2. EXPERIMENTAL:

This work is concerned with the study of dye wastewater collected from Carpet industries, Bhadohi, and Uttar Pradesh, India. The physico-chemical characteristics of dye wastewater were studied. The soil samples collected from different sites of dye contaminated soil utilized for the isolation of different bacterial strains. The isolated bacterial strains were utilized for dye degradation. There are various parameters optimize for efficient degradation of dye wastewater. The dye and its degraded products were studied using UV-Visible Spectrophotometer, FTIR and HPLC. The degradation pathway was studied by using GC-MS spectra. The mixed consortia isolated from dye contaminated soil and utilize in packed bed reactor for complete mineralization of dye wastewater. Details of the chemicals and procedures employed are given in the following paragraphs.

2.1 Collection and Characterization of Dye Wastewater

The dye wastewater collected from dye contaminated area, Carpet industries, Bhadohi, Uttar Pradesh, India were large amount of different dye effluents are being discharged was collected and use for characterization of dye wastewater. The dye wastewater was collected from effluent coming from various Carpet Industry, Bhadohi, and Uttar Pradesh, India and stored in ice berth to maintain its properties. The wastewater samples were filtered through GFA glass-fiber filters before the absorbance was measured on spectrophotometers (Systronics-2202) during the first part of the study with synthetic wastewater. The physico-chemical analysis of the dye wastewater was

shown in *Table 2.1*. The wastewater filtered through screen filter to remove suspended solids. The pH of each solution was measured by using microprocessor based pH meter (ELICO LI614). The temperature was measured by using thermometer. The chemical oxygen demand (COD) of wastewater was measured by using COD digester system (UNIPHOS D-0107). Total alkalinity and Total Suspended Solids (TSS) were determined according to the procedures outlined in standard methods [APHA, (1998)]. The color of influent and effluent was determined spectrophotometrically by UV-Visible Spectrophotometer (Systronic-2220). The samples were filtered by micro-fiber filter and centrifuged at 10000 rpm for 10 min prior to absorbance measurements [Rui *et al*. (2001) Mendez-Paz *et al.* (2005)]. Color removal was determined based on the maximum absorbance of UV-Visible spectrum.

Table 2.1: Showing physico-chemical characteristics of dye wastewater collected from different sites from Carpet Industries, Bhadohi and Uttar Pradesh, India.

Parameters	Average Range
pH	6-11
Temperature	30-45 °C
Total suspended Solids	250-28000 mg/l
Total Dissolved Solids	2900-18000 mg/l
Chemical Oxygen Demand	350 – 65000 mg/l

2.2 Collection of soil sample from dye contaminated sites

Bhadohi - Mirzapur belt is located at 25.3873° N and 82.5680° E in the southern part of Uttar Pradesh (*Figure 2.1*). There are more than 3000 carpets manufacturing units recorded in India of which more than 1000 are located in this region. This industry is

endowed with a large and diversified production base and accounts for around 80% of activities pertaining to production and export. Soil collected from dye contaminated area, Carpet Industries, Bhadohi, Uttar Pradesh, India were large amount of different dye effluents are being discharged for isolation of bacterial colonies for degradation of dyes wastewater. The bacterial consortia for degradation of Scarlet 4BS and RED G dye were isolated from the soil from the drain caring dyeing effluent of various industries.

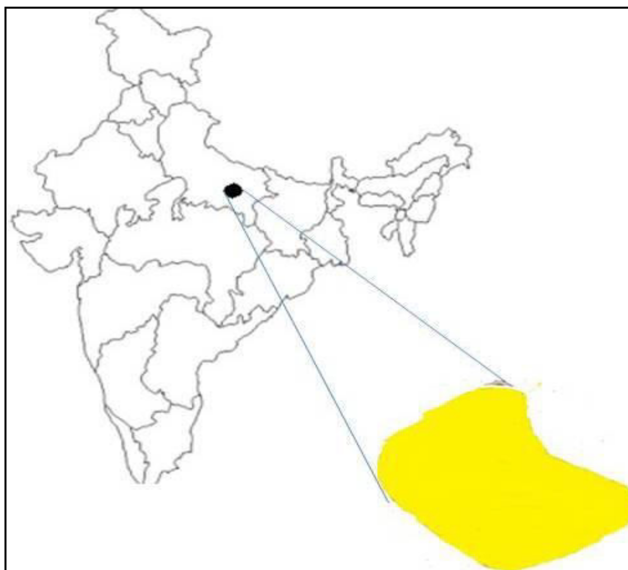


Figure 2.1: Geographical location of the study site.

