were obtained and these isolates were used for decolorization studies.

Plate assay method screening of Dye Decolorizing Microorganism

Decolorizing ability of the 7 isolates were identified by using the Reactive Yellow 81 dye which is a commercially important dye, with a wide range of applications across the textile and leather industries.

Dye solution inoculated with the isolates was maintained in both stationary and agitated conditions separately.

Optical density of the dye solution inoculated with the isolates for both stationary and agitated conditions was taken separately and are given in table 4 and 5 respectively.

The percentage of dye decolourization of the isolates in both stationary and agitated condition was calculated with a standard formula and are given in the table 6.

S.No	Isolates	% of Decolourization	
		Under Stationary Condition	Under Agitated Condition
1	Pseudomonas sp	37.5 %	25 %
2	Staphylococcus sp	25 %	12.5 %
3	Kurthia sp	12.5 %	12.5 %
4	Legionella sp	37.5 %	37.5 %
5	Sulfidobacillus sp	37.5 %	25 %
6	Bacillus sp1	50 %	37.5 %
7	Bacillus sp2	12.5 %	25 %

Table 1 :Decolourization of Reactive Yellow 81 by the Isolates

DISCUSSION

A total of 17 isolates were obtained from textile effluent samples. Among which five isolates belonging to the genera Bacillus (from sample 1,2,5,6,3) and four isolates are Staphylococcus (from sample 1,3,5,6). Three isolates belonging to the genus Sulfidobacillus (from sample 7,8) and one genera of Kurthia (from sample 5) and Legionella (from sample 7,8) three genera of Pseudomonas(from 7.8.5) were isolated from the textile effluents.

From the dye contaminated soil, Bacillus was isolated dominantly by Leena et al., (2008). The other bacteria such as Pseudomonas, Arthrobacter, Alcaligenes were also isolated.

Thus among the all isolates, Bacillus is the Predominant one, which is largely isolated from the textile effluent. Other than this Bacillus is also an effective biodegrader of various dyes such as pigmented red 208 Saraswathi et al., (2009). Reactive Black-B Leena et al., (2008), Acidblue 113 Gurulakshmi et al., (2008), Reactive red (RR) Barakat et al., (2009), Cibacronblack PSG, cibacron red P4B Ola et al., (2010).

In this study, the decolourisation ability of the isolates against reactive yellow 81 was demonstrated. The optical density was measured at 570nm with two days interval for 12 days. On the Second day Pseudomonas showed 25% decolourisation, Bacillus 1 showed 12.5%. Whereas other isolates did not show any decolourisation. Legionella showed 25% decolourisation during the fourth day and the other isolates such as Pseudomonas (25%) Bacillus 2, Sulfidobacillus, Bacillus 1 showed 12.5% decolourisation. Staphylococcus showed 12.5% and Bacillus 1 has 37.5% on sixth day. Followed by further incubation on eight day except pseudomonas (37.5 %) sulfidobacillus (25%) other isolates does not show any decolourisation on Ten day 50 % decolourisation was showed by Bacillus 1, Kurthia shows 12.5 % only on this day. Finally 25 % decolourisation was observed in Staphylococcus, Legionella and sulfidobacillus has 37.5 %, Bacillus 2 remains the same as it was in the sixth day. These conditions were observed under stationary condition.

Bacillus 2 has 12.5% decolourisation from second, fourth, sixth, eighth, day and it has 25% decolourisation on tenth and 12th day.

Compare to stationary and agitated condition the isolates exhibited effective dye decolorizing activity only when incubated under the stationary conditions. Whereas negligible decolourisation was noticed under the agitating conditions. the further incubation of these isolates on reactive yellow 81 did not improve decolorization. Anaerobic or stationary conditions were necessary for bacterial decolourisation though the cell growth was poorer than that under aerobic conditions. (Gurulakshmi et al.,2008). Among all the isolates Bacillus 1 is the first effective degrader of reactive yellow 81, secondly by Legionella next to them are Pseudomonas, Sulfidobacillus, Bacillus 2 and Staphylococcus, Kurthia sp. has the lowest decolourisation ability(Gurulakshmi, et al.,2008) stated that there was no increase in decolourisation of Kurthia whenever the Inoculum size is increased.

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