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Decolourisation and Degradation of Synthetic textile dyes by Pseudomonas spp

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Abstract

Textile industry is one of the most demanding and growing industry in India, particularly concentrated in Maharashtra and Gujrat. As tones of textile fabrics and dyeing units are working continuously, huge amount of highly various toxic chemicals are released in the natural water bodies and land. This creates notorious effects on natural ecosystem. Illeffects includes reduction in light penetration, bioaccumulation of toxic dyes in living system leading to various genetic defects and may be responsible for creation of cancer like dreadful conditions, increasing the mortality rate in silent manner.
Four textile dyes including Proc Lemon H4G, Reactive red, Reactive black and Reactive blue were selected for degradation by the selected dye degrading strain. Textile dye degrading microbial strains were isolated by usual microbial technique by taking sample of textile effluents. Isolated strain was identified as Pseudomonas spp. Each textile dye was taken at 50 ppm level and checked for its microbial degradation by Pseudomonas spp. This paper reflects applicability of most efficient textile dye degrading strain Pseudomonas spp. Degradation of textile dyes was recorded as 29 to 89.28 %. Impact of degraded sample was tested on growth of microbial strains and seed germination.

Keywords: Bioremediation, Textile dyes, Microbial degradation, Percent degradation, Screening

INTRODUCTION

Dyes is a colouring agent used for general purpose. Thus colorant imparts its colour to specific substrates. Generally dye is an organic compound which is soluble in water. Dyes have chromophore and auxochrome groups linked to benzene rings. Chromophore are the colouring molecules which may be containing basic chromophore groups, including the azo group, azide group and indamine group. Azo group is found in all azo dyes. The dyes belonging to this group may be considered as azo benzene due to attachment of nitrogen atom to benzene ring (A.J. Salle,1993).There are two types of dyes, natural and synthetic dyes. Natural dyes are extracted from natural materials including tree barks, flowers, fruits etc. Natural pigments can also be extracted from micro-organisms like *Serratia spp., Caulobacter* spp. etc. In 1856 Sir William Henry Perkin discovered first purple synthetic dye. This lead revolutionary activities

in research and development of textile dyeing industry. Nowadays anthrquinone, acridin, triarylmethane, reactive dyes are major groups. Due to these different shades of dyes textile dyeing industry has grown up to most demanding one by customers.

Literature Review:

Water pollution is major threatening issue due to increased population and industrialization. Textile industry is most demanding financial sector worldwide due to fashion and increase in awareness among population in beauty industry. Effluents generated from various textile industries have toxic textile dyes, as a principle source and is one of the most environmental pollution factor(Cripps et al 1990 and Chanwala et al 2019).Worldwide annually there is 7 lakh tons of textile dyes produced , out of which 12% dyes are disposed in water resources as effluents (Artifon et al 2021).It has been studied that about 200 billion liters of effluents are produced annually worldwide which might contains about 72 different toxic chemicals .Maximum amount of water is needed for dyeing and printing.1.6 million liters of water is used for production of 8000 kg of fabric daily , out of which 16% is used for dyeing and 8% in printing (Kant et al 2012).Out of 80 % of worldwide of total production, 1,30,000 tons of dyestuffs production is contributed by Indian sectors due to increasing demand for polyester and cotton (Naik et al 2013).Released textile effluents in water bodies are showing highly detrimental effects causing severe environmental ill effects. Due to highly coloured effluents generated from textile effluents, there is less amount of sun lightenters in these water bodies leading to fatal effects on aquatic environment (Ghaly et al 2014). Along with recalcitrant textile dyes metal ions like Cr,As,Au and Zn are also contributing ill effects on environment. These heavy metals are non -degradable and may accumulated in food chains and gets resulted in creation of severe health conditions (Khan and Mallik et al 2017).When such dyes are accumulated in food chain , they may lead to diarrohea, liver neuromuscular hemorrhage, dermatitis, central nervous system disorders and kidney malfunctioning (USEPA 1999).This lead to increased demand for biological oxygen, reduced photosynthesis, reduced re-oxygenation which needed to be treated immediately (Holkar et al 2016) .Textile wastewater also pollutes soil .This leads to declination of soil fertility affecting the overall crop yield. Chances of soil pollution is much more in lower levels of lands than higher lands (Uddin 2018). Taking into consideration of all these hazardous effects of dyestuff recalcitrant, various physicochemical and Biological applications have been suggested. Literature survey shows that adsorption, coagulation or flocculation as common physicochemical processes for treatment of dye containing wastewater. Biological methods include use of variety of yeast, fungal and bacterial strains for removal of toxic chemicals from effluents. Microbial strains are available and handled easily. This makes use of micro-organisms as cheaper and ecofriendly approach. Use of enzymatic method is also one of the best method for textile effluent treatment but it is expensive as enzymes needs maintenance.

