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Original Research Article

Scenario of Native Microorganisms Used to Cure Textile Dyes in a Microbiological Manner

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Abstract:

Background: Contamination to this aquatic system brings serious threat overall socio-economic pattern of the study. So, proper analysis is needed to assess the pollution level also for the protection of environment and natural resources.

Aim: o assess azo dye decolourising ability of the wild strains/native flora (Kovilpatti) of bacteria isolated from effluent of textile industry.

Materials and Methods: Effluent Sample from textile industry effluent discharge sites, Remazol yellow, Bushnell Hass medium were used to conduct our research.

Results: Textile mills uses acid based dyes which are able to damage ecosystem. Peoples are using various methods to remove that dyes but they are not efficient. To understand the efficiency of microbes on dye degradation this work was undertaken using wild strains isolated from dye contaminated effluent.

Conclusion: These created pollution to water bodies and its surrounding ecosystem. Various physical, chemical and biological methods are available to curtail the problem but still there is no opt method to overcome this problem. In this study trial was done using wild / native dye degradative microorganisms for the decolourization of pure azo dye. If these microorganisms decolourize this synthetic azo dyes effectively then it will be taken to field for the bioremediation process. By considering ecological impact of azo dye the present study was undertaken with specified aim and objectives.

Keywords: Dye, Decolurising, Pollution, Ramazole, Effluent, Ecosystem, Microorgasim.

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Introduction

A textile industry is classified into three types based on the fibers used. They are cellulose fibers (cotton, rayon, linen, ramie, hemp and lyocell), protein fiber (wool, angora, mohair, cashmere and silk) and synthetic fiber (polyester, nylon, spandex, acetate, acrylic, ingeo and polypropylene). Now a day's more than 80% of textile industry uses synthetic fibers. These synthetic fibers are coloured by making use of acid based synthetic dyes. These acid dyes are forming an insoluble complex on fiber. Most common synthetic fiber dyes are azo dyes, xanthenes dyes and anthraquinone dyes. Acid dyes are found to contain diazo groups [-N-N-] in the centre and are called azo dyes.

Synthetic dyes such as azo dyes, xanthenes dyes and anthraquinone dyes are very toxic to living organisms. Azo dyes constitute a major class of environmental pollutants. Breakdown products of the azo dyes are known to be highly toxic and mutagenic on living organisms (Kumar Pravin et al., 2012). Textile and dyeing industrial effluents may cause alteration of the physical, chemical and biological properties of aquatic environment by continuous change in temperature, odour, noise, turbidity etc. that is harmful to public health, livestock, wildlife, fish and other biodiversity. Also it causes many waterborne diseases and severe irritation of respiratory tract. Contamination to this aquatic system brings serious threat overall socio-economic pattern of the study. So, proper analysis is needed to assess the pollution level also for the protection of environment and natural resources.

The inhibition of seed germination may be due to the high dissolved solids in the effluent that disturb the osmotic relation of the seed. Increase in the effluent concentrations affected the germination of paddy and the dry matter production. The dry matter accumulation reflects the actual physiological status of the plant and therefore an indicator parameter of plant growth. Having known the importance of azo dyes, its ecological impact and degradative ability of the

microorganisms, this research work was undertaken with the following aim and objectives.

Materials and Methods

Kovilpatti is one of the industrial city of Thoothukudi, Tamilnadu, India. It is a second largest city in this district, which connect Chennai and Kanyakumari through NH44. It is one of the industrial city having loyal textiles and Lakshmi mills. This city is famous for match factories and fireworks industry. Kovilpatti is famous for its unique candy kadalai mittai (Ground-nut candy) and is referred as land of homemade sweets and savouries. Textile mills uses acid based dyes which are able to damage ecosystem. Peoples are using various methods to remove that dyes but they are not efficient. To understand the efficiency of microbes on dye degradation this work was undertaken using wild strains isolated from dye contaminated effluent.

Effluent Sample from textile industry effluent discharge sites

- Remazol yellow
- Bushnell Hass medium
- Nutrient agar medium
- Effluent basal medium
- Biochemical media
- Biochemical Reagents
- UV- VIS spectrophotometer
- Carbon Sources: Glucose, sucrose, lactose and mannitol
- Nitrogen sources: Yeast extract, peptone, ammonium sulphate and ammonium chloride
- Plant seeds: Green gram (Vigna radiate), Kidney beans (Phaseolus vulgaris), Fenugreek (Trigonellafoenum)

Reactive Dye Preparation

The textile dye (Remazol Yellow) was collected from a dyeing industry in sankarankovil. A stock solution of the dye (1000mg L') was prepared in distilled water and used for all studies.

Effluent sample was collected from the disposal sites and transported to laboratory for the isolation of dye decolourizing bacterial species.

Sample Collection

The Effluent samples were collected from textile dye effluent run off site of textile unit located in Kovilpatti, Thoothukudi district, Tamil Nadu, India using sterile containers. All the samples were transported to the laboratory for the isolation of efficient azo dye degraders.

Isolation of dye degrading bacteria (Ponraj et al., 2011)

The dye decolourizing bacteria was isolated from the effluent sample of textile dye effluent run-off site by serial dilution and plating appropriate dilutions on modified Bushnell Hass agar medium.

Serial dilution technique

About l0ml Effluent sample were taken and mixed in 90 mL of sterile distilled water. Samples were serially diluted upto lO7and plated l0 onto sterile medium to screen the specific dye degrading organisms. The media was supplemented with Remazol Yellow dye separately.

Enumeration of Bacteria (Primary Screening)

All the inoculated plates were incubated at 37°C for 24 h. Following incubation, clearance of dye colour around the colonies were selected and enumerated. These selected colonies were considered as predominant dye degraders. Non-dye degrading bacteria also counted to obtain Total Viable Count (TVC).

Storage of organisms

The selected predominant dye degraders were subcultured onto nutrient agar slants and subcultured for every 15 days. The cultures were stored at 4°C for further degradation studies.

Enrichment technique

All the isolated cultures were studied by inoculating them in an enrichment medium (effluent basal medium). The inoculated medium was incubated at 30°C for 3 to 6 days under shaking in an orbital shaker at 120 rpm.

Secondary Screening of dye decolourizers (Spot inoculation)

A total of 95 strains were selected in the primary screening and enumeration method. Spot inoculation method was performed to isolate effective dye degraders. Dye added Bushnell hass medium was inoculated with test organisms as spot were incubated at 37°C for 72 hrs. Following incubation, clearance of dye colour around the colonies was selected. One the basis of higher zone of clearance a total of five test organisms were selected and named as KTE7, KTE28, KTE48, KTE64 and KTE83.

Dye decolourization evaluation (Ponraj et al., 2011)

The dye decolourizing evaluation of the isolated bacteria were done using the method proposed by Ponraj et at., (2011). Decolourization activity was performed in 90 mL of Bushnell Hass medium containing 0.02g of Remazol Yellow dyes inoculated with 10% (v/v) inoculum of each isolate.

Uninoculated medium with dyes at similar concentration (0.O2g) served as separate controls. Inoculated medium and control was incubated at 37°C for 3 to 12 days under shake culture

condition. About 10 mL samples were withdrawn aseptically and centrifuged at 8,000 rpm for 15 mm. The clear supernatant was used for measuring absorption at 590 nm for Remazol Yellow dye using UV-VIS spectrophotometer. The percentage decolourization of dyes was determined by using the formula

$D = [(A_0-A_1)/A_0] \times 100$

Where,

D, decolourization in %; A0, initial absorbance; A1, final absorbance

Identification of Dye Degrading Bacteria (Koneman et al., 1994)

KTE7, KTE28, KTE48, KTE64 and KTE83 strains were individually stored as pure culture. Subculture was done periodically. Pure culture of the test organisms were subjected for the following tests to identify the nature of organisms as per bergeys manual of Determinative Bacteriology.

Colony Morphology

The colony morphology of dye degrading isolates was observed for their size, pigmentation, form, margin and elevation on the Nutrient agar plates. All the isolates were inoculated on selective cum differential media like MacConkey agar, Blood agar, EMB Agar, MRS medium and Cetrimide agar for primary differentiation.

Microscopic observation

Gram Staining

The bacterial smear was prepared, air dried and heat fixed. The slides were flooded with crystal violet for 1 minute and the smear was washed with tap water for and then the smear was flooded with grams iodine for 30 seconds and the slide was washed with tap water.

Next the smear was decolourized using decolourized and the smear was washed with tap water. Finally the slide was flooded with counted stain safranin for 30 seconds and washed with tap water. The slide was air dried and examined under microscope.

Spore Staining

The bacterial smear was prepared, air dried and heat fixed. The smear was flooded with malachite green. Then the slide was exposed to steam of boiling water for about 5 minutes, followed by washing and counter stained by safranin. Then it was observed under the microscope.

Motility Test

Hanging drop method was used for observing motility of organisms.

Biochemical characterization of indigenous bacterial isolates

All the screened dye degrading isolates were subjected for biochemical identification to confirm the organisms till genus level. Significant biochemical tests listed below were performed to identify the dye degraders.

Indole production test

The indole production test was performed to determine the production of indole as the end product of tryptophan catabolism. Tryptone broth was prepared separately for each screened dye degraders. All the isolates were inoculated and incubated for 24 h at 37°C. Following incubation, 0.2 mL of the Kovac's reagent was added to observe the formation of cherry red coloured ring for positive reaction and yellow colour for negative reaction.

Methyl Red test

The test was done to determine the ability of microorganisms to produce acidic end products. MR — VP broth was prepared, sterilized, inoculated separately with each of the dye degraders. All the tubes were incubated at 37 °C for 18 to 24 h. After incubation, few drops of methyl red indicator was added and observed for the presence or the absence of red colour.

Voges — Proskauer test

The test was to determine the ability of microorganism to produce neutral end products of carbohydrate fermentation. MR — VP broth was prepared, sterilized, inoculated separately with each of the dye degraders. All the tubes were incubated at 37°C for 18 to 24 h. Solution-A and Solution-B of Barritt's reagents were added after incubation and then observed for the presence or the absence of red colour.

Citrate Utilization test

Test was done to detect the ability of the dye degraders to utilize citrate as the sole carbon source. Simmon's citrate agar slant was prepared, sterilized, inoculated and incubated at 37 °C for 24 h. In each tube dye degrading isolates were stabbed and streaked onto butt and slant. All the inoculated tubes were incubated at 37°C for 18 to 24 h. After incubation, the presence or the absences of colour change from green to Prussian blue colour was observed.

Oxidase test

The oxidase test is a test used to determine the ability of the isolate to produce certain cytochrome c oxidases. The oxidase disc was placed into the cultures. The presence or absence of blue colour was observed after few seconds.

Catalase test

Catalase test was performed to determine the ability of dye degraders to release free oxygen after breaking down hydrogen peroxide. All the dye degrading isolates were tested individually in a sterile glass slides. About lmL of each of the isolates was exposed to hydrogen peroxide solution to observe the gas bubbles. The formation of gas bubbles indicates the release of free oxygen by the production of enzyme catalase.

Urease Production test

The urease test was used to determine the ability of an organism to break urea by production of the enzyme urease. Christensen's Urea agar slant was prepared, sterilized and inoculated with dye degrading isolates. All the inoculated tubes were incubated at 37 °C for 18- 24 h. The colour change from yellow to pink indicated the production of urease and breakdown of urea in media.

Nitrate reduction test

The ability of dye degraders to reduce nitrate in the media which was considered to be a regular metabolic function of soil isolates was tested. Nitrate broth was prepared, sterilized and inoculated with each of the dye degrading isolates aseptically. All the inoculated tubes were incubated at 37 °C for 18-24 h. After incubation, few drops of solution A and solution B was added and observed for the presence or the absence of red colour.

Starch hydrolysis test

Starch hydrolysis test was performed to determine the ability of the dye degrading isolates to produce polysaccharide hydrolyzing enzyme, amylase. Starch agar media was prepared and poured onto sterile petriplates. Each of the dye degrading isolates was streaked onto the centre of the media as single line. All the inoculated plates were incubated at 37 °C for 24 to 48 h. After incubation the plates were flooded with lugol's iodide solution to observe the zone of hydrolysis around the line of streak on the starch agar media. The zone around the bacterial growth indicates the hydrolysis of starch by the dye degraders.