2.3 Isolation, Screening and Identification of Dye Degrading Bacteria

2.3.*1 Media and Chemical*

The various dyes Navy N5RL1 , Acid Red G (C.I. Acid Red 1) and Scarlet 4BS (C. I. Direct RED23), was procured from Amit Carpet Industries Limited, Sant Ravidas Nagar (Bhadohi), Uttar Pradesh, India. The mineral salt media (MSM media) contained the following compounds (mg/L): $MnSO_4$ H_2O (0.155), $CuSO_4$ $5H_2O$ (0.285), $ZnSO_4$

$7H_2O$ (0.46), $CoCl_2$ $6H_2O$ (0.26), $(NH_4)_6$ Mo_7O_{24} (0.285), $MgSO_4$ $7H_2O$ (15.2), $CaCl_2$ (13.48), $FeCl_3.6H_2O$ (29.06), NH_4Cl (190.9), KH_2PO_4 (8.5), $NaHPO_4$ $2H_2O$ (33.4), K_2HPO_4 (21.75). The synthetic nutrient media used for culturing the bacteria was purchased from Hi-Media, India. Nutrient broth and nutrient agar were obtained from Hi-Media, India. All the solvents used in the HPLC and FTIR were of HPLC grade (Fisher Scientific). All other chemicals used were of analytical grade. The seeds Vigna mungo, Phaseolus aureus, Sorghum vulgare and Phaseolus mungo used for phytotoxicity studies were obtained from local market. NADH, NADPH and 2, 6-Dichloro-phenol indophenol (DCIP) were procured from Sigma Aldrich for enzyme study. Anthraquinone-2, 6-disulfonate (AQDS) (Aldrich) was used as redox mediator model compound, without additional purification.

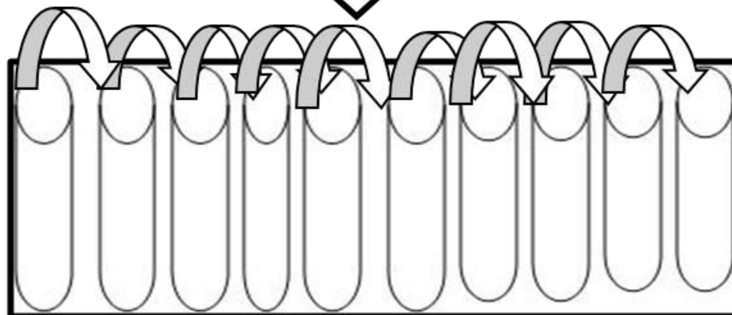
2.2.2 Isolation of bacterial strains

Soil collected from dye contaminated area, Carpet industries, Sant Ravidas Nagar (Bhadohi), Uttar Pradesh, India were large amount of dye effluents are being discharged for isolation of bacterial colonies for degradation of dye wastewater. For bacterial isolation, 1g of soil was mixed in 100 ml in basal media contained the following compounds (mg/L): $MnSO_4$ H_2O (0.155), $CuSO_4$ $5H_2O$ (0.285), $ZnSO_4$ $7H_2O$ (0.46), $CoCl_2$ $6H_2O$ (0.26), $(NH_4)_6$ Mo_7O_{24} (0.285), $MgSO_4$ $7H_2O$ (15.2), $CaCl_2$ (13.48), $FeCl_3.6H_2O$ (29.06), NH_4Cl (190.9), KH_2PO_4 (8.5), $NaHPO_4$ $2H_2O$ (33.4), K_2HPO_4 (21.75) per litter for isolation of bacterial strains. The nutrient was supplemented with 500 mg/l Navy N5RL1 dye and RED G dyes incubated at 30 ± 2 °C in an orbital shaker (100 rpm) as well as under static condition for 48 h to enrich the dye-degrading bacterial

population (*Figure 2.2*). One milliliter of the culture was withdrawn after one week of incubation and it was serially diluted. 0.1 ml samples were withdrawn from various 10^{-1} to 10^{-10} dilution and these were incubated on nutrient agar plate containing 500 mg/l NavyN5RL1 dye and other various dyes. These plates were incubated till appearance of morphologically distinct bacterial colonies. The selected colonies were further streaked on fresh nutrient agar plates for isolating the pure strains. Selected fast-growing bacteria were again streaked over the fresh nutrient agar plate. For further utilization, these bacterial colonies were stored in nutrient agar slant at 4 °C and glycerol stock at -20 °C. The bacterial strains were identified on the basis of morphologically, biochemically as well as through 16S rRNA gene sequence analysis. Gram staining and biochemical test of isolated bacterial strains were performed according to the Bergey's manual and on the basis of 16S rRNA gene sequence analysis.