MATERIALS AND METHODS:

MATERIALS:

All textile dyes required for textile dyeing are procured from textile dyeing market from local area of Surat (Gujarat). Highly coloured textile effluents are also collected from small textile dyeing unit outlets from dyeing units of Kolhapur region. Soil samples were collected from nearby area of textile effluents in sterile containers, labelled and immediately stored under refrigeration conditions for screening of textile dye degrader strains.

METHODS:

Physico-chemical Characterization of textile effluents:

Textile effluents from Mangave textile M.I.D.C., Jaysinghpur, Kolhapur was aseptically collected in aseptic condition within sterile 3 liter container. Collected sample was stored under refrigeration condition for further analysis. Various physico-chemical parameters like Colour, pH, temperature, absorbance, conductivity and refractive index were analyzed. Same effluent sample was also further tested for presence of dye degrading microbial strains.

Screening, Enrichment and isolation of dye degrading microbial strains:

Aseptically 5ml sample of highly turbid textile effluents was inoculated in sterile 100ml nutrient medium supplemented with four different dyes at 50ppm concentration. This flask was kept for incubation at 48 to 72 hr at 37 ^{0}C. Periodically it was tested for dye degradation ability by streaking a loop ful of enriched broth on sterile dye containing plate.

Collected soil sample from textile effluents that were stored under refrigeration conditions were screened for presence of dye degrader strains. 1 gm soil sample was aseptically diluted up to 10^{-8} and 10^{-9}dilutions and pour plate technique with 10ppm each dye sample was followed and microbial colonies showing clear zone around were selected and preserved for further experimentation.

Identification of dye degrading isolates:

Preliminary identification like Grams staining, motility and biochemical tests were performed as per Bergeys Manual of Determinative Bacteriology. Further identification of strain was confirmed from National center for Microbial Resource for MALDI-TOF MS analysis.

Determination of dye degradation profile of selected dye degrading strains:

Four textile dyes including Proc Lemon H4G, Reactive red, Reactive black and Reactive blue were selected for degradation by the selected dye degrading strain. Synthetic wastewater with composition (gm/l) contains glucose 5 gm, urea 0.4 gm, KH2PO40.099 gm, $Cacl_2$ 0.5 gm and $MgSO_4$0.001 gm with 1ml of trace solution (pH =7) ($CuSO_4.7H_2O$: 0.08 gm, $MnSO_4.7\ H_2O$: 0.05 gm, $ZnSO_4.7H_2O$:0.04 gm).5% inoculum was added separately in each dye (50ppm and 100 ppm) containing artificial wastewater and such flasks were incubated at room temperature. Decolourization and degradation studies were recorded after 24 hr, 48 hr, 72hr and 96 hr of incubation period. Percent decolorization was calculated by following formula:

% Decolourization = Initial O.D. – Final O.D. / Initial O.D. x100

Confirmation of dye degradation:

Degradation of synthetic waste water containing each textile dye were tested for thin layer chromatography. For this purpose degraded dye samples were centrifuged and supernatant was tested for thin layer chromatography. To confirm degradation, uninoculated dye samples were also tested along with degraded samples for thin layer chromatography. TLC silica gel 60 F254 25 aluminium sheets were used for analysis. Hexane: acetone (3:2) was used as a solvent system. Rf values were calculated for each respective dyes.

Decolourisation of original textile effluent with mixture of textile dyes at 100 ppm:

Degradation of each textile dye at 50 ppm level at laboratory level was studied. In order to study, mixture of textile dyes in sterile artificial waste water was studied. Both the strains

showing maximum decolourisation of dyes were inoculated at 5% inoculum and incubated from 24 hrs to 96 hr. At each incubation level percent decolourisation was studied.