Gelatin hydrolysis test

The gelatin hydrolysis test is used to detect the ability of dye degraders to produce the enzyme gelatinase. Gelatin tubes were prepared, sterilized, inoculated and incubated at 37 °C for 24 h. After incubation, all the gelatin agar tubes were placed in refrigerator at 4 °C for 30 mm. After incubation, inoculated tubes were observed with reference to its state (whether it is liquid or solid).

Casein hydrolysis test

Casein hydrolysis test was performed to determine the ability of the dye degrading isolates to produce protein hydrolyzing enzyme. Casein agar media was prepared and poured onto sterile petriplates. Each of the dye degrading isolates was streaked onto the centre of the media as single line. All the inoculated plates were incubated at 37°C for 24 to 48 h. After incubation the plates were flooded with mercuric iodide solutions to observe the zone of hydrolysis around the line of streak on the casein agar media. The zone around the bacterial growth indicates the hydrolysis of protein/casein by the dye degraders.

Carbohydrate Fermentation Test

This test determines the ability of organisms to ferment different sugars. The results were confirmed by acid and gas production along with the release of co2 and colour change. Sugar broth was prepared using glucose, sucrose, lactose. Durham's tube was put into the broth and ioop full of test culture were inoculated and incubated at 370 C for 24- 48 hrs. Changes in colour and production of gas were observed after incubation period.

Optimization of dye decolourization using different parameters (Shah et al, 2013)

Effect of various carbon sources on dye decolourization

Bushnell Hass media was prepared with 1% of different carbon sources such as glucose, sucrose, lactose and mannitol separately with pH 7. All the carbon sources were sterilized separately and added to pre-sterilized and cooled Bushnell Hass broth. To this, filter sterilized Remazol Yellow dye at the concentration of 50 mgIL was added separately. The Bushnell Hass broth supplemented with Remazol Yellow dye was inoculated with KTE7, KTE28, KTE48, KTE64 and KTE83 separately. All the flasks were incubated at 37°C for 72 to 96 hrs. The medium without inoculum was maintained as control. After the incubation period, 10 mL of the culture filtrate was centrifuged at 8000 rpm for 15 minutes. The OD value for the obtained supematant was recorded at 590 nm for Remazol Yellow dye using UV-VIS spectrophotometer. The percentage decolourization was determined by using the formula as mentioned earlier.

Effect of various nitrogen sources/substrates on growth and decolourization

Bushnell Hass media was prepared with 1% of different nitrogen sources such as yeast extract, peptone, ammonium sulphate and ammonium chloride separately with pH 7. All the nitrogen sources were sterilized separately and added to pre-sterilized and cooled bushnell Hass broth.

To this, filter sterilized Remazol Yellow dye at the concentration of 50 mg/L was added separately.

The Bushnell hass broth supplemented with Remazol Yellow dye and Indigo Blue dye was inoculated with KTE7, KTE28, KTE48, KTE64 and KTE83 separately. All the flasks were incubated at 37°C for 72 to 96 hrs. The medium without inoculum was maintained as control. After the incubation period, l0mL of the culture filtrate was centrifuged at 8000 rpm for 15 minutes. The OD value for the obtained supematant was recorded at 590 nm for Remazol Yellow dye using UV-VIS spectrophotometer. The percentage decolourization was determined by using the formula as mentioned earlier.

Effect of various temperatures on growth and decolourization

Bushnell Hass media was prepared separately supplemented with Remazol Yellow dye with pH 7.0. Filter sterilized Remazol Yellow dye at the concentration of 50 mg/L were added. The Remazol Yellow dye supplemented medium was inoculated with KTE7, KTE28, KTE48, KTE64 and KTE83 separately and incubated at different temperature separately (25°C, 37°C, 40°C and 45°C) for 72 to 96 hrs. The medium without inoculum was maintained as control. After the incubation period, 10 mL of the culture filtrate was centrifuged at 8000rpm for 15 minutes from each culture flasks. The OD value for the obtained supernatant was recorded at 590 nm for Remazol Yellow dye using UV-VIS spectrophotometer. The percentage decolourization was determined by using the formula as mentioned earlier.

Effect of various pH on growth and decolourization

Bushnell Hass media was prepared at different pH (5, 6, 7, 8, 9) and sterilized at 1 atmospheric pressure. To this, filter sterilized dyes Remazol Yellow dye at the concentration of 50 mg/L was added respectively. The Remazol Yellow dye supplemented medium was inoculated with KTE7, KTE28, KTE48, KTE64 and KTE83 separately and incubated at different temperature separately (25°C, 37°C, 40°C and 45°C) for 72 to 96 hrs. The medium without inoculum was maintained as control. After the incubation period, 19 mL of the culture filtrate was centrifuged at 8000 rpm for 15 mm from each culture flasks. The OD value for the obtained supernatant was recorded at 590 nm for Remazol Yellow dye using UV-VIS spectrophotometer. The percentage decolourization was determined by using the formula as mentioned earlier.

Effect of various inoculum sizes on growth and decolourization

Bushnell Hass media was prepared at pH 7 and sterilized at 1 atmospheric pressure. To this, filter sterilized dyes Remazol Yellow dye at the concentration of 50 mg/L was added respectively.

The Remazol Yellow dye added medium was inoculated with KTE7, KTE28, KTE48, KTE64 and KTE83 separately. [noculum size with varying concentrations of 2%, 4%, 6%, 8% and 10% was added in each flask. All the inoculated flasks were incubated for 72 to 96 hrs at room temperature. The medium without inoculum was maintained as control. After the incubation period, 10 mL of the culture filtrate was centrifuged at 8000 rpm for 15 mm. The OD value for the obtained supernatant was recorded at 590 nm for Remazol Yellow dye using UV-VIS spectrophotometer. The percentage decolourization was determined by using the formula as mentioned earlier.

Time course of decolourization (Ponraj et al, 2011)

The time course of decolourization was carried out under optimum conditions obtained from the above studies. The Bushnell Hass broth supplemented with Remazol Yellow dye and Indigo Blue dye was inoculated with KTE7, KTE28, KTE48, KTE64 and KTE83 separately with the optimized factors. To study the time course of decolourization, inoculated flasks were incubated up to 12 days. For every 24 h the samples were removed and analyzed for decolourization activity. The medium without inculum was maintained as control. After the incubation period, 10mL of the culture filtrate was centrifuged at 8000 rpm for 15 mm. The OD value for the obtained supernatant was recorded at 590 nm for Remazol Yellow dye using UV-VIS spectrophotometer. The percentage decolourization was determined by using the formula as mentioned earlier. All analysis was conducted in triplicate and the results were presented as the mean of triplicate ± Standard Deviations (SD).

Dye decolouration using mixed culture and Microbial consortium (Palanivelan et al., 2012).

Mixed culture was prepared in ten combinations as following

- Lacto and Escherichia coli
- Staphylococcus aureue and E. coli
- Staphylococcus aureus and Lacto and E. coil and Lacto and Staphylococcus aureus
- Pseudomonas and E. coil
- Pseudomonas and Lacto
- Pseudomonas and Staphylococcus
- Pseudomonas

Consortium is prepared with the mixture of all the five isolates (KTE7, KTE28, KTE48, KTE64 and KTE83).

Bushnell Hass media was prepared with pH 7 with all the optimized conditions and sterilized at 121°C for 15 minutes. To this, filter sterilized dyes

Remazol Yellow dye at the concentration of 50 mg/L was added. The Remazol Yellow dye supplemented medium was inoculated with mixed cultures and consortium separately and incubated at 37^0C for 12 days. The medium without inoculums was maintained as control. After the incub f the culture filtrate was centrifuged at 8000 rpm for 15 mm from each culture flasks. The OD value for the obtained supernatant was recorded at 590 nm for Rernazol Yellow dye using UV-VIS spectrophotometer. The percentage decolourization was determined by using the formula as mentioned earlier.

FTIR analysis

FTIR analysis of Pseudomonas inoculated filtrate and Bacillus inoculated filtrate were done at National College, Tiruchirappalli. Samples were mixed with 200mg of spectroscopic grade KBr. FTIR Spectra were recorded using a Nicolet 520P Spectrometer with detector at 4cm-I resolution and 20 scans per sample.

Bioassay for dye toxicity/phytotoxicity: Seed germination test (Durve et al., 2012)

In this experiment, the effect of Remazol Yellow dye at the concentration of 20 mgIL was evaluated on germination of seeds of 3 different plants, Green gram (Vigna radiate), Kidney beans (Phaseolus vulgaris), Fenugreek (Trigonella foenum). The seeds were germinated in pots (Pot Assay) using paddy field soil. For pot assay 10 kg of paddy soil was used in each pot.

Three sets of 20 seed each of Green gram (Vigna radiate), Kidney beans (Phaseolus vulgaris) and Fenugreek (Trigonellafoenum) were treated every 24 h with 10 mL of dye solutions, degraded Remazol Yellow dye solutions separately. Seeds germinated in pots treated with distilled water were used as a control. All pots were kept under shade near sunlight for the period of 6 days.

Germination of seeds treated with dye and degraded dye solutions was calculated after comparing with control. The seed germination percentage was calculated using the formula given below;

No. of seeds used — No. of ungerminated seeds

Percentage of = ----------X 100

Seed germination No. of seeds used

At the end of the germination experiment, the shoot length and root length of seedlings was measured separately for dye, degraded dye and control samples. All analysis was conducted in triplicate and the results were presented as the mean of triplicate ± Standard Deviations (SD).

Results

Kovilpatti is one of the industrial city of Thoothukudi, Tamilnadu, India. It is a second largest city in this district, which connect Chennai and Kanyakumari through NH44. It is one of the industrial city having loyal textiles and Lakshmi mills. This city is famous for match factories and fireworks industry. Kovilpatti is famous for its unique candy kadalai mittai (Ground-nut candy) and is referred as land of homemade sweets and savouries. Textile mills uses acid based dyes which are able to damage ecosystem. Peoples are using various methods to remove that dyes but they are not efficient. To understand the efficiency of microbes on dye degradation this work was undertaken using wild strains isolated from dye contaminated effluent.

Isolation and Identification of Microorganisms

Totally 36 textile mill effluent samples were collected to isolate dye degrading bacteria and the samples are named as KTE1a, b, c KTEI2 a, b, c (Monthly one sample in triplicate). Dye degrading bacteria were selected based on zone of clearance around the colonies. Yellow colour discolouration indicated discolouration ability of the isolate. Bacteria degrade azo dye by enzymatic cleaving of central segment (Diazo group) of the dye.

Below chart showed total bacteria isolated vs dye degrading bacteria from the textile effluent. Results revealed that effluent is one of the rich sources of bacterial biodiversity. Bacterial flora present in the textile effluent are varied depends on the month by which it was isolated. Totally twelve sample in triplicates were collected throughout the year (One month one sample in triplicate). Collection of sample started in April 2016 and ends in March 2017. Higher bacterial load was expressed in April 2016 month followed by June 2017. Least bacterial load was noted in the month of August 2017. During summer months higher total viable count was noted. Similarly isolation rate of dye degrader's incidence was highly noted in April and May months. Figure 1 also clearly illustrated the recovery rate of dye degrader's vs total viable count with reference to month of its isolation from textile mill effluents. Higher number of Azo dye degraders was noted in February 2017 followed by 11 each in April 2016 and January 2017.