Soil (1gm) + Dye (500 mg/l concentration)
+ Mineral Salt Media (100 mg/l)

After Degradation (one week)



10^{-1} 10^{-2} 10^{-3} 10^{-4} 10^{-5} 10^{-6} 10^{-7} 10^{-8} 10^{-9} 10^{-10}

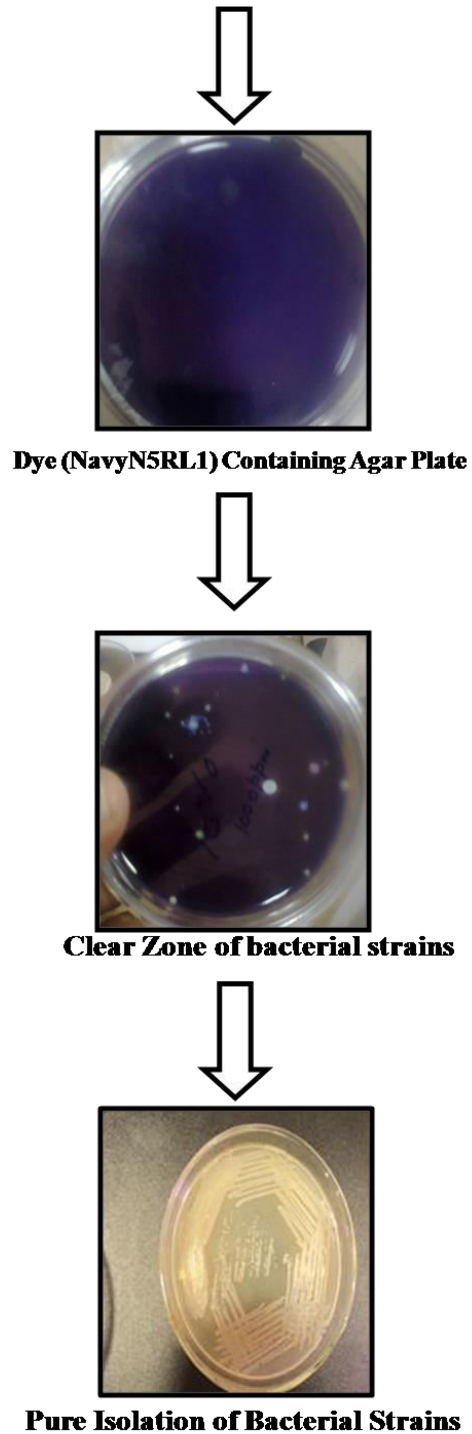
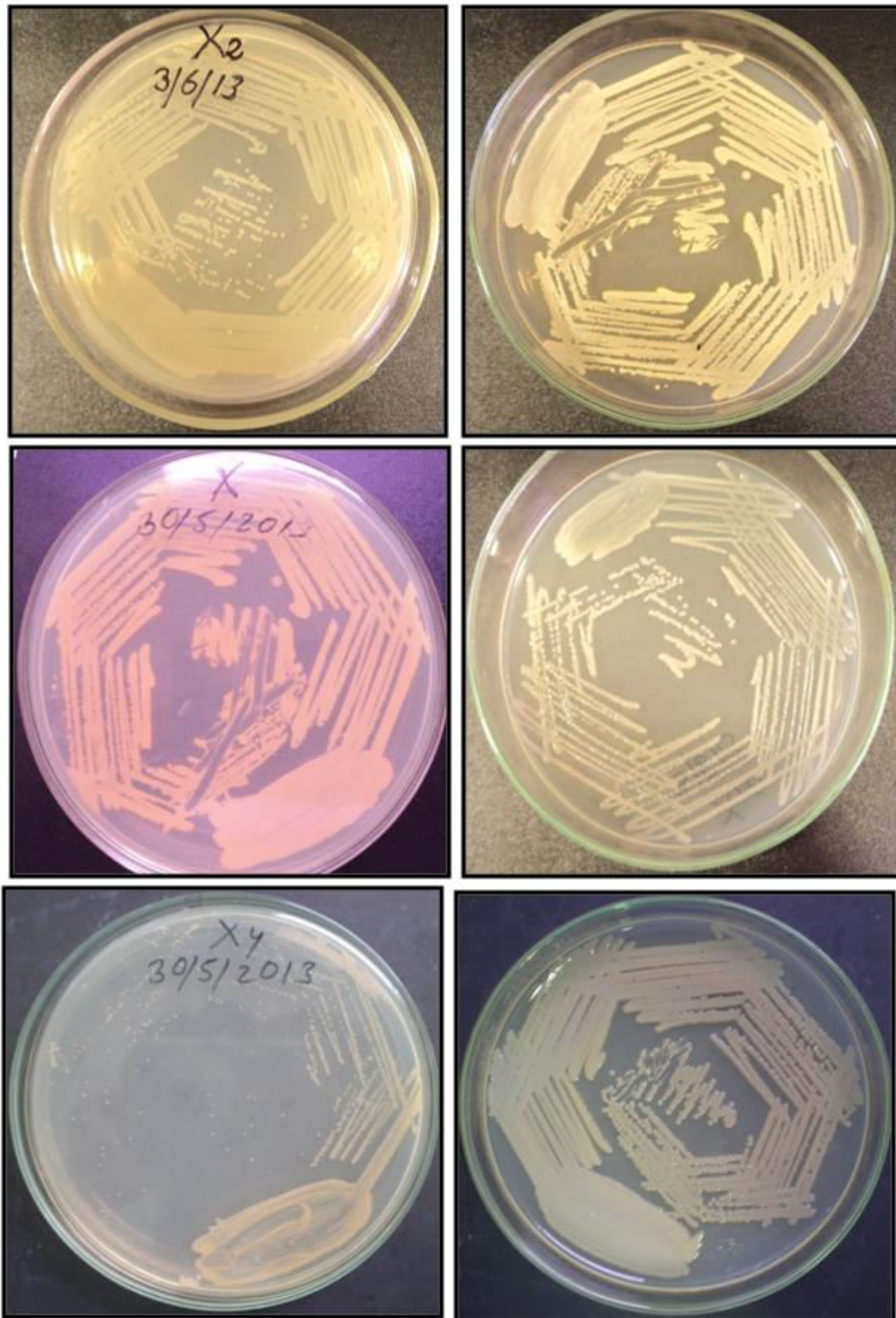