Impact of decolorized dye wastewater on microbial growth:

The effect of microbial treated wastewater was determined on agriculturally useful microflora like *Azotobacter ,Rhizobium* and *Bacillus cereus spp.* Disc diffusion technique was followed for testing effect of dye containing control and dye degraded sample .Plates were incubated at 37^0C for 24 to 48hr and zone of inhibition was examined .

Impact of decolourised dye wastewater on seed germination rate:

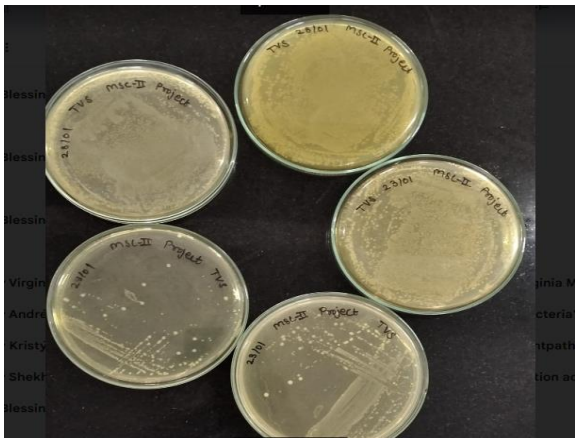
For study of toxicity assay of degraded textile effluent samples, healthy grains *Triticum aestivum* (Wheat) were selected. Ten wheat seeds in three sets were washed with sterile saline and were placed on sterile petri plates covered with filter paper. These seeds were provided with 2ml distilled water (Reference), control (untreated synthetic waste water with dyes) and test (synthetic waste water treated with selected microbial strain).All plates were placed at room temperature for a week and were provided with 2ml of respective solution after each consecutive day. The impact of reference, control and test sample on length of root and shoot was recorded and germination rate of *Triticum aestivum* was calculated by using formula:
% germination = Number of seeds germination / Number of seeds sown x100

Results and Discussion:
As huge amount of waste water get generated from domestic sites as well as from various industries, including textile effluents. Management of such wastewater generated is a very difficult task. This wastewater creates lots of environmental problems. To minimize effects of these wastewater effluents on surrounding environment, treated of waste water is mandatory for textile dyeing units before disposal in water bodies. Waste water sample collected from Jaysighpur , Kolhapur site was analyzed for its various physicochemical characteristics.

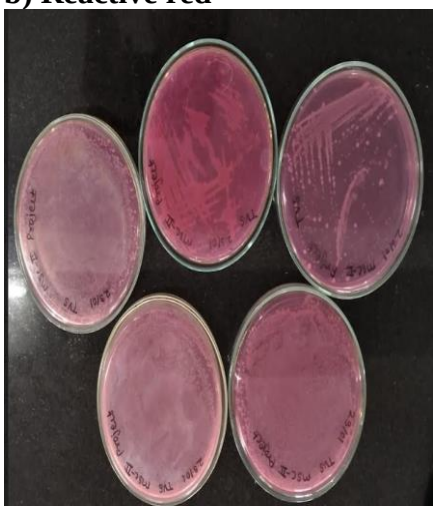
Screening, Enrichment and isolation of dye degrading microbial strains:
Each enriched flasks with 50 ppm textile dye sample, pour plate technique on 10 ppm textile dye containing media was followed. Rapid depletion of dye sample from plate was seen after 24 hrs of incubation .Such plates having microbial colonies were re-streaked on dye containing slants repeatedly for studies of degradative activities of selected strains. Seven such cultures were properly preserved under refrigeration conditions for further experimentation of textile dye degradation.

***Fig 1*: Screening and isolation of textile dye degrading strains**

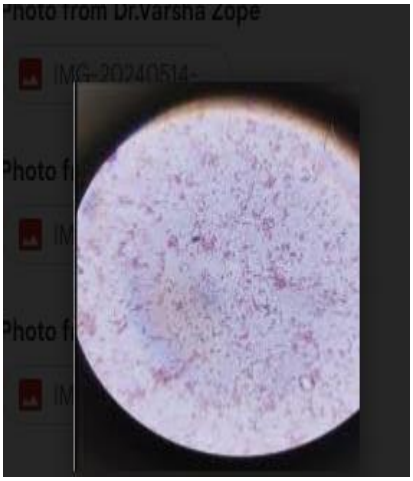
a) Proc Lemon Yellow H4G **c) Reactive Blue**



b) Reactive red



Grams Staining of the isolate



Identification of dye degrading isolates:

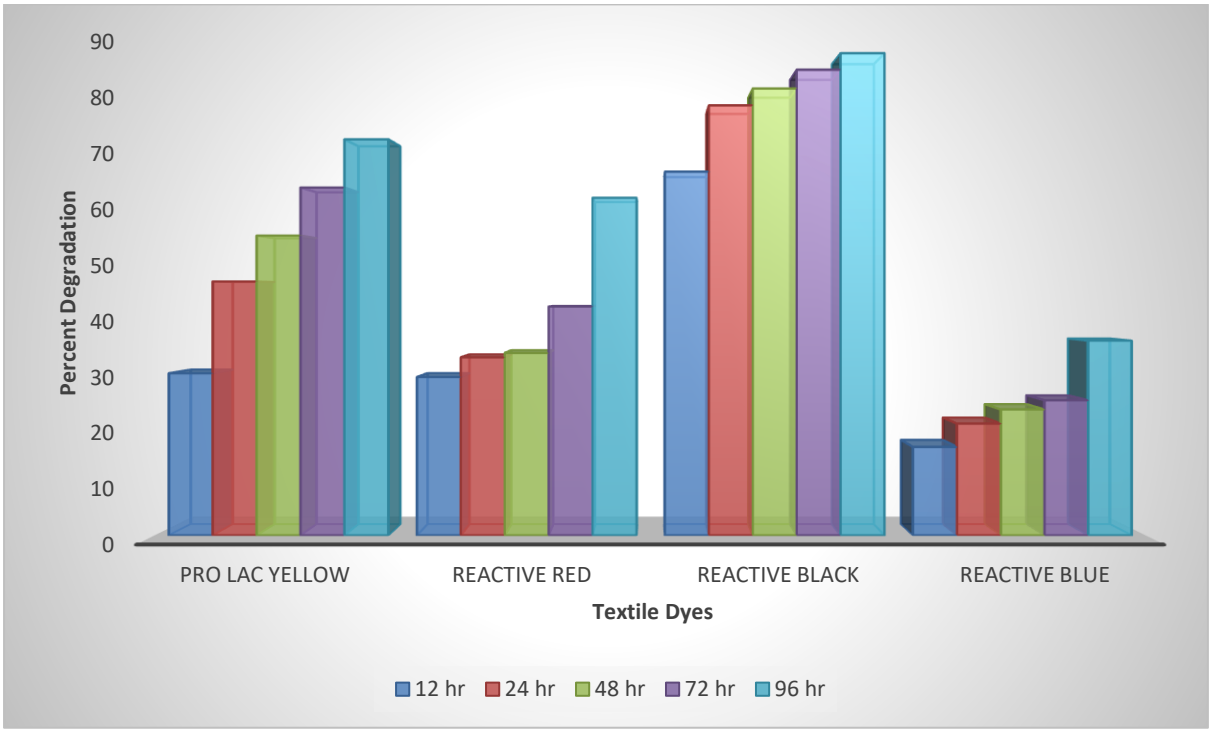
For preliminary identification of two isolates out of all total seven isolates, different lab techniques such as morphological characteristics, Grams staining, motility and various biochemical tests were performed. For this standard format of Bergys Manual of Determinative Bacteriology was followed. These two samples were further identified from National center for Microbial Resource for MALDI-TOF MS analysis.After following identification protocols, sample 1 and sample 2 were identified as *Enterobacter cloacae* 13159_1CHB. The second sample was was analysed as *Pseudomonas spp.*

Confirmation of dye degradation:

Dye degradation of *Pseudomonas* species was confirmed with the help of spectrophotometer. Each dye sample was inoculated at 50 ppm concentration in minimal media artificial synthetic waste water. Un-inoculated flasks having 50 ppm and 100 ppm each dye was used as control. Inoculum at 5 % concentration was added and after 24 hr, 48hr, 36hr and 96 hr of incubation. After incubation is over at each stap 10 ml sample was applied for centrifugation at 10000 rpm for 15 minute. Supernantant was analysed for spectrophotometric analysis. O.D. of control and degraded proc Lemon Yellow H4G reading was taken at 450nm, Reactive red at 505 nm, Reactive black at 505 nm, Reactive blue at 630nm.