Table 1: Incidence rate of dye degrading bacteria Vs Total Viable Count

S.NO.	Month and Year	Total Viable Count	Number of Dye degrading isolates
1	April 2016	$144x10^6$±8.02	$11x10^6$±0.09
2	May2016	$128x10^6$±4.76	$09x10^6$±0.06
3	June2016	$132x10^6$±2.32	$07x10^6$±1.07

4	July2016	$030x10^6±3.03$	$04x10^6±1.16$
5	August2016	$027x10^6±5.68$	$06x10^6±I.05$
6	September 2016	$067x10^6+7.84$	$04x10^6±0.18$
7	October 2016	$05\ 1x10^6±4.02$	$07x10^6±0.68$
8	November 2016	$036x10^6±3.40$	$08x10^6±1.09$
9	December2016	$028x10^6±6.65$	$05x10^6±1.78$
10	January 2017	$062x10^6±5.21$	$11x10^6±2.37$
11	February20l7	$085x10^6+2.68$	$14x10^6±0.18$
12	March2017	$094x10^6±9.87$	$09x10^6+3.65$

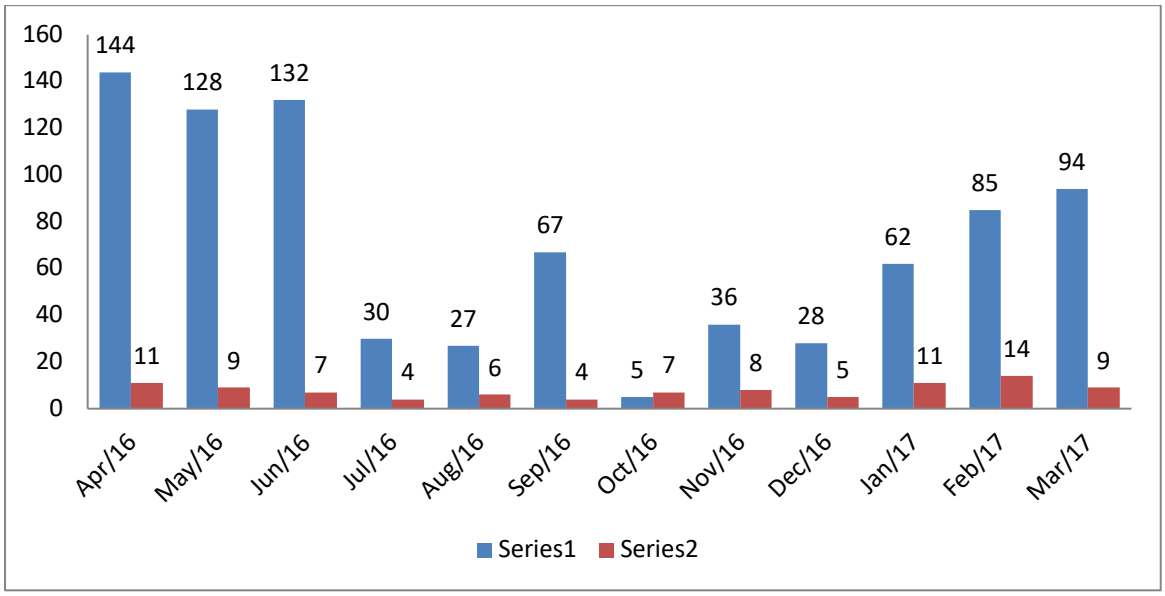


Figure1: Isolation of dye degrading Bacteria from Effluents

Month wise isolation incidence of dye degrading bacteria was presented in Figure 2. Higher percentage of dye degraders were isolated in August 2016 and November 2016 (22.2% each) followed by 17.8% incidence in December 2016. Lowest incidence of dye degraders were noted in June 2016 (5.3%).

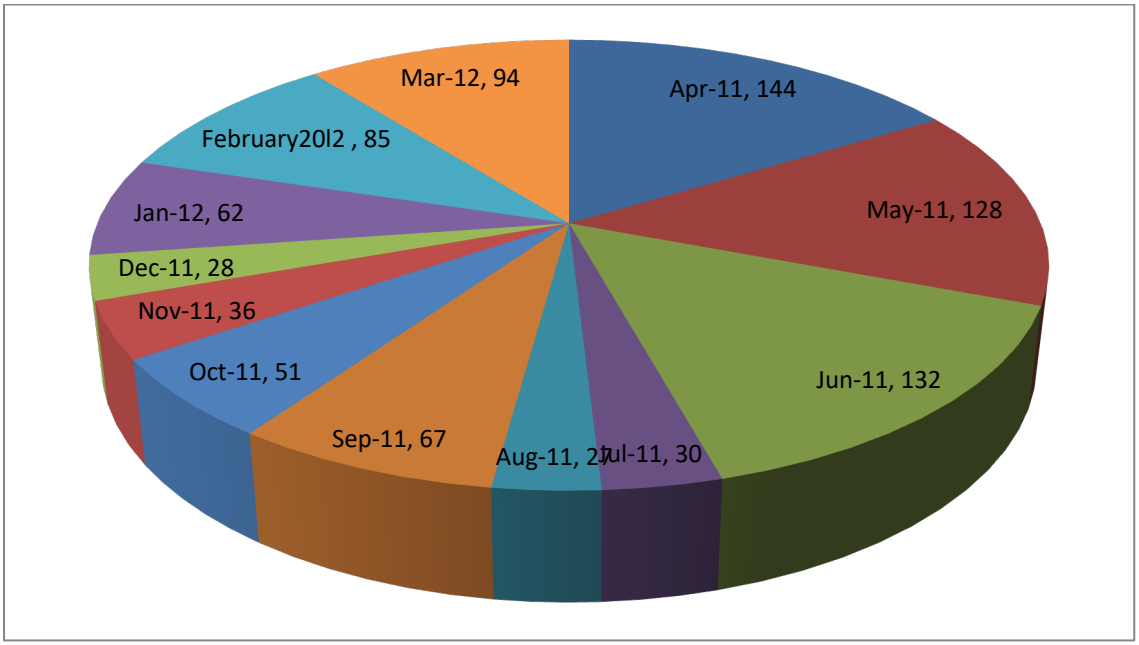


Figure 2: Month wise percentage of Dye degrading Isolation

Totally 95 azo dye degrading strains were isolated from the textile effluents. These isolates were numbered as KTE1…..KTE95. Variable numbers of gram positive as well as gram negative dye

degraders were isolated in different months respectively (Figure 3).Overall when we analysed all the dye degrading bacteria isolated in primary screening is subjected for the assessment of its grams nature. Results revealed that about 52% of bacteria were belongs to gram positive groups (Figure 4). Grams nature based assessment did not show any significant results. Previous reports stated that both gram negative as well as gram positive organisms are efficient in dye degradation.

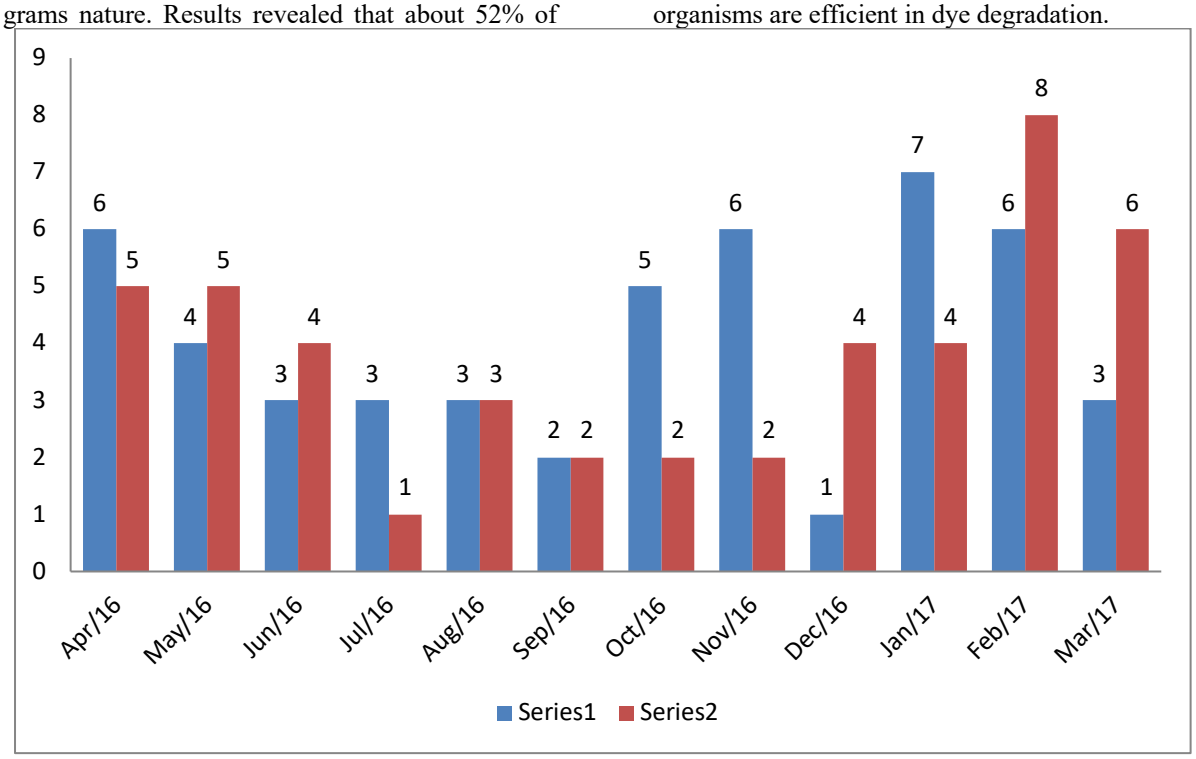


Figure 3: Categorization of Dye degraders based on Grams Nature

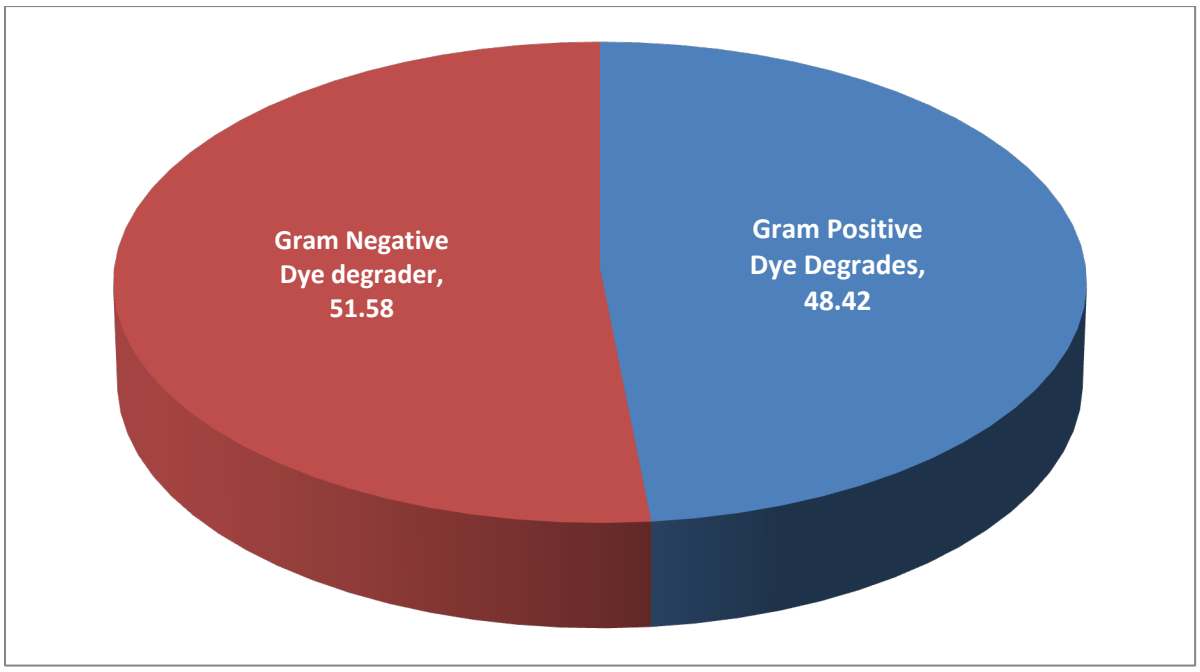


Figure 4: Incidence of Dye Degrading bacteria with reference to Grams nature

Spot inoculation test was performed to select best dye degrading bacteria. Among the isolates five isolates were selected for further studies.

On Bushnell Hass medium 20Otg/ ml Azodye was added and prepared plate. Test organism was inoculated as spot on the medium and incubated at 37°C for 72 hours. On the base of zone of discolouration five test organisms were selected. They arc KTE7, KTE28, KTE48, KTE64 and KTE83. When analyzing zone of clearance it was

indicated that KTE7 and KTE28 produced 36mm zone of clearance after 72 hours.

This was followed by 22mm by KTE64 strain given below. This indicated that both gram positive Bacillus and Gram Negative Pseudomonas are efficient in dye degradation.

Table 2: Selection of effective strain with reference to Spot test

S. No.	Strain No.	Zone of Clearance in mm
1	KTE7	36
2	KTE28	36
3	KTE48	18
4	KTE64	22
5	KTE63	20

Selected strains like KTE7, KTE 28, KTE48, KTE64 and KTE83 were subjected to characterization as per Bergeys manual systematic Bacteriology. Selected strains are subjected to microscopy, culturing on selective cum differential media and biochemical tests. Result indicated that KTE7 strain belongs to Bacillus cereus (Plate I — P.- Fig.1, 2), KTE 28 was identified as Pseudomonas fluorescence (Plate II — P. Fig. 3, 4 & 5), KTE 48 as Staphylococcus aureus (Plate III — P. Fig. 6, 7 & 8), KTE64 as Escherichia coli (Plate IV — P. Fig. 9, 10, 11, 12 & 13) and KTE83 as Lacto (Plate V-P. Fig. 14 & 5). On blood agar medium Staphylococcus aureus produced beta haemolytic colonies, similarly E. coli produced metallic sheen colonies on EMB agar, LactoBacillus produced colourles colonies on MRS medium, Pseudomonas fluorescence produced greenish colour colonies on cetrimide agar, Bacillus produced rough colourless colones on nutrient agar and produced endospore. On the basis of growth pattern and biochemical features isolates were identified.