Figure 2.2: Showing the step for isolation of bacterial strains from dye contaminated soil.


X2
3/6/13
X
X4
30/5/2013

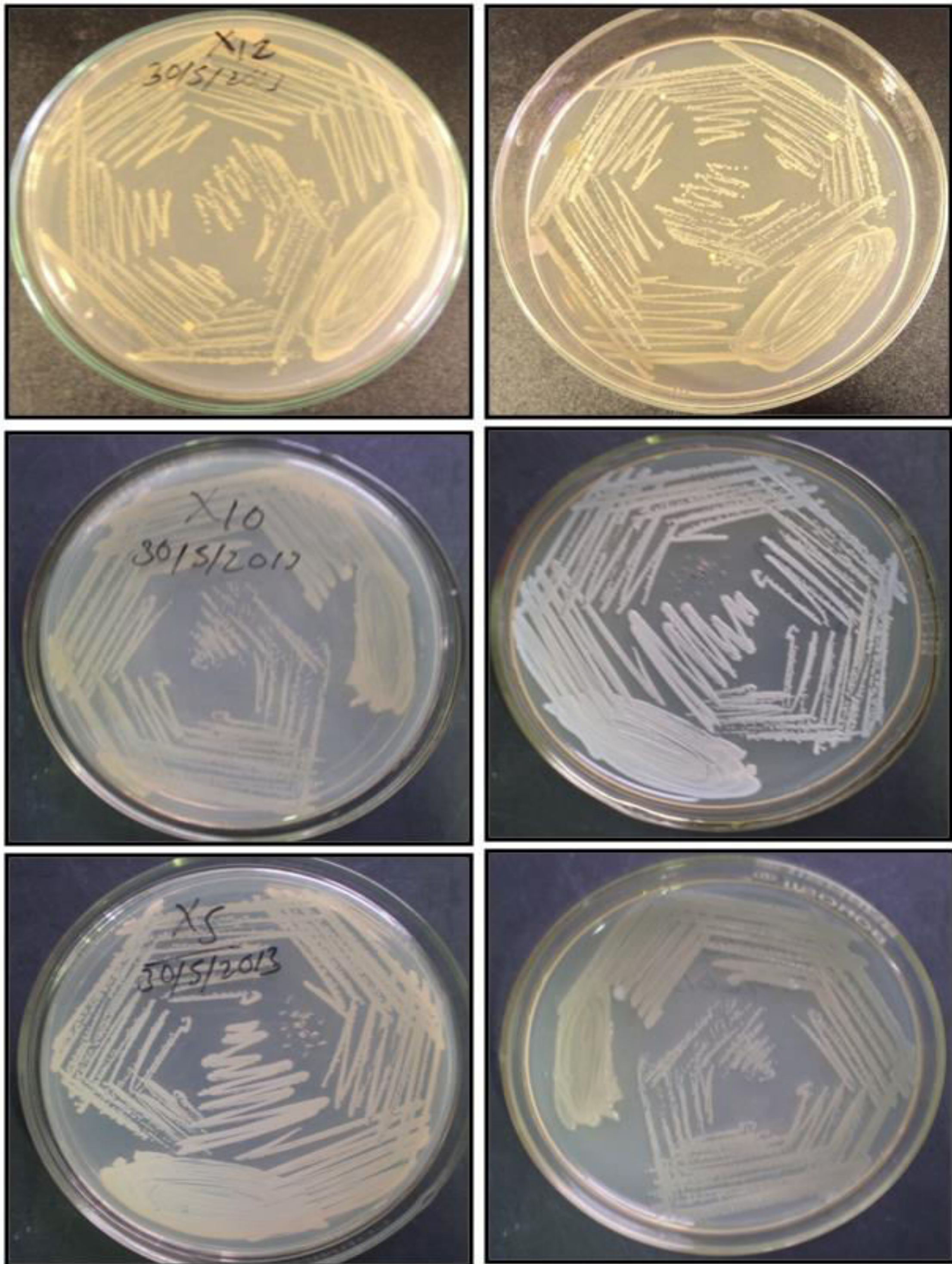

X12
X10
X5
30/5/2013

Figure 2.3: Showing the different bacterial strains isolated from different dye contaminated soil.

2.2.3 PCR amplification and DNA sequencing of the 16S rRNA gene

The PCR amplification and DNA sequencing of 16S rRNA of the isolated bacterial strains was carried out as follows. For 16S rRNA gene sequence analysis, overnight grown bacterial cultures in nutrient broth were centrifuged at 10,000 rpm for 10 min. The pellets were washed with 1% Triton-X buffer and treated with proteinase K for digestion (5 µl in 1 % SDS, 55 °C for 1 h). The bacterial DNA isolation was done by CTAB method. For this, bacterial cell pellets were mixed with 1% CTAB and 0.7 M NaCl and incubated in water bath at 65 °C for 10 min. The mixture was cooled and mixed with phenol:chloroform:IAA (25:24:1) in vortex for 15 sec and then centrifuged at 10,000 rpm for 10 min. The supernatant thus collected was mixed with equal volume of chloroform:IAA (24:1). One milliliter of isopropanol was added to it and resultant

solution was kept for 10 min at room temperature (25 °C). The pellets thus obtained were washed with 70% ethanol, dissolved in TE buffer and stored at -20 °C for amplification of DNA using PCR. PCR was performed using Taq DNA polymerase and 16S universal primers ('NF5GGCGGCAKGCCTAAYACATGCAAGT3') and('NR5GACGACAGCCATGCASACCTGT3'). The PCR amplified products were purified by utilizing the purification kit (Hi-Media) prior to DNA sequencing. The PCR sequence was used for the analysis of 16S rRNA gene sequence analysis (CDRI