Readings of control and test samples were taken at these respective wavelengths and percent degradation was calculated. All the experiments were carried out in triplicates. It was found that after 12 hrs of incubation Proc lemon yellow sample percent degradation was 30 percent. After 24 hrs showed 47 % degradation at 50 ppm concentration after 48hrs degradation was 55.5 %. After 72 and 96 hrs of incubation proc lemon yellow was degraded by *Pseudomonas spp*.at 64.4% and 73.33% respectively (Fig 2).

***Fig.2*: Percent degradation of four textile dyes after 12 hrs, 24 hrs, 48hrs and 72 hrs of incubation**



Reactive red sample dye was degraded after 12 hrs, 24hrs, 48 hrs, 72 hrs and 96 hrs of incubation with *Pseudomonas spp*... Percent degradation was found to be 29.3 % 32.94 %, 33.75 %, 42.35 % and 62.5 % respectively. Reactive black dye sample, after inoculation with the test organism *Pseudomonas spp.*, percent dye degradation was found to be 67.34%, 79.62 %, 82.75 %, 86.20 %, and 89.28 % respectively after 12hrs, 24 hrs, 48 hrs, 72 hrs and 96 hrs of incubation. After 12 hrs, 24 hrs ,48 hrs, 72 hrs and 96 hrs of incubation with *Pseudomonas spp*. reactive blue dye was found to be 46.57 %, 50.63%, 51.89 %, 58.44 % and 68.83 % degraded. Reactive blue after 12, 24, 48, 72 and 96 hrs shows 16.36, 20.68, 23.3,25 and 36.03 percent.

All spectrophotometric assays of degradation percent were found to be in between 29.3 % to 89.28 %.This confirmed degradation of textile dyes.

Confirmation of textile dyes degradation was also done with the help of thin layer chromatography. Each dye control sample and incubated dye sample centrifuged was applied on TLC silica gel 60 F 254 plates with the help of small capillary. These silica plates were DC kieselgel 60 F254, CCM gel de silica 60 F 254 plates made in Germany. Hexane: acetone at 3:2 concentrations was used as solvent system for each dye. Rf value was calculated for each textile dye with its degraded sample. Table indicates Rf values of each dye control and its degraded test sample. For Proc lemon yellow sample Rf value was 0.81 and its test sample showed Rf value of 0.72.Rf value of reactive red control was 0.77 and its test sample was 0.69.

Rf value of reactive black control was 0.85 and its degraded sample Rf value was 0.79. While Reactive blue control Rf value is 0.84 and its test sample was 0.42. This indicates confirmation of degradation of selected textile dyes samples.

Decolourisation of original textile effluent with mixture of textile dyes at 50 ppm:

After 12 hrs 24 hrs, 48hrs, 72 hrs and 96 hrs of incubation of 50 ppm each textile dye in mixture with sterile artificial waste water, percent degradation was studied. For confirmation of degradation spectrophotometric analysis was carried out. For studies of degradation λ max of artificial waste water containing textile dyes was determined. It was found to be 505 nm. Decolourised sample was checked for its optical density and percent decolourisation was calculated. After 12 hrs , 24 hrs decolourisation was 10 and 18.8 percent,48 hrs decolourisation was 23.9 percent, after 72hrs of incubation decolourisation was 30.9 percent and after 96 hrs of incubation decolourisation percent was found to be 33.8 percent.

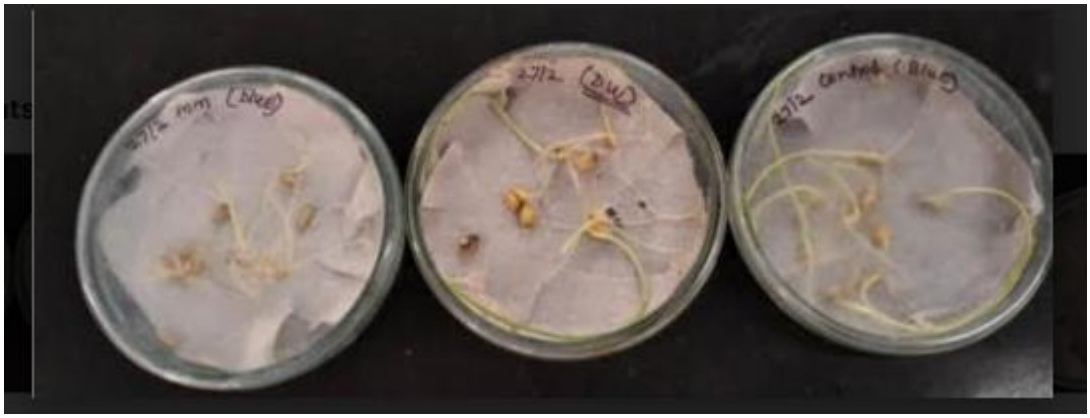
Impact of decolorized dye wastewater on microbial growth:
The impact of *Pseudomonas* sample treated each textile dye sample with its control was determined on agriculturally important microflora including *Azotobacter spp., Rhizobium spp.* and *Bacillus spp.*Disc diffusion method was followed on sterile nutrient agar plate inoculated each with *Azotobacter*, *Rhizobium* and *Bacillus spp.* Although the control samples showed zone of inhibition after 24 hrs of incubation, *Pseudomonas* treated each dye sample when tested on nutrient agar plates, absence of zone of inhibition indicates that degraded textile dye sample is not harmful to agriculturally important microflora.