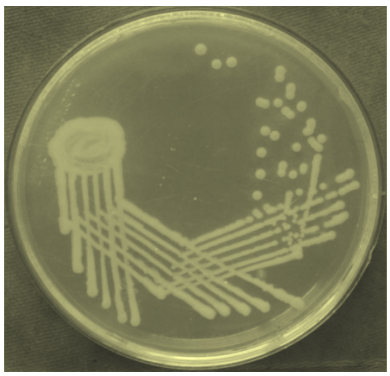
Table 3: Characterization of azo dye degrading bacteria isolated from textile mill effluent

S No	Tests	KTE7	KTE2	KTE4	KTE6	KTE8
			8	8	4	3
1	Simple staining	Rod	Rod	Cocci	Rod	Rod
2	Grtam staining	+	-	+	-	+
3	Spore staining	+	-	-	-	-
4	Motility Testing	+	+	-	+	+
5	Catalase test	+	+	+	+	-
6	Oxidase test	-	+	-	-	-
7	Nitrate reduction test	+	-	+	+	+
8	H2S production test	-	-	-	-	-
9	Indole Producation test	+	-	-	+	-
10	Methyl Red test	-	-	+	+	+
11	Voges proskauer test	-	-	+	-	-
12	Citrate utilization test	+	+	-	-	+
13	Urease Test	-	-	-	+	
14	Catbohydrate fermentation test Glucose	AIG	AIG	-	AJG	A
	Sucroe	A/G	-	-	A	A
	Xylose	A/G	-	A/G	A	-
15	Triple sugar iron agar test	-	-	-	A/A	-
16	Gelatin hydrolysis	-	-	-	-	-
17	Lipid hydrolysis	-	-	-	-	-
18	Starch hydrolysis	+	+	-	-	+
19	Casein Hydrolysis	+	-	+	-	-

- KTE7 — cereus
- KTE28 — Pseudomonas aeruginosa
- KTE48 — Staphylococcus aureus
- KTE64 — Escherichia coli
- KTE83 — Lacto sp.,

Plate I

KTE7 – Cereus



P.Fig.1: Colony on Nutrition Agar P

Fig.2: Biochemical Features

Plate III: Identification features of Staphylococcus aureus

Baired Parker Agar Blood Agar

P.Fig-6

P.Fig.7

Beta Haemolytic Colonies

P.Fig. 8: Biochemical Features

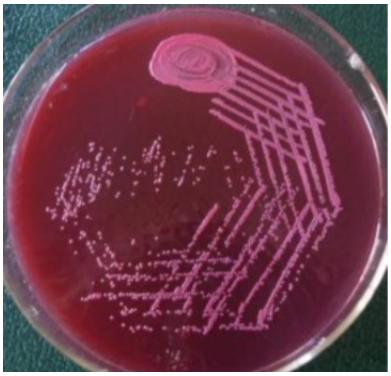
Plate IV: Identification features of KTE64

Escherichia Coil

XLD Agar MacConkey Agar

P.Fig-9: Yellow colour colony

(Pink) Colony

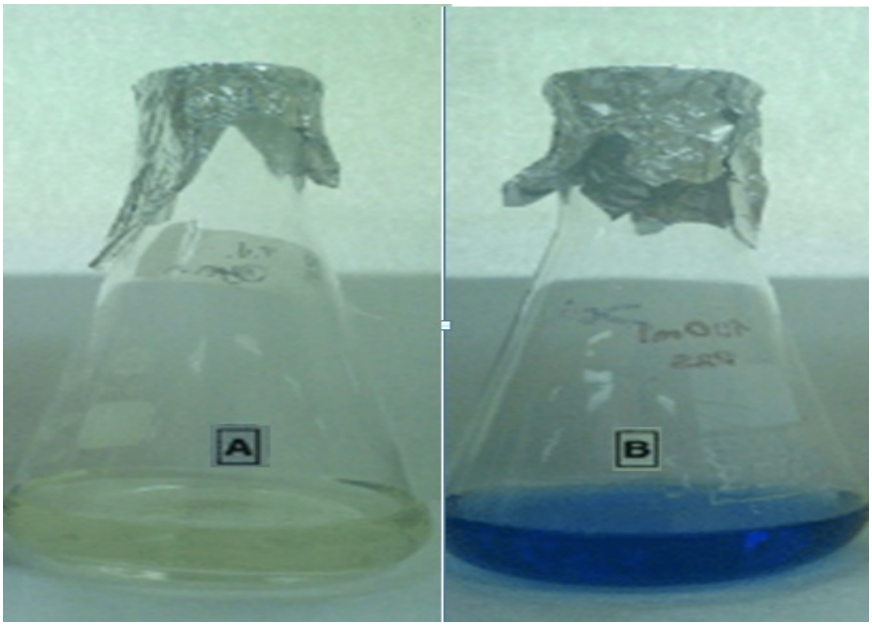


P.Fig.10: Lactose fermenting

P.Fig. 11-Haektoein Enteric Agar, Salmon colour Colony

P.Fig. 12 – EMB Agar

Plate VI: Remazol Yellow dye degradation



P.Fig. 17: Before Degradation **P.Fig. 18: After Degradation**

Dye degradation is largely dependent on the condition of growth of the culture and composition of nutrient broth. The present study aims at optimization of medium components which have been predicted to play a significant role in enhancing the degradation of reactive dyes. Carbon source supplement gives increased percentage of dye decolourization in nutrient broth. Since the dyes are deficient in carbon source, it seems necessary to supplement additional carbon to assist biodegradation of dyes by the bacterial consortium (Bibi et al, 2012). Where as in this study bushnell hass medium was used in place of Nutrient agar.

Optimization study revealed that sucrose was found to be a suitable carbon source for the isolate Bacillus (KTE7), Lactose was suitable for KTE28 (Pseudomonas) & KTE64 (E. coli). Similarly decolouration of KTE48 (Staphylococcus) and 83 (LactoBacillus) were high in mannitol given below to decolourise Remazol Yellow dye. Maximum decolourization of 92.6 0.10 & 90.2 0.29% was obtained during the optimization studies of carbon sources for the selected isolate KTE28 and KTE7 respectively. In general LactoBacillus uses lactose as a major carbon source whereas in this study it was maximum utilization of Mannitol.

Table 4: Effect of Various Carbon sources on dye decolourization

S.No.	Carbon Sources	Percentage of decolourization (%)				
		KTE7	KTE28	KTE48	KTE64	KTE83
1	Glucose	89.3±0.09	82.3±0.015	62.3±1.23	87.6±6.75	79.8±0.32
	Sucrose	90.2±0.29	83.2±0.42	76.2±0.30	76.2±2.32	66.3±0.78
	Lactose	86.5±1.00	92.6±0.10	70.6±1.14	89.6±1.81	67.2±0.34
	Mannitol	87.2±1.73	81.4±0.58	89.4±1.72	46.4±2.87	74.6±0.18

All values are presented in triplicates (Mean ±SD)

Effect of various nitrogen sources on dye decolourization

The optimization study of nitrogen source extrapolated that peptone was suitable for maximum decolourization of Remazol Yellow dye by all the isolates tested. Maximum decolourization of 89.6 ± 0.53% was exhibited by Psedudomonas aeruginosa as given below. Decolourization rate of Remazol Yellow dye by all the microorganisms were reduced when yeast extract, ammonium sulphate and ammonium chloride used as a co-substrate. Literature also indicated the importance of peptone as a major nitrogen source for effective dye discolouration.

Table 5: Effect of various nitrogen sources on dye decolourization

S.No.	Carbon Sources	Percentage of decolourization (%)				
		KTE7	KTE28	KTE48	KTE64	KTE83
1	Yeast Extract	86.9±0.10	85.9±0.64	62.4±1.23	6.3±1.23	67.3±6.58
	Peptone	88.5 ± 0.26	89.6 ± 0.53	87.2±0.25	88.6±0.58	80.6±3.52
	Ammonium Sulphate	82.8 ± 0.67	86.6 ± 0.71	59.8±0.66	78.2±0.42	68.2±0.46
	Ammonium chloride	82.9 ± 0.15	86.9 ± 0.10	62.5±2.68	56.2±0.22	73.2±0.22

All values are presented in triplicates (Mean ±SD)

Effect of various temperature on dye decolourization

Remazol Yellow dye was decolourized upto 96.7±3.40% by Pseudomonas aeruginosa as given below at 37°C. Interestingly, all the gram negative bacteria were effectively performed its decolouration at 37°C whereas a gram positive bacterium performs its activity at variable temperatures. Remazol Yellow dye was effectively degraded by Bacillus cereus (KTE7) at variable temperatures (37°C to 45°C). Temperature at 37°C was optimum for the decolourization of almost all textile dyes by various microbial species, which is also supported by different literatures from different countries.

Table 6: Effect of different temperatures on dye decolourization

S. No.		Temperature		Percentage of decolourization (%)		
1		KTE7	KTE28	KTE48	KTE64	KTE83
	25°C	88.7±0.1	67.2±0.1	32.6±1.2	46.8±3.4	38.6±1.1
		0	5	3	0	5
	37°C	91.6±0.9	96.7±3.4	83.2±3.8	88.3±2.6	76.3±2.6
		0	0	6	7	7
	40°C	92.2±0.1	85.3±0.4	81.4±2.5	71.2±0.5	67.5±0.7
		2	6	9		3
	45°C	95.4±0.0	76.3±0.3	69.6±1.8	61.4±0.4	54.3±0.3
		0	8	0	7	4

All values are presented in triplicates (Mean±SD)

Effect of different pH on dye decolourization

pH plays a effective role in the growth of microbial cells as well as in its metabolism. pH of the effluents may vary depends on the type of textile processing. pH is greatly depends on the generation of hydroxyl radicals. Azo dyes are generally processed at acidic pH. Selected strain Pseudomonas aeruginosa (KTE28) able to decolourize effectively at pH7 with 91.7± 0.57% decolourization effect.

Two different pH were found suitable for decolourization of Remazol Yellow dye by Pseudomonas aeruginosa (KTE28) and Bacillus cereus (KTE7). Bacillus sp., also decolourize azo dyes at pH 7. LactoBacillus decolourize azo dyes at pH6 also with 90.3±0.10% decolourization effect.

Table 7: Effect of different pH on dye decolourization

S.No.		KTE7	KTE2X	KTE48	KTE64	KTE83
1		KTE7	KTE2X	KTE48	KTE64	KTE83
	5	59.6 ±0.46	79.1±0.55	48.3±1.08	61.5±0.0	79.8±2.9
					5	8
	6	68.6±0.58	73.8±0.57	67.6±2.08	71.9±0.1	90.3±0.1
					5	0
	7	80.7±0.64	91.7± 0.57	74.8±1.11	83.2±0.2	86.2±1.6
	1				5	7
	8	79.1±0.84	87.6±0.52	64.6±0.43	68.4±1.6	40.3±0.3
					4	4
	9	77.6±0.51	69.6±0.10	23.6±1.59	58.6+1.2	28.8±6.5
					1	2

All values are presented in triplicates (Mean+SD)

Effect of different inoculum sizes on dye decolourization

Inoculum size had significant effect on dye decolourization of dyes (Daneshwar et at., 2004). Textbooks and references indicated role of inoculum on various metabolic process.

Similar to textual reports here also all the test strains decolourized azo dyes at 10 % inoculums size concentrations. At this inoculum concentration Pseudomonas aeruginosa decolourize azo dyes upto 72.4±0.68% level, similarly LactoBacillus sp., decolourized azo dyes upto 92.4*0.68%. Eight percentage inoculums size also decolourize azo dyes at the significant level as given below. But better efficiency with 10% inoculums.

Only few reports showed increased percentage of inoculation increases decolouration effect whereas most authors indicated 2-10% inoculum for efficient dye degradation that is also depends on the type of strain used.