Hyderabad, India). The resultant 16S rRNA sequence was analyzed using BLAST of NCBI for identifying closest relative of the bacteria and the sequence was then deposited in Gene Bank. The sequence was compared using BLAST programmed at NCBI server to identify bacteria. Amplified PCR products after purification were sequences aligned with those of reference microorganisms existing in the same region of the closet relative strains available in the Gene Bank database using the BLAST facility and were also tested for possible with the Neighbor-Joining phylogenetic tree were constructed with the MEGA version 6 software. A bootstrap analysis with 500 replicates was carried out to check the robustness of the tree. The 16S rRNA sequence of isolated bacterial strain and related sequences of NCBI were aligned using Cluster W, and phylogenetic tree was made using neighbor-joining methods of MEGA (Version 6).

2.2.4 Nucleotide Sequence Accession Number

The various sequences were submitted to the NCBI of Nucleotide Sequence Data Base (Gene Bank Public Database) for the accession number.

2.3 Inoculum Preparation

100 ml of MSM and one loopful bacterial strain were incubated at 100 rpm and 30°C for 24 h. 4% (v/v) actively growing culture having 10^7cells/mL were used as inoculums in all further experiments. 0.1 ml was subsequently used as inoculum to study the effect of various factor conditions for decolorization studies. The un-inoculated flask kept under identical conditions was used as control. Samples (2 ml) were withdrawn at regular interval (2hr) and centrifuge (10000g, 15 min). The supernatant was analyzed for dye decolorization by double beam UV-Visible spectrophotometer (Systronics-2202).