Impact of decolourised dye wastewater on seed germination rate:

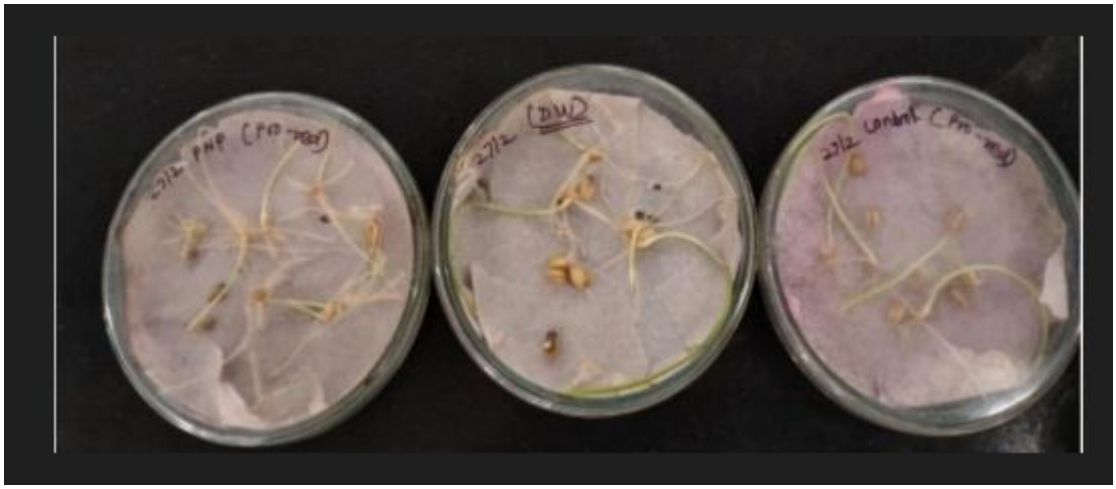
For toxicity assay of degraded textile dye sample wheat grains were tested. % germination of these wheat grains was calculated. *Pseudomonas* treated sample showed percent germination of seeds in between the range of 30 to 70%. Pro-lac yellow (Fig 3 A) and reactive black (Fig. 3 C) degraded samples shows 30% seed germination. Reactive blue degraded sample showed 50% seed germination (Fig 3 B) while reactive red treated sample showed 70% seed germination (Fig 3 C).

***Fig.:3*: Impact of decolourised dye wastewater on seed germination rate:**

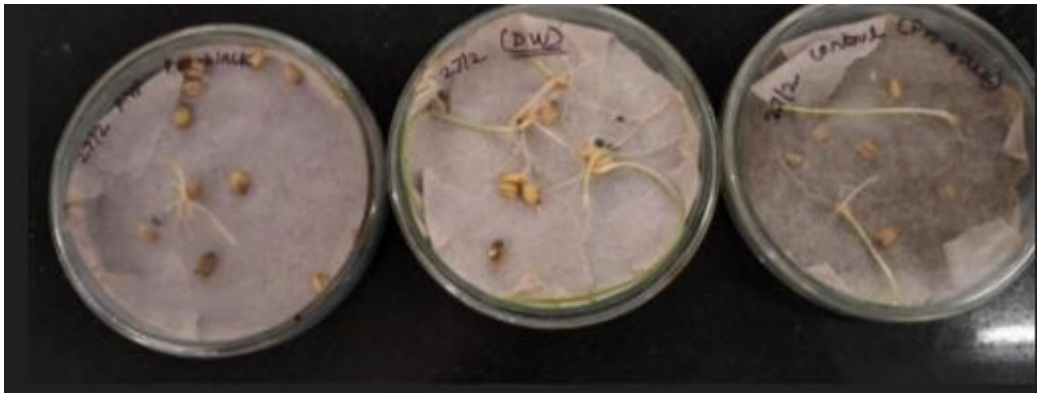
A) Seed germination assay with degraded product of Pro Lac Yellow