Table 8: Effect of different inoculum size on dye decolourization

S. No.	Inoculums	Percentage of decolourization (%)				
		KTE7	KTE28	KTE48	KTE64	KTE83
1	2%	44.6±0.52	59.5 ± 0.47	42.3±0.69	48.7±0.68	36.5±0.22
	4%	76.4±0.08	61.7±0.55	46.8±0.28	50.8±1.32	58.1±0.29
	6%	83.8±0.32	89.3±0.65	50.8±0.23	57.5±1.89	64.6±0.85
	8%	83.7±0.37	85.9 ±0.36	51.4±1.78	64.8±0.73	68.5±1.82
	10%	89.1±0.17	92.5±0.08	59.6±3.39	69.8±1.89	72.4±0.68

All values are presented in triplicates (Mean±SD)

Effect of time course on dye decolourization The Bushnell Hass broth supplemented with Remazol Yellow dye was inoculated with five test wild strains isolated from textile effluent were inoculated in optimized medium and optimized conditions for obtaining optimized time required for complete azo dye degradation. To study the time course of decolourization, inoculated flasks were incubated upto 12 days.

Optimized Maximum decolourization of Remazol Yellow was observed on 2th day of incubation. Bacillus cereus and Pseudomonas aeruginosa decolourized 90.8±O.34and 95.4±0.28% of Rernazol Yellow dye respectively on 10th day itself as given below.

Table 9: Effect of incubation time course on dye decolourization

S. No.	Day of Incubation	Percentage of decolourization (%)				
1		KTE7	KTE28	KTE48	KTE64	KTE83
		48.5±0.5	22.8±0.2	22.3±4.8	10.8±2.85	02.8±1.2
	2	0	9	2		8
		53.4±0.5	37.2±0.2	23.2±2.1	13.1±0.33	03.2±2.1
	4	3	5	8		5
		59.4±0.5	39.3±0.2	45.6±1.2	23.6±3.59	09.0±0.8
	6	3	6	6		2
		62.3±0.2	48,8±0.1	51.8±0.5	36.8±1.58	22.5±0.3
	8	0	2	9		8
		90. 1±0.4	94.6±0.6	53.6±0.8	48.9±0.64	59.8±1.3
	10	2	6	2		0
		90.8±0.3	95.4±0.2	60.9±4.8	52.8±0.69	64.2±0.8
	12	1	8	5		2

All values are presented in triplicates (Mean±SD). On the basis of above optimization study the following optimized condition was derived and was subjected for azodegradation process of individual organism as well as consortium of wild species isolated from the effluents of textile industry of Kovilpatti as given below.

Table 10: Optimized conditions of wild strains on azodye degradation

S.No	pH	KTE7	KTE28	KTE48	KTE64	KTE83	
1	Basal Medium	Bushnell Hass Medium					
2	pH	7	7	7	7	6	
3	Temperature	37°C	37°C	37°C	37°C	37°C	
4	Carbon	Sucrose	Lactose	Mannito	Glucos	Glucos	
				1	e	e	
5	Nitrogen	Peptone	Pepton	Peptone	Peptone	Peptone	
			E				
6	Inoculum Size	10%	10%	10%	10%	10%	
7	Days Of incubation	10	10	12	12	12	
8	Percentage of Degradation on 12th day	90.8	95.4	60.9	52.8	64.2	

Overall Pseudomonas aeruginosa produced 95.4% azo dye degradation in the presence of lactose, peptone with 10% inoculum, pH 7, incubation Temperature 37°C on 12th day of incubation. Bacillus cereus also produced 90.8% dye discolouration with the similar condition used for Pseudoinonas aeruginosa with sucrose as a carbon source as given above. E. coli produced least dye discolouration ability with 52.8% ability. Nature of microorganisms influence on bioremediation process in an ecosystem. KTE7 and KTE28 strains

attained 90% dye degradation on 1 0th day of incubation itself.

Percentage	Microbial Combination
Lactobacillus and Escherichia coli	40.66
Staphylococcus aureue and E.coli	78.59
Staphylococcus aureus and Lactobacillus	75.68
Bacillus and E.coli	79.13
Bacillus and Lactobacillus	82.08
Bacillus and Staphylococcus aureus	82.83
Pseudomonas and E.coli	90.18
Psudomonas and Lactobacillus	90.43
Pseudomonas and Staphylococcus	86.31
Pseudomonas and Bacillus	97.84

Combination of microbial species also used to study discolouration ability of the azo dye. Ten different combinations were done to assess dye discolouration. Among combination, Pseudomonas + Bacillus produced 97.84% of dye discolouration within 12 days of inoculation. Similarly more than 9Q% dye discolouration was done by Pseudomonas + LactoBacillus and Pseudomonas +Escherichia coli I within six days. LactoBacillus + Escherichia coli combination produced only 40.66% dye discolouration effect (Figure 5) on 6th day of incubation. Hence incubation stopped on 6th day for all test organisms and assessed dye degradation activity.

Consortium of gram positive as well as gram negative microorganisms also done using + Staphylococcus+ Lacto and Pseudomonas + E. coli.

Results revealed that gram positive group produced 93.97% dye discolouration (figure 6) when compared to gram negative group (90.18%). Here incubation was done upto 8 days.

Table 11: **Bioassay of Remazol Yellow dye and decolourized dye toxicity -Pot Assay**

Seed type	Samples	Percentage of germination							
		y	y	y	y	y	y		
		1	2	3	4	5	6	Average shoot length (cm)	Average root Length (cm)
Vigna Radiate	Control (Water)	-	28	64	76	100	100	17.4+1.193	3.7+0.436
	Decolourized dye-1	-	24	52	56	76	80	16.4±0.451	2.9±0.551
	Dye-1 (yellow)	-	20	40	48	42	52	14.1+0.100	2.7±0.513
Phaseolus Vulgaris	Control (Water)	-	20	80	88	92	96	24.3±0.571	2.4+0.431
	Decolourized dye-1	-	0	64	68	88	88	19.8±0.681	4.9±0.551
	Dye-1 (yellow)	-	0	36	40	48	54	10.7±1.955	3.6±0.513
Trigonella foenum	Control (Water)	-	24	52	62	84	90	6.6±0.764	4.0±0.436
	Decolourized dye-1	-	16	48	52	72	85	6.0±1.193	4.0±0.513
	Dye-1 (Yellow)	-	0	12	16	50	56	4.9±1.300	2.3±0.551

All values are presented in triplicates (Mean±SD)

FTIR Analysis in Dye Discolouration

FTIR analysis gives idea about the type of chemical functional groups present in the compounds. The FTIR analysis of the pure dye indicates that there was strong peak at 3234.62 cm-I indicating the presence of hydroxyl (-OH) and secondary amine (-NH) stretching vibrations in the pure dye.

SAMPLE A: (Pseudomonas aeruginosa)

The sample A (Pseudomonas) degraded by 90% the dye, showed peaks at 3500 1/cm in 3234.62, 3086.11, 2933.73, 2873.94, and 2500 1/cm in 1639.49, 1570.06,1396.46, 1045.42, 700.16, 615.29, 528.50. In sample A (Pseudomonas) 3234.62 to 3086.11 indicates the OH stretch, free hydroxyl band, N-H stretch, OH-stretch. And the 2933.73 to 2873.94 it's indicates the HC=C Stretch, C (triple bond) N-Stretch, C (triple bond) C- stretch. And peaks ranges in 1639.49, 528.50 strongly indicates the -C=C-stretch, N-H bend, C-C stretch in rings , N-O asymmetric stretch, C-H bend, C-O stretch, -C (triple bond) C-H: C-H bend, C-Br stretch.

Sample B (Bacillus cereus)

The sample B (Bacillus) showed the peaks at indication of degradation in dye at 3500 1/cm in 3226.91, 3066.82, 2935.66, 2873.94, and 2500 1/cm in1639.49, 1579.70, 1396.46, 1330.88, 1068.56, 1043.49, 698.23, 613.36, 520.78.

The multiple ranges of peaks indicate the -OH stretch, free hydroxyl band, N-H stretch, OH-stretch. The strongly indicates the peaks at the ranges in 1639.49 to 520.78 in - C=C- stretch, N-H bend, C-C stretch in ring, N-O assymmentric stretch, C-H bend C-O stretch, -C(triple bond), C-H: C-H bend, C-Br stretch.

From the FTIR analysis it was concluded that the decolourization of azo dye. It was by the means of degradation which causes changes in the molecular orientation of the pure dye molecule, it results in the formation of the different fragments indicated by the formation of new peaks in FTIR spectra. It was concluded that the action of the selected Pseudomonas species and Bacillus on dye molecules resulted in to the formation of dye products, which may be nontoxic to the ecosystem.

Discussion

The sludge and soil samples were collected from the textile dye effluent run off site of different textile units located in Kovilpatti, Thuthukudi district, Tamil Nadu, India. They were used for screening the dye degrading organisms.

The present study has focused on decolourization of textile azo dyes using wild strains. The bacterial isolates were screened for dye decolourizing capability of Remazol Yellow dye. Primary screening of dye degradation study recovered 95 isolates of Remazol Yellow dye degrading bacteria from effluent soil samples.

Results of secondary screening revealed that only five strains have good degrading ability when compared to other strains. Rate of dye degrading .bacteria may vary depending on the environment that creates stress and adaptability of the wild strain of the particular ecosystem. Survival of the organism in the stress full environment depends on the tolerability. All the 70 isolates were considered to be stress-tolerant strains. Our results were also supported by the scientists from all parts of India as well as from abroad.

Olukanni et al. (2005) isolated eighteen textile effluent adapted bacterial isolates belonging to the genera, Bacillus, Acinetobacter, Staphylococcus, Legionella and Pseudomonas. These isolates were investigated for the potential of decolourizing the textile effluent. Bacillus and Legionelia were found to be better degraders of the textile effluent. Ajibola et al. (2005) checked the ability of Staphylococcus aureus, Bacterioides fragilis, Bacillus subtilis, Bacillus cereus, Clostridium perfringens, Escherichia coli and Peptostreptococcus sp., to reduce and stabilize textile effluents containing predominantly Indigo Blue. Mahmood et al. (2011) isolated 200 isolates from water as well as sludge samples from Paharang drain near Bawa Chak, Faisalabad, which degraded proteins and tested for their potential to remove Remazol Black-B azo dye. Five bacterial isolates capable of degrading Remazol Black-B azo dye efficiently were screened through experimentation on modified mineral salt medium.

Aftab et al. (2011) collected water samples from Kias Textile Industrials effluents, Lahore, They isolated 18 microorganisms out of which the best isolate was screened and identified as Cyanobacteria sp. The species had the capability to decolourize and degrade dyes into non-toxic form. Sriram et al. (2013) collected soil samples from the surroundings of dye decolourization industry, Tirupur region, Tamil Nadu, India.

The researchers isolated three different bacterial isolates Bacillus sp., Escherichia coli and Pseudomonas fluorescens. The isolated organisms were used for the degradation study. They also found that all the isolated microorganisms were efficient decolourizers of Reactive textile azo dyes. Mohan et al. (2013) collected effluent samples from different textile dyeing units in Tirupur and Erode Districts of Tamil Nadu, India. They identified these five isolates as Bacillus subtilis, Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella pneumoniae and Escherichia coli. Bacillus subtilis showed highest dye degrading capacity (76.3%) when compared with the other organisms.

Ponraj et al. (2011) isolated Bacillus sp., Klebsiella sp., Salmonella sp., and Pseudomonas sp., that decolourized textile dye-Orange 3R collected from a dyeing industry located at Elampillai, Salem (TN). The dye decolourizing bacteria were isolated from textile dye effluent by serial dilution and plating appropriate dilutions on modified Zhou and

Zimmermann (ZZ) agar medium. The researchers concluded that Bacillus sp., was found to be more efficient in dye decolourization. Karthikeyan and Anbusaravanan (2013) isolated five different colonies B1, B2, B3, B4 and B5 from untreated textile effluents, released into Amaravathy river by small-scale dyeing industries in Karur. In their studies they found that Pseudomonas sp., and Bacillus cereus degraded azo dyes at maximum rate of 78% and 82% respectively.

Hassan et al., (2013) collected dye effluent samples from 3 discharge points of Qualitex Industries (BD) Ltd., CEPZ, Chittagong, Bangladesh. The researchers isolated 11 bacterial strains from textile dye effluents based on their distinct colony characteristics like form, colour, elevation, margin and surface. Ramalingam and Shobana (2011) collected soil samples from nearby locations of Kumaraguru College of technology, Coimbatore. The researchers found that Bacillus cereus, Bacillus megateriwn and Pseudomonas fluorescens decolourized the dye upto 68%, 70% and 81% respectively.