2.4 Optimization of various parameters for efficient degradation of dye (Batch Mode under Static condition)

The decolorization experiments were carried out in 250 ml Erlenmeyer flasks containing 100 ml nutrient broth supplemented with dye (200 mg/l). The media were inoculated with respective bacterial strains by addition of inoculums with uniform cell density (O.D. 0.5). The processes parameter optimization for Navy N5RL1 dye by *Staphylococcus saprophyticus* BHUSS X3 were static/shaking (100 rpm) condition, pH, initial dye concentration and temperature. The pH was adjusted by using 0.01N NaOH and 0.01N HCl in the range of (6-11) to study the effect of pH. The decolorization study using different initial dye concentrations (50-1000 ppm) and temperature (15-45°C, 5°C interval), initial dye concentration (100–1000 mg/l), various different carbon and nitrogen sources, and inoculums size (1, 2, 3, 4, 5, 6, 7, 8, 10 %) were optimized. Each experiment was performed in triplicate. The un-inoculated flask was kept under similar conditions for control. These investigations were also used for control. Aliquots of 2.0

mL from experimental and control medium were withdrawn at regular intervals of 2 h upto 24 h or till complete decolorization was observed and centrifuged at 10000 rpm for 15 min at 4 °C to obtain a clear supernatant. The supernatant was analyzed for dye decolorization using a double beam UV-Vis spectrophotometer (Systronic 2202). The percentage of decolorization was calculated from the difference between initial and final absorbance using equation 2.1.

$$\% \textbf{ Decolorization} = \frac{\textbf{Initial OD} - \textbf{Final OD}}{\textbf{Initial OD}} \times \mathbf{100} \qquad (\mathbf{2.1})$$

2.4.1 Decolorization at static and shaking conditions

The isolated bacterial strains were grown for 24 h at 35 °C in 250 ml Erlenmeyer flasks containing 100 ml nutrient broth to study the effect of static and shaking conditions (100 rpm) on decolorization performance of microbial culture. After 24 h, 100 mg/l dye was added in each and incubated at static as well as shaking condition at 35 °C for 100 rpm on orbital shaker. The aliquot (3 ml) of the culture media was withdrawn at different time intervals, centrifuged at 5000 rpm for 15 min at 4 °C. Decolorization was monitored by measuring the absorbance of culture supernatant at various dye wavelengths by using UV-Visible Spectrophotometer (Systronic 2202).

2.4.2 Effect of pH

To determine the effect of pH on decolorization by isolated bacterial strain was cultivated for 24h in conical flaks containing 100 ml nutrient broth of varying pH (5-11) was amended with 100 mg/l of dye concentration at temperature 35 °C and under static condition.

2.4.3 Effect of Temperature

To study the effect of various temperatures for efficient dye degradation was determined by evaluating the dye decolorization at 20, 25, 30, 35, 40 and 45 °C.

2.4.4 Effect of initial dye concentration

In order to examine the effect of initial dye concentration on the decolorization in static condition as well as shaking condition, nutrient medium was added with 100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000 mg/l of various dyes. The percentage of decolorization was measured at different time interval. All decolorization experiments were performed in triplicates. Abiotic controls (without microorganism) were always included.

The percentage decolorization was calculated as follows:

$$\% \ \mathbf{Decolorization} = \frac{\mathbf{Initial\ Absorbance - Final\ Absorbance\ \times 100}}{\mathbf{Initial\ Absorbance}} \qquad (\mathbf{2.2})$$

2.4.5 Effect of various carbon and nitrogen sources

Mineral Salt Medium (MSM) used for this particular study to check the dye decolorization efficiency of microorganism in the presence of various different carbon and nitrogen substrates. The MSM media supplemented with yeast extract to study the effect of carbon (glucose, starch) and nitrogen (yeast extract, ammonium chloride, urea, and peptone) sources at the concentration of 5.0 g/L on the decolorization of (200 mg/l). In addition to effect of synthetic carbon and nitrogen sources, agricultural waste extracts

were studied on decolorization of RED G in MSM medium (5 mL extract of 10 g/ L boiled agricultural residue).

2.4.6 Investigation on capability of isolated microbe for repeated use

A repeated batch of 100-mg/l Red G dye was repeatedly treated at 35 °C under static conditions by using bacterial stain BHUSSp X2. The process was repeated for ten times.

2.5 Preparation of cell-free extract and enzyme assays:

The isolated bacterial strain BHUSSp X2 was incubated with Red G (200 mg/l) and without dye for 24 h. The resultant biomass was collected by centrifuging the respective broths at 8000 rpm and 4 °C for 15 min. The resultant cell pellets were sonicated using ultrasonication probe in 50-mM () sodium phosphate buffer (pH 7.4) at 4 °C, with 12 strokes of 30s, for 1 min intervals each. The sonicated cells were centrifuged and supernatant was used as the source of intracellular and extracellular enzymes. The enzymes activities were assayed spectrophotometrically for cell-free extracts with and without sonication at room temperature. One unit of enzyme activity is the amount of enzyme required to convert 1μmol of substrate per min. Azoreductase assays were performed by taking 200 μl of 2 mM NADH, 100-mM sodium phosphate buffer (7.5), 150-μM substrate concentration, and 100-μl enzyme solution. The change in color intensity was monitored by UV-Visible spectrophotometer (Systonic 2022) at 500 nm. The activity of NADH-DCIP reductase was determined by processes reported earlier by [Lade *et al*. (2012)]. NADH-DCIP reductase activity that was analysis of 2 ml reaction mixture was assayed at 500 nm by adding 250 mM NADH, 5 ml reaction mixture