B) Seed germination assay with degraded product of Reactive blue



C) Seed germination assay of degraded product of Reactive red



D) Seed germination assay of degraded product of Reactive black

DISCUSSION:

Subhashini and Thamizhmani Vivek (2020), studied textile dye direct orange 102 by *Pseudomonas flouroscence* at concentration between 1ml/100ml to 5 mg/100ml showed degradation under agitation condition between 71 to 78 % while same dye under static condition showed dye degradation to be in between 88 to 92 %. After our experimentation, it was found that after 12 hrs of incubation Proc lemon yellow sample percent degradation was 30 percent. After 24 hrs showed 47 % degradation at 50 ppm concentration after 48hrs degradation was 55.5 %. After 72 and 96 hrs of incubation proc lemon yellow was degraded by *Pseudomonas spp.*at 64.4% and 73.33% respectively. Reactive red sample dye was degraded after 12 hrs, 24hrs, 48 hrs, 72 hrs and 96 hrs of incubation with *Pseudomonas spp...* Percent degradation was found to be 29.3 % 32.94 %, 33.75 %, 42.35 % and 62.5 % respectively. Reactive black dye sample, after inoculation with the test organism *Pseudomonas spp.*, percent dye degradation was found to be 67.34%, 79.62 %, 82.75 %, 86.20 %, and 89.28 % respectively after 12hrs, 24 hrs, 48 hrs, 72 hrs and 96 hrs of incubation. After 12 hrs, 24 hrs ,48 hrs, 72 hrs and 96 hrs of incubation with *Pseudomonas spp*. reactive blue dye was found to be 46.57 %, 50.63%, 51.89 %, 58.44 % and 68.83 % degraded. Reactive blue after 12, 24, 48, 72 and 96 hrs shows 16.36, 20.68, 23.3,25 and 36.03 percent.

Bera S. and Tank S.K. (2021) also demonstrated phytotoxicity assay of dye effluent and its degraded product against germination capacity of mung beans and Bengal gram. After experimentation they found the germination rate of both seeds as 80 % to 90% respectively. *During* our experimentation *Pseudomonas* treated sample showed percent germination of seeds in between the range of 30 to 70%. Pro-lac yellow and reactive black degraded samples shows 30% seed germination. Reactive blue degraded sample showed 50% seed germination while reactive red treated sample showed 70% seed germination. We studied only dye decolourisation and its phytotoxicity and microbial toxicity was tested under laboratory condition. In terms of future prospectus, work on actual nature of metabolites, their shelf life should be studied in details in order to strengthen the textile dye removal technology.

CONCLUSION:

Microbial degradation of various textile dyes was studied for treating textile effluents by economical and efficient method. After collecting textile effluents its basic physicochemical characteristics were studied. Colour of effluent sample was faint pink colour with normal temperature of 27^0C, pH :7.3, conductivity is 5,11 and 20 ms with refractive index of 0.334.This was indicative to work for removal textile dyes from textile effluents. Screening and isolation of textile dyes was carried out. Seven isolates were analyzed. Out of seven two were selected for identification due to their maximum dye degradation abilities. They were analysed as *Enterobacter spp,* and *Pseudomonas spp.* Due to pathogenic nature of *Enterobacter spp.*, it was not used further for degradation studies. *Pseudomonas spp.* was selected for further textile dye degradation studies. All the textile dyes were taken at 50 ppm individually as well as in artificial wastewater with mixture of all dyes. Degradation of textile dyes was recorded as 29 to 89.28 %. Percent degradation was confirmed with Spectrophotometric analysis as well as thin layer chromatography techniques. Studies on effect of degraded dye samples on agriculturally important micro-organisms shows that degraded sample is nontoxic towards these microbial strains. It shows good results for germination of seeds. Thus present experimentation after further studies on chemical nature of metabolite studies could be helpful for removal of textile dyes from textile effluents

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