Decolourization activity was assessed in ZZ medium containing Remazol Yellow dye with an inoculum of each isolate used separately. These dye degrading bacteria inhabited in textile effluent utilized the dyes as their Source of energy, thereby cleared the dye incorporated in the media.

Kumar et al., (2013) isolated bacterial isolates from a textile industrial ground at Mettuppalayam, Tamil Nadu, India. The isolate decolourized Disperse Red F3B to 94% at 100 mg' concentration within 5 h under microaerophilic condition at 50°C. The bacterial isolate was determined to be Enterococcus faecalis based on the biochemical characteristics and 1 6S rRNA sequence analysis.

Barragan et al., (2007) isolated three different bacterial isolates namely Enterobacter, Pseudomonas and Morganella sp. The researchers illustrated that kaolin, bentonite and powdered activated carbon (PAC) when added to the media along with these organisms showed high azo dye degradation capacity. They proved that these particles adsorb the dye into their surface and facilitate the organisms to degrade these adhesive particles (Acid Orange 7 dye). Neelam and Rao (2013) isolated Pseudomonas putida, Bacillus lichen jformis and Alcaligens faecalis that showed good degradation activity against Remazol Red Dye collected from effluent samples of four different dyeing units located at Mangalagiri Town, Guntur District in A.P. India.

Tripathi and Srivastava (2011) isolated 33 bacterial isolates from various textile industries at Pune, India and identified as Pseudomonas putida, Bacillus cereus, Pseudomonas fluorescens, Bacillus subtilis, Alcaligens sp., and Staphylococcus aureus. Among the isolates Pseudomonas putida emerged out to be the most potent decolourizer.

Similarly this study also confirmed the isolation of five efficient strains for the decolouration of azo dye. Bacillus cereus, Pseudomonas, Staphylococcus aureus, E. coli and Lactobacilli. Our results are in line with the above reports. Both gram positive and gram negative bacteria are able to decolourize azo dyes (Figure 1, 2, 3).

The five isolates were selected based on its ability on dye degradation and identified based on their microscopic and biochemical characterization. The most common biochemical tests used to identify bacterial isolates are IMViC test, urease test, nitrate reduction test, catalase test, oxidase test, gelatin hydrolysis test and starch hydrolysis test. Among the five isolates, KTE7 was found to be Gram-positive rods with subterminal spores. KTE28 was a Gram-negative rods and motile. Biochemical tests like Voges-Proskauer, citrate and indole were positive for KTE7. The isolate KTE23 showed significantly positive results for citrate, oxidase, catalase tests with negative hydrolysis of lipid and casein in the media after specific incubation period (Table 3).

Pseudomonas fluorescens is a common gram negative rod shaped bacterium. Cabrefiga et at., (2014) suggested that 4-hydroxy acetophenone monooxygenase enzyme found in Pseudoinonas aeruginosa that transforms piceol, NADDH, H and 02 into 4 hydroxy pheny, acentate acetate, NADP and H20. In the present study P.Jluorescens was isolated from contaminted dye soil (Plate II). Bacillus cereus is a gram positive rod shaped, facuitatively anaerobic commonly found in soil. Hoten et al., (2005) indicated that Basillus cereus showed a degradation ability of dyes, it is a plasmid borne ability. In the current work Bacillus cereus was isolated and identified by various biochemical tests (Phñe 1). Staphylococcus aureus is a gram positive organism. Masalha et al., (2001) explained some genes are Isolated from soil, in the present study S.aureus was isolated from efflent (Plate III). Lacto Bacillus of gram positive facultative anaerobic organism, they are major part of the lactic acid bacterial group. According to France et at., (2016) Lacto Bacillus act as an ecological drivers. In the current work focused on Isolation and identification of focused on Isolation and identification of Lacto Bacillus from poluted soil (Plate IV).

Escherichia coli is a gram negative organism. It is expelled into the environment within fecal matter. Russel et at., (2001) explained mostly the organism were come from faecal matter and contaminated soil. In the present study revealed this organism was isolated from dye polluted area.

Morphological and biochemical features of the test organism revealed that the best dye degrading strain KTE7 belongs to the order bacillales and the genus Bacillus whereas KTE23 was belongs to the group beta proteobacteria and the genus Pseudomonas. These bacteria were found in stress full environment. Some reports suggested that these organisms were the plant growth promoting rhizobacteria and some described as antagonistic strain. Our results suggested that this organism could be considered as a good dye degrading strain, suitably used for pollution control.

Parvathi et al., (2009) isolated Bacillus sp., from the coastal area of Cochin, India. The researchers isolated 25 different species and idenified using morpho-physiological and biochemical characterization. Similarly Pandian et al., (2012) isolated Bacillus sp., from feather dumping soils collected from Madurai, India. The isolate showed positive results to Voges — Proskauer test, citrate utilization test, triple sugar-iron test, carbohydrate fermentation test and starch hydrolysis test. Ishak et al., (2011) isolated Bacillus megaterium from the soil samples from Indah Water consortium, Malaysia. Vinod and More (2013) isolated and purified Bacillus sp., from the soil and water samples collected from Lonar Crater Lake, Maharashtra. The purified Bacillus sp., showed positive results for Voges - Proskauer test. Panda et al. (2013) isolated Bacillus sp., from soil samples of hot spring of Tarabalo, India. The researchers recorded morphological and physiological characteristics of Bacillus sp., using biochemical tests. The isolate showed positive results for citrate utilization test and starch hydrolysis test. Ann (2013) isolated Bacillus sp., from marine locations of Malaysia. The researchers selected the best one among the 10 species and characterized using certain biochemical tests. The isolate showed positive results for citrate utilization test, starch hydrolysis test and triple sugar iron test. Kayasth et al., (2012) isolated Bacillus subtilis from saline soils in the region of Haryana, India. The best among the isolates were characterized using biochemical and morphological studies. Shah and Bhatt (2012) isolated Bacillus subtilis from soil samples at Gujarat region, India. Reddy et al., (2009) isolated Bacillus cereus from different soil samples collected from the alkaline metal industries, Hyderabad, India. The isolated bacteria were characterized based on the biochemical tests. They showed positive results for Voges — Proskauer test and starch hydrolysis test. Jin et al, (2012) isolated Bacillus altitudinis from marine soil samples collected from East Chinese Sea, close to Nanao island, Shantou, China.

Optimization of dye decolourization using different parameters

Effect of various carbon sources on dye decolourization

Dye degradation is largely dependent on the condition of growth of the culture and composition of nutrient medium. The present investigation aims at optimization of medium components which have been predicted to play a significant role in enhancing the degradation of reactive dyes. Carbon source supplement gives increased percentage of dye decolourization in nutrient broth. Since the dyes are deficient in carbon source, it seems necessary to supplement additional carbon to assist biodegradation of dyes by the bacterial consortium (Bibi et al., 2012). In this study sucrose acts as a carbon source for Bacillus and Lactose for Pseudomonas. Similarly mannitol for Staphylococcus aureus, Glucose for E. coil and Lactobacilius (Table 4).

The obtained results were found in agreement with the research findings cited in the following literature. Gupta and Sukas (2009) reported that Bacillus sp., used carbon sources as main source for degrading the Reactive dyes. Besides, theresearchers observed that decolourization percentage was higher (54.6%) in Bacillus sp., as compared with Pianococcus sp. Moosvi et al. (2005) proved that decolourization percentage increased by supplementing the effluent medium with other cheaper effective carbon or energy source such as sucrose, starch and hydrolyzed starch. The researchers also commented that the ability of the microbial isolates to utilize sucrose as a co-substrate could be encouraging from commercial point of view. Gupta et al. (2005) concluded that effluent-adapted Bacillus sp., gave 35.68% reduction in Orange 3R dye colour whereas the non-adapted isolate of the same species showed 30.04% colour removal in the presence of sucrose as carbon source. Maier et al., (2004) commented tlat Pasteurella sp. exhibited 41.73% of decolourization, Pseudomonas sp. (39.03%) of decolourization, whereas Kluyvera ascorbata showed only 19.76% decolourization of dye in the presence of maltose and mannitol as carbon source.

Daneshvar et al., (2004) investigated that sucrose resulted in better decolourization efficiency of VITABRI3 by Bacillus endophyticus with 91% followed by dextrose (78%) and manriose (62%) at the end of 24 h incubation period. The decolourization efficiency decreased with dulcitol (56%), mannitol (42%), lactose (37%) and d-xylose (34%). Besides the researchers indicated that least decolourization was observed with maltose (11%).

Effect of various nitrogen sources on dye decolourization

The optimization study of nitrogen source extrapolated that peptone was suitable for maximum decolourization of Remazol Yellow dye

by the isolate all the test isolates (KTE7, KTE23, KTE48, KTE64 and KTE83). Decolourization rate of Remazol Yellow dye by Bacillus cereus (KTE7) strain dropped sharply when yeast extract, ammonium sulphate and ammonium chloride used as a co-substrate (Table-5).

Sudha and Balagurunathan (2013) isolated Bacillus licheniformis from textile effluent contaminated site. During their research they found that ammonium nitrate was the best additional nitrogen source for the decolourization of textile dye effluent. Joshi et al. (2013) described ammonium chloride to be the suitable nitrogen source for the textile dye decolourization followed by ammoniurn hydroxide, sodium nitrate, urea, while the peptone supported the least among the five different types of nitrogen sources used in the study.

Modi et al., (2010) reported peptone as the ideal nitrogen source for efficient decolourization of Reactive Red 195 by Bacillus cereus, recording 97% reduction of dye colour in the effluent. Another study by Ola et al., (2010) reported decolourization of Cibacron red P4B and Cibacron black PSG to the levels 81% and 75% respectively. Further they reported that different nitrogen sources were optimized for maximum decolourization of the two dyes. Shah et al., (2013) reported the effect of different nitrogen sources on decolourization of azo dye by Bacillus cereus and Bacillus megaterium. Decolourization of the dye recorded for Bacillus cereus when beef extract was used as nitrogen source was 10% and that for Bacillus megaterium was 15%. The percentage of decolourization of azo dye with peptone as nitrogen source was found to be 82% and 74%, for Bacillus cereus and Bacillus inegaterium respectively. When the yeast extract was used as nitrogen source 68% decolourization of the dye was recorded with both the bacterial species. From the above data the authors observed that Bacillus cereus is more efficient in decolourizing azo dye than Bacillus megaterium and peptone is the ideal nitrogen source for its activity under in vitro condition.

Zhang el al., (1999) and Nosheen et al. (2010) used urea and ammonium nitrate as inorganic nitrogen sOurces for optimizing the maximum decolourization of azo dyes Reactive Black B and Reactive Orange 16. As far as nitrogen sources are concerned none of them could be proved as an enhancing factor for decolourization rate of dyes under study. A strong inhibitory effect was recorded at higher levels for all additional nitrogen sources under investigation. These results are in harmony with those of Tatarko and Bumpus (1998) and Sanghi et al., (2006) who also reported inhibition of dye clccolourzation with supplemental nitrogen. The facts responsible for these effects are different for three nitrogen compounds under study. The addition of inorganic nutrients like nitrogen does not always enhance degradation of organic compounds, because there are many other factors which may decrease microbial activity (Steffensen and Alexander, 1995).

Research by Srinivas et al., (2012) was done with isolated dye degrading organism from effluent of environmental treatment plant, Naroda. The organism was inoculated in media supplemented with different nitrogen sources like yeast extract, meat extract, peptone and urea for decolourization of RB 250. Analysis revealed that yeast extract promoted higher decolourization efficiency. Dye RB 250 showed maximum percentage of decolourization (96.68%) and decolourization rate (12.08 mg/LIh) within 8 h. Kumar et al. (2012) studied the effect of different nitrogen sources on deo1ourization of Red 3BN by B. cereus and B. megaterium.

Percentage decolourizafiqn of the dye recorded for B. cereus when beef extract was used as nitrogen source was 7.69% and that for B. megateriurn was 11.54%. The percentage of decolourization of Red 3BN with peptone as nitrogen source was found to be 66.67% and 57.41%, respectively for B. cereus and B. megateriurn. When the yeast extract was used as nitrogen source 62.5% decolourization of the dye was recorded with both the bacterial species. From the above data it is observed that B. cereus is more efficient in decolourizing Red-3BN than B. megaterium and peptone is the ideal nitrogen source for its activity under in vitro condition. From the above outcome of different studies, it can be inferred that the metabolic flux of B. cereus alters with the type nitrogen sources available in the surrounding environment and efficiency of decolourization of the azo dyes is greatly dependent on these factors. Among gram positive organisms Bacillus cereus (KTE7) efficiently decolourize azo dye with peptone as a nitrogen source. Similar kind of result was noted in this study also.