containing 25 mM 2,6-Dichloro-phenol indophenol (DCIP) and 200-µl enzyme solution in potassium phosphate buffer (50 mM, pH 7.4). Tyrosinase activity was measured as described elsewhere [Duckworth and Coleman (1970)]. Laccase and lignin peroxidase activities were assayed using spectrophotometer in the cell-free extract. Laccase activity, measured as an increase in optical density at 420 nm, was determined in 2 ml mixture containing ABTS (10 %) in 0.1 Macetate buffer pH 4.9 [Hatvani and Mecs (2001); Saratale *et al*. (2009); Kalyani *et al*. (2008)]. Lignin peroxidase (LP) activity was determined by monitoring the propanaldehyde formed at 300 nm in a reaction mixture of 2.5 ml containing 100 mM n-propanol, 250 mM tartaric acid, and 10 mM H_2O [Telke *et al*. (2009)]. All enzyme assays were carried out at room temperature. All enzyme assays were run in triplicate, and the average activity was calculated.

2.6 Phytotoxicity studies

The investigations were made to estimate the phytotoxicity of dye effluents prior to and after degradation. The germination characteristics of gram seeds in pure water, effluents and extracted metabolites were observed and summarized. The phototoxicity of Navy N5RL1 dye was assessed before and after bacterial degradation of the dye in the concentration range of 100 and 1,000 mg/l using Phaseolus aureus seeds. A piece of sterilized filter paper was soaked in selected dye concentration prior and after the degradation with bacterial strain. The surface-sterilized seeds were placed over it and incubated in the seed germination chamber. Filter papers were moistened with the same amount of degraded and undegraded dye solution intermittently. The control was run by keeping the paper pad wet with tap water. Length of plumule and radical was measured

after 6 days and percentage of germination was calculated. The similar process done for RED G dye and its effect on Vigno radiate seeds.

2.7 Analytical Investigation

2.7.1 UV-VIS Spectrophotometer

The color measurement of the samples was performed spectrophotometrically at particular wavelength according to particular dye (the maximum absorbent wavelength of AR18) using UV–Vis spectrophotometer (Systronics 2202). One milliliter of inoculum was incubated in 100 ml nutrient broth for 24 h and then 1 ml of dye stock containing 10,000 mg/l dye was added to it. Two-milliliter samples were withdrawn at regular interval, centrifuged (10,000 g for 15 min), and supernatant thus obtained was analyzed for estimation of degradation efficiency using double beam UV–Visible spectrophotometer in 200–700 nm range (Systronics 2202). The absorption spectrum of each sample was recorded over the range 200-700 nm and the absorbance at the λmax was registered. The absorption spectrum of each sample was recorded over the range 200–700 nm and the absorbance at the λ_{max} was registered.

2.7.2 HPLC and FTIR analysis

The HPLC and FTIR were performed for estimation of degradation efficiency and degradation products. The degraded samples (100ml) after 24h were centrifuged at 10,000 rpm for 15 min at 25 ± 1 °C, and mixed with equal volumes of ethyl acetate and kept in the desiccators for water removal using anhydrous sodium sulphate. The solution was further dried using rotary evaporator. The control was also exposed to similar step.

The residue thus obtained was dissolved in 5 ml of methanol and subjected to HPLC analysis (Water HPLC, Model no. 2690) on a reverse phase C18 column (5 mm, 4.6, 250 mm) at 35 °C. Acetonitrile at 10–90 % gradient was used as the mobile phase and the flow rate was adjusted to 1.0 ml/min. Analysis was carried out for 20 min and the peaks were identified using a photodiode array detector at 294 nm. The mobile phase was a gradient started with 91% water, 5% acetonitrile and 4% methanol. The gradient was changed linearly to 69% water, 27% acetonitrile and 4% methanol over 25 min. The detection was performed at 254 nm.

The FTIR spectra of pure and degraded dye samples were analysed between wave number 4,000 and 500 cm by mixing them in KBr pellet using FTIR spectrophotometer (Perkin Elmer, version 10.03.05).

2.7.3 GC-MS analysis

Decolorization under static condition by bacterial strains was carried out for complete degradation dye followed by successive biotransformation for 48h under static condition. Both decolorized and further biotransformed samples were centrifuged at 5000 rpm for 15min. the supernatant was transferred to separating funnel and extracted with an equal volume of ethyl acetate. The top layer was collected and concentrated to about 10 mL using a rotary evaporator. Samples were filtered through a 0.2µm nylon filter prior to GC –MS.