Effect of different temperatures on dye decolourization

Microbial growth is temperature specific and decolourization is catalyzed by the enzyme. Variation in temperature decreases or increases the enzyme activity. In microorganisms, the environmental temperature directly establishes organisms temperature, as the microbial cell responds to temperature changes by adaptation via biochemical or enzymatic mechanisms. Consequently, temperature is a factor of paramount importance for all processes associated with microbial vitality, including the remediation of water and soil (Giwa et al., 2012).

Mohan et at., (2013) proved that Planococcus sp., and Bacillus sp., at temperature 37°C showed maximum decolourization of Mordant Orange I dye

when compared to other temperatures (25°C, 30°C, 45°C). Prasath et al., (2013) concluded that Pseudomonas sp., and Bacillus sp., showed maximum decolourization of textile dyes at 40°C. The researchers revealed that temperature plays an important role in microbial growth and enzyme activity and one of the most important parameter taken into consideration for the development of bio-decolourization processes. Similarly in this study also all the five test strains disscoloured azo dye at 37°C. Only Bacillus cereus discolourd yellow dye at 37°C, 40°C and 45°C (Table 6). This could be due to thermophilic spore forming nature of Bacillus cereus (KTE7).

Hassan et al., (2013.) reported that Nocardia atlantica caused almost complete decolourization of Blue FNR and Red FNR at 37°C when compared to other dyes tested (Yellow FN2R, Yellow FN2R). Thirupathi and Srivatsava (2011) studied the decolourization of the dye, Acid Orange 10 with temperature ranging from 20°C to 50°C. The researchers also extrapolated that the optimum temperature for Pseudomonas putida MTCC 102 was 37°C. Kumar and Sumangala (2013) revealed that percentage decolourization of the Azo dye and Red 3BN for Bacillus cereus and Bacillus megaterium were 93.64% , 96.88% at 37°C respectively.

Shahvali et al., (2000) studied various environmental parameters on decolourization of textile wastewater using Phanerochaete chrysosporium and found 35°C as optimum temperature for maximum decolourization.

Effect of different pH on dye decolourization

The effluents from textile industries usually have a wide range of pH values. Further, the generation of hydroxyl radicals is also a function of pH. Thus, pH plays an important role both in the characteristics of textile wastes and generation of hydroxyl radicals. pH effects can be related with changes in the specification of the dye. That is, protonation or deprotonation of the dye can change its adsorption characteristics and redox activity.

Kumar and Sawhney (2011) reported that optimum concentration of dyes in both acidic and alkaline pH seems to decrease the percentage decolourization of the dyes besides the inhibitory effect seems to be more pronounced in the alkaline range (pH 11—13). At high pH values the hydroxyl radicals are so rapidly scavenged that they do not have the opportunity to react with dyes. Saratale et al. (2009) extrapolated that Bacillus sp., showed maximum activity (70.86%) at pH 7.0. The researchers observed that the decolourization showed approximately four fold rise with increasing pH 2.0 to 7.0 and further increase in pH of the media led to decline in decolourization 93% with minimum activity (7.0%) at pH 10.

Swati and Meena (2012) commented that the rate of decolourization of Fast Red by Bacillus sp., was very low in high acidic pH range lower than pH 3.5. As pH increases rate of degradation also increases. When pH reached to basic range the rate of degradation increased fast and at the pH range 7.5 to 9, very good rate of degradation was observed. On further increase in pH the rate of degradation decreased. Above the pH range of 10 or above, the rate of degradation was less and continually decreased as pH increased. Hence the researchers concluded that rate of degradation in basic medium is higher than acidic medium. Our result also coincides with most of the authors. All the test strains efficiently decolourize azo dye at pH 7 (Table 7).

Mehra and Sharma (2012) observed that the decolourization efficiency of azo dyes by Bacillus sp., increased with increase in pH exhibiting maximum rate of degradation (97.32%) at pH 8. The researchers revealed that higher degradation of dye occurred in basic region than in acidic solution. Sakthivel et al., (2003) concluded that the rate of photo decolourization increased with increase in pH, exhibiting maximum efficiency (98.5%) at pH S. beyond which the rate of degradation remained constant.

The researchers also stated that the adsorption of dye molecules are low at alkaline pH, the possible reason for this behavior may be the formation of more OH radicals. Mohabansi et al., (2011) revealed that pH is most important parameter to study because wastewater containing dyes are discharged at different pH ranges. The decolourization percentage was highest (64.34%) at alkaline pH 9.0 for Bacillus sp. when compared to other pH ranges.

Effect of different inoculum sizes on dye decolourization

Decolourization activity of Bacillus sp., was studied by Ponraj et at., (2011). Authors indicated that Bacillus sp., was high (86.72%) in 4% of inoculum. Jadhav et al. (2007) reported that about 2% of inoculum was found to be suitable for decolourizing blue dye. Maximum of 91.3% decolourization was observed in the blue dye media inoculated with 2% of Bacillus sp., Choudhari and Singhal (2007) suggested that the increase in inoculum size up to 5.5 % (v/v) increases the dye decolourization, the inoculum size above 5.5% decreases the dye decolourization upon further augmentation. Maximum decolourization was obtained at pH 6.9. our results showed variable result (Table 8).

Alinsafi et at., (2005) founded that Bacillus sp., showed a maximum of 95.59% of decolourization when the inoculum size reached 10% (v/v). Maier et at., (2004) investigated that Kiebsiella sp., has

high decolourization potential (67.19%) in 6% of inoculum. Decolourization activity of Salmonella sp., was high (53.91%) in 6% of inoculum.

Mittal et al. (2005) depicted that the decolourization activity of Bacillus cereus was high (67.19%) in 6% of inoclum size. Saratale et al., (2009) conluded that the decolourization rate of sp., increased with increase in the inoculums size, reaching maximum 20% (v/v) inoculum size. There was no proportionate increase I the percentage of decolourization with increase in the inoculum size of Kurthia sp., when inoculated in textile effluent. This indicated that depends on the nature of species and the environment, the requirement of inoculums size may vary. In this study all the isolates need 10% inoculums for better efficient discolouration of azo dye.

Kumar and Sawhney (2011) observed that the decolourization percentage was highest at 10% inoculum concentration for Bacillus sp., (48.15%) and Planococcus sp., (57.68%) and low decolourization was obtained in other inoculum concentration.

Chen et al., (2003) extrapolated that Klebsiella sp., resulted in higher decolourization rate (96.24%) when the inoculum size increased to 1 O% (v/v). Aksu et at., (2003) proved that the Bacillus sp. shows the highest decolourizaticn of 86.72% at pH 7 and inoculum size of 8% (v/v).

Effect of time course on dye decolourization

Coughlin et at., (2002) reveled that pure culture degraded more than 99% of Orange 7 dye within72 h. The researchers also concluded that mixed culture degrade more than 98% but in a time span of 119 h. Mabrouk and Yusef (2008) observed that 99% decolourization of Fast Red was achieved in 6 h representing 1.14 fold increase compared to unoptimized medium after 14 h. Nachiyar et al., (2012) found that the bacterial consortium (Citrobacter jreundii, Moraxella osloensis, Pseudomonas aeruginosa) degraded 90% of the dye by 22 h in 80% diluted textile effluent supplemented with glucose and ammonium nitrate.

Milikli and Rao (2012) proved that Bacillus subtilis degraded 92% of Bromophenol blue dye (5 mg/L) within 12 h at optimized conditions such as pH 8, temperature 40°C, and carbon source (glucose). Prasad et al., (2013) concluded that all Bacillus sp showed maximum dye degradation of 99.5% after 8 days of incubation. Rudakiya and Pawar (2013) showed that Comamonas acidovorans decolourized Reactive Orange 16 at 37°C, pH 6.8 within 24 h and gave 99.03±0.5% dye decolourization under optimum environmental conditions.

Sharma et al., (2010) investigated that maximum colour reduction was near about 76%, recorded after the incubation period of five days under shaking conditions. The researchers also concluded that the decolourization pattern indicated degradation process was highly time dependent. Saharan and Ranga (2011) studied that Bacillus subtilis SPR42 decolourized azo dyes: Vaxent Red HE7B (Reactive Redl4l) and Vaxent Yellow HEGR at a concentration of lOOmgL1 up to 73% and 92%, respectively within 24 h at 37°C and pH 8.5 during static conditions. All the results cited from literature were found in agreement with the present research findings. Above studies illustrated the efficiency of azo dye discolouration of microbial cells were within 72 hours only where as in this study all the strains needs 12 days of incubation for the better dye degradation. Bacillus cereus and Pseudomonas aeruginosa decolourize azo dyes upto 90% within 12 days. Time taken for decolouration was high but the efficiency was too good (Table 9).

Decolourization of textile dye effluent is serious environmental problem, which is evident from the magnitude of research done in this field in the last decade. Nehra et at.,., (2008) indicated that treatment of textile effluent by physical and chemical methods have a high cost potential and a high sludge problem, whereas biological process convert organic compounds completely into water and Co2 have low cost and are easy to use. Hitz et al., (1978) stated that the presence of sulfonates in reactive dye structures results in low levels of colour removal. However, this is not applicable to direct dyes that usually exhibit high levels of colour removal independent of the number of sulfonate groups in the dye structure, reinforcing the idea that sterile hindrance and the number of azo bonds are responsible for the different discolourization times.

The wastewater released from the textile industries containing dyes are highly coloured and are therefore visually identifiable The complex aromatic structure of the dyes is resistant to light, ozone and other degradative environmental conditions. Therefore, conventional wastewater treatment remains ineffective. Also, anionic and non- ionic azo dyes result in toxic amines due to the reactive cleavage of azo groups, (Kilic et at., 2007). Generally the decolourization of azo dyes occurs under conventional anaerobic, facuhative anaerobic and aerobic conditions by different groups of bacteria. The mechanism of microbial degradation of azo dyes involves the reductive cleavage of azo bonds (-N55N-) with the help of azoreductase enzymes under aerobic conditions that resulted in the formation of colourless solutions containing potentially hazardous-aromatic amines (Chang and Kuo, 2000). The resulting intermediate metabolites (e.g., aromatic amines) are further degraded aerobically (Joshi et al., 2008).

Many recent studies focus on the utilization of microbial biocatalysts to remove dye from the effluent Extensive studies have been carried out to determine the role of the diverse groups of bacteria in the decolourization of azo dyes. The bacterial decolourization and degradation of these dyes has been of considerable interest since it can achieve a higher degree of biodegradation and mineralization, is applicable to a wide variety of azo dyes. Recently a substantial amount of research on the subject of colour removal has been carried out using single bacterial cultures like Bacillus and Pseudomonas sp (Khehra et al., 2005). This study also uses five individual isolates isolated from effluent of dye deposited area.

Haugh et al., (1990) demonstrated that decolourization of synthetic is the result of the clear age of the chromohoric group which genrerates which colourless metabolic intermediates. Agarry et al., (2011) observed a maximum of decolourization at 25 ppm initial concentration of dye after 18 hours of incubation using of culture Pseudomonas sp. The current report produced best result of 93.2% declourization while using 50 ppm concentration of dye over 24 hours of incubation and 94% of decolourization with the similar incubation at 5oppm concentration.

Bogan et al., (2001) described that the remediation process is usually limited by different culture condition. The optimum pH for complete decolourization of dye decolourized by Pseudomonas sp was shown to be efficient on the dyes. Cripps et at., (1990) detected that the deco louization of azo dye was being enhanced in nitrogen limited than in nitrogen suffifient culture. Ren et at., (2006) studies revealed that colour removed of triphenylmethane dyes was due to soluble cytosolic enzymes and the enzyme was an NADH/NADPH-depents oxygenase. Thus, the majority of research is directed towards biological treatment which provides reliable results, less sludge and more ceo-friendly treatment (Ramalingam et at., 2010).

Although the majority of decolourization studies employ white rot fungi as the sole and efficient group of fungi capable of colour removal yet, brown rot fungi was also reported to have the ability to decolourize diverse dyes (Ramalingam et at., 2010). Our study uses only bacteria.

Jiunkins et at., (1982) stated that industrial effluent is not stable and it varies often in a wide range depending upon the process practised. This phenomenon is very common where the polluting industries like textile dyeing, leather tanning, paper and pulp processing, sugar manufacturing, etc. thrive as clusters. Among these the textile industries are large industrial consumers of waters as well as producers of wastewater.Bacteria have been reported.to be highly substrate specific to the dye to which they are adapted.

Haugh et al., (1991) demonstrated that decolourization of synthetic dyes is the result of the clear age of the chromophoric group which generates colourless metabolic intermediates. Coughlin et al., (1997) reported that azo and triphenylmethane dyes suits the carbon, nitrogen and energy sources for bacterial growth that correlates with the present degradation study. Khehra et al., (2005) studied the percentage of crystal violet degradation by Bacillus subtilis that showed 90% decolourization within 24 hours of incubation. Agarry et al.,(201 1) observed a maximum of 98% decolourization at 25 ppm initial concentration of dye after 18 hours of incubation using the culture of Pseudomonas sp. The current report produced best result of 93.2% decolourization while using 5Oppm concentration of crystal violet dye over 24 hours of incubation and 94% of decolourization with similar incubation time using malachite green at 50ppm concentration.

According to Parshetti et al.,(2011) with the increase in inoculums concentration of Agrobacterium radiobacter the decolourization of crystal violet (100 mg/L) was increased with 2%, 5%, 7%, 10% and 15% inoculum the time required for decolourization of crystal violet was 40, 30, 25, 18, 15 hrs respectively. The inoculums concentration varied in the present study exhibited maximum decolourization of crystal violet and malachite green while adding 10% inoculums. Showed 62% of decolounzation, Bacillus sp showed 50% of decolourizatiqn followed by Lacto Bacillus sp showed 47% of decolourization and E.coli showed 31% of decolourization ability.

The microbes utilized dye as a carbon source, the percentage of decolourization ability as Pseudomonas sp used 62% of carbon as it sole energy, followed by Bacillus sp 46%, Lactobacilius sp 35% and E. coil 29%. Another work for dye decolourization using bacteria Bacillus sp 89%, Pseudomonas sp 89% LactoBacillus deibrueckii 51% (NurHazirah et ai.,2014).Effect of anaerobic, aerobic and microaerophilic conditions on decolourization of DB 38 and CR azo dyes by E. coil and Pseudomonas sp culture (Mustafa and Delia, 2003). In my study that four strains of Bacillus sp, Pseudomonas sp, Lactobacilius sp and E.coli have capability to utilize the dye as a sole source without any external carbon in the trace amount of oxygen (microaerophilic) condition was found. When compared with four bacterial isolates Pseudomonas sp gave best result in dye decolourization.

Wastewater treatment using biological approach is one of the technologies apply in textile wastewater, through physical and chemical approach

(NurHazirah et al., 2014). The ubiquitous nature of bacteria makes them invaluable tools in effluent biotreatment. The genus Bacillus and Pseudomonas, which were beneficial for the degradation of toxic constituents present in the effluents, was confirmed by the decolourization bioassay with least value of the final colour.

The continued develojment and application of biotechnologies for the biodegradation is limited primarily by physicalfactors such as pH, temperature and substrate concentration (Stephy et al., 2014). Palanivelan et ai., (2012) pointed that the most of the strains shows clearing zone is formed surrounding the bacterial culture which grown on LB agar plates for dye concentration of 100 mg/l. In my study Pseudomonas sp showed high level degradation in 20Oil textile dye, Bacillus sp and LactoBacillus sp showed moderate level degradation and lastly E. coli has low level degradation at normal temperature and pH 7. Bacterial degradation of these dyes was carried out by their intracellular uptake while the fungi degrade these by extra cellular enzymes. The organisms used in most of the study were Staphylococcus sp., E.coli, Bacillus sp., Clostridium sp., and Pseudomonas sp (Balaji etal., 2012).

In 2014 Stephy Lucious et al., revealed that the decrease in dye absorbance was in the range of 26 to 48% (Bacillus subtilis) and 31 to 45% (Pseudornonas aeruginsa). In this study the lml of textile dye in liquid medium, Pseudoinonas sp showed 90% decolourization, Bacillus sp showed 82% decolourization, LactovBacillus sp showed 70% decolourization and E.coli showed 60% decolourization ability.

The culture showed negligible decolourization in the presence of sucrose (25%) whereas moderate activity was shown in presence of glucose (55%), lactose (80%) and maximum decolourization was reported in presence of starch (95%). In contrast, addition of carbon sources seemed to be less effective to promote the decolourization probably due to the preference of the cells in assimilating the added carbon sources over using the dye compound as the carbon source (Maulin et al., 2013).

Bioassay for dye toxicity or phytotoxicity: Seed germination test

The textile industry is one of the important and rapidly developing industrial sectors. It has a high importance in terms of its environmental impact. India has large network of textile industries of varying capacity. 'Ministry of Environment and Forests' stated that textile industries are the most polluting industries. Many reports indicates that textile dye and effluent have toxic effect on the germination rate and the biomass of several plant species showed highly inhibitory effect on Sorghum seed germination in 100 % concentration of effluent (Priya et al., 2005). Textile effluent contains many constituents which are phytotoxic at higher concentration to plants. However, some of the constituents at lower concentration are also beneficial for growth and development of plants (Kannan and Raj, 2008). The present study was undertaken keeping in view the environmental significance of the potential influence of toxic textile effluents.

Effect of Remazol Yellow dye at the concentration of 20 mg was evaluated on germination of seeds of 3 different plants, Green gram (Vigna radiate), Kidney beans (Phaseolus vulgaris), Fenugreek (Trigonella foenum). The seeds were germinated in pots (Pot Assay) using paddy field soil.

The bioassay for dye toxicity or phytotoxicity was based on measuring the effect of Remazol Yellow dye on seed germination, plant shooting and root elongation. The phytotoxicity of the dyes was estimated by measuring the ability of dye and decolourized dye to germinate the green gram, kidney beans and fenugreek seeds as test plants. The results showed tbat the selected concentration of both the dyes was not considered toxic to germination of seeds; since good plant shooting and root elongation was observed when compared to that of control.

More number of seeds was germinated when the seeds exposed to decolourized Remazol Yellow dye samples, which was evidenced in pot assay. The seed germination percentage was found less for the untreated dye samples. Similarly, maximum shoot length and root length percentage was observed for the seeds exposed to decolourized dye samples when compared to the untreated dye samples (Plate VII).

Similar test was carried out by Shahin and Murugesen (2013) using green gram, red gram and black gram. They reported that the lower concentration of textile effluent did not inhibit seed germination in three seed varieties. The exposure of the untreated effluent and dye mixture was highly toxic to the plantlets as the root and shoit lengths were also inhibited when compared to treated effluent and dye mixture samples by different sets of reactors. Green gram showed 94.5% seed germination at 6.25% effluent concentration after 96 h. Red gram showed 100% at 6.25% effluent concentration; and black gram showed least percentage of germination rates. The report of present study is different from the available reports.

In another similar experimental set up carried out by Garg and Kaushik, (2008) also revealed that at lower concentration, the textile milLeffluent (untreated and treated) did not inhibit seed germination of Pioneer jowar and Desi jowar. in Pioneer jowar.

100% seed germination was observed at 6.25% effluent concentration after 120h, whereas Desi jowar seed germination was 100% in untreated effluent and 96.7±5.8% in treated effluent at 6.25% effluent concentration. The germination was inhibited in both the varieties when effluent concentrations exceeded 12.5% in irrigation water. Sheela et al., (2013) analyzed the physico-chemical properties of textile effluents after treating with Aspergilius Niger. The treated effluent was analyzed for its bioremediation potential by using it as foliar sprays on Solanum nigrum Linn. After 3Odays parameters such as height of the plant, length of the leaf, protein profiles were determined. This also showed slight variation (Table 11).

Summery & Conclusion

Kovilpatti municipality is well known for textile mill and its allied industries. Textile industries releases dye based waste to the water column. These created pollution to water bodies and its surrounding ecosystem. Various physical , chemical and biological methods are available to curtail the problem but still there is no opt method to overcome this problem. In this study trial was done using wild / native dye degradative microorganisms for the decolourization of pure azo dye. If these microorganisms decolourize this synthetic azo dyes effectively then it will be taken to field for the bioremediation process. By considering ecological impact of azo dye the present study was undertaken with specified aim and objectives.

Remazol yellow dye is subjected for microbial decolouration using native microbialspecies isolated from textile mill effluent.

About 95 strains were isolated with dye degrading ability in primary screening from textile mill effluent. Five strains with higher decolourizing capabilities were selected in secondary screening. Decolourization activity was confinned in Bushnell Hass medium containing Remazol Yellow dye. On the basis of dye degradation ability strains like KTE7, KTE28, KTE48, KTE64 and KTE83 were selected and subjected for further analysis.

All the test isolates were subjected to characterization by making use of standard microbiological techniques like microscopy (gram staining, simple staining, Spore staining and Motility), growth on selective cum differential agar and biochemicals tests. All these tests confirm the identity of the test organisms.

On the basis of microbiological test and in comparison with bergeys manual of determinative bacteriology the isolates were identified as Bacillus cereus (KTE7), Pseudomonas fluorescence (KTE28), Staphylococcus aureus (KTE48), Escherichia coli (KTE64) and LactoBacillus (KTE83).

All the test isolates were subjected for dye decolourization optimization study. The decolourization was optimized under different parameters like pH, temperature, inoculum size, and nutritional supplementation. Bacillus cereus (KTE7) decolourized Remazol Yellow dye at the following optimized conditions.

- pH - 7.0
- Temperature - 37°C
- Inoculum size -10%
- Carbon sourse – Sucrose
- Peptone
- Incubation period - 12 days
- Efficiency of discolouration -90.8%

Pseudomonas strain (KTE28) decolourized Remazol Yellow dye at the following optimized conditions.

- pH -7.0
- Temperature - 37°C
- Inoculum size - 10%
- Carbon sourse - Lactose
- Nitrogen source - Peptone
- Incubation period - 12 days
- Efficiency of decolouration - 95.4%

All the isolates were subjected for dye decolouration under optimized condition. Results revealed that isolate KTE 28 showed high percentage of decolouration of Remazol Yellow dye (95.4%). Similarly KTE 7 showed 90.8% azo dye degradation ability. Microbial consortium and mixed culture are also used to assess azo dye degradation. Among the mixed culture Pseudomonas and Bacillus group performed well and decolourized upto 97.84% of azo dye. Similarly gram positive group consortium produced 93.67% dye decolouration. Consortium of all microbial wild species produced 83.2% efficiency with in four days.

This indicated that consortium with effective strains decolourize dye effectively within shorter duration. Individual microbial strains like Pseudomonas and Bacillus produced morethan 80% dye decolouration whereas cultures like E. coli, LactoBacillus Staphylococcus aures decolourized dyes only less than 70%. Similarly mixed culture and consortium efficiently decolourized dye within 4-6days.

FTIR data revealed that bioremidiated pure azo dye indicated absence of —N-N — stretch, which indicated that dye decoluration is directly proportional to dye degradation. The present study was undertaken keeping in view the environmental significance of the potential influence of toxic textile dyes. Effect of Remazol Yellow dye at the concentration of 20 mg/i was evaluated on

germination of seeds of 3 different plants, Green gram (Vigna radiate), Kidney beans (Phaseolus vulgaris) and Fenugreek (Trigonella foenum). The seeds were germinated in pots (Pot Assay) using paddy field soil.

The bioassay for dye toxicity or phytotoxicity was based on measuring the effect of Remazol Yellow dye on seed germination, plant shooting and root elongation. The phytotoxicity of the dyes was estimated by measuring the ability of dye and decolourized dye to germinate the green gram, kidney beans and fenugreek seeds as test plants.

The results showed that the selected concentration of the dye was considered toxic to germination of seeds whereas bioremediated dye are not toxic to plant since good plant shooting and root elongation was observed when compared to that of control.

Conclusion

It is concluded that Pseudomoans fluorescence and Bacillus cereus were able to decolourize Remazol Yellow dye upto 97%. Bioremediated dye containing effluent doesnot prevent germination of seed and growth of the plant. Our results suggested that these organisms could be considered as a good dye degrading strains, suitably used for pollution control. They are also considered as an ecofriently strains as these strains are considered to be a plant growth promoting rhizobacteria. Good combination Microbial consortium may be developed with these strains and utilized properly for bioremediation process.

Further ecosystem based study should be conducted to check toxicity of bioremediated dye and also check pathogenic nature of test microorganisms.

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