GC-MS analysis was carried out in a GC-174 coupled to a QP 5000 mass spectrometer from Shimadzu (Shimadzu corporation, Kyoto, Japan). ADB-5 column with

30 × 0.25 id and coating thickness of 0.25 μm was used. The temperature program was set initially at 70 °C with 1 min hold time then ramped to 245°C at a rate of 25 °C min^{-1} followed by an increase to 290 °C at 10 °C min^{-1} with 1 min hold time. The total analysis time was 13.5 min injector and interface temperature was set at 28 °C. The software used for data treatment was GC-MS real time analysis.

2.8 Rectors studies:

2.8.1 Procedure for isolation of bacterial Consortia BC1 from soil used in aerobic dye degradation

Soil collected from dye contaminated area, Carpet industries, Bhadohi, Uttar Pradesh were large amount of different dye effluents are being discharged for isolation of bacterial colonies for degradation of dye wastewater. For isolation of mixed consortia, 1g of soil containing 1000mg/l dye with nutrient broth (mg/l) peptone, yeast and glucose under shaking condition at temperature 35 °C and the solution is changed by every week. Then the solution diluted from 10^{-1} to 10^{-10}. Aliquots of 100μl the resulting were placed in the nutrient agar. The microbial consortium was obtained from soil and later adapted for dye degradation. In addition they have good bioflim forming ability and have high resistance to higher concentration of dye. The enrichment was made by successive changes of liquid medium in flasks. The bacterial consortia were developed in the laboratory and used for aerobic degradation of dye in batch methods. It consists of five bacterial strains that were isolated from dye contaminated soil. The microbial consortia were inoculated into nutrient media and incubated for 48 h at 35°C and used for seed culture and used for immobilization.

2.8.2 Optimization of various parameters for efficient degradation (Batch mode under aerobic condition)

Bacterial consortia BC1 was inoculated at 1% (w/v) into MSM containing 100 mg/l dye concentration and incubated at 35 °C for 24h in shaking and static condition. Various parameters such as pH, temperature, shaking condition, glucose concentration and initial dye concentration were optimized for efficient degradation of dyes under study by bacterial consortia BC1. Varying initial pH ranging from 5-10 was applied in the culture media in shaking condition (120 rpm) to study the effect of pH on dye degradation. At every successive step supernatant of the centrifuged aliquot was used to determine the degradation at regular time intervals. Each of the analytical experiments was performed in triplicate using their average values for calculation.

2.8.3 Experimental Set up

Two types of Packed Bed Reactor were used for investigation, (a) Attached culture Fixed bed Reactor in (Bioflim Packed Bed Reacto) *Figure* (2.4), (b) Packed Bed Reactor having TiO_2 coated clinkers in *Figure* (2.5). The first reactor was used for biodegradation, whereas second one has offered adsorptive treatment.

2.8.3.1 Biodegradation through Attached culture Fixed bed Reactor

As seen in Figure 2.4, a rectangular reactor of capacity 4.5 l (10× 10× 40 cm^3) was indigenously design and fabricated. An aquarium at the bottom of the reactors was used for supply of oxygen to maintain required aerobic conditions and also to facilitate recirculation. Three sampling ports were provided along the height of the reactor for

withdrawal of the desired samples. These ports were also used to create desired recirculation through the connection of any of two ports. The washed and sterilized uniform size (4-6 mm) clinkers procured from brick kilin Vishnupura village of Chiari-Gaon block of Varanasi district were used as packing material for generation of desired bioflim. Two reactor of similar specification were used in investigation, one as a control (without inoculums, Cc) and another as test column (with inoculums, Tc), the specification of these reactor are given in Table 3.1. These reactors were also equipped with temperature controller having accuracy ±1%. The operating condition used were temperature at 35 °C, pH of 8 using 12h cycle in sequential batch reactor mode. In every cycle settling time used was 10 min, another 10 min were used for effluent withdrawal in addition to 10 min feeding time. The remaining 11hr 30 min was used as reaction time for biodegradation. The oxygen concentration was maintained at and above 5 mg/l.

The simulated feed to the bioreactor contains dye concentration in the range of 50-500 mg/l. The nutrient such as Glucose (1500) mg l^{-1}, Peptone (1500) mg l^{-1}, Yeast (116.5) mg l^{-1}, KH_2PO_4 (23.3) mg l^{-1}, K_2HPO_4 (30) mg l^{-1} and $NaHCO_3$ (1500) mg l^{-1} were added to feed reactor.

Table 2.2 Characteristics of Packed Bed Reactors:

Working volume is 4.5 l (10× 10× 40 cm^3)

Specification	Dimension/ quantity
Outer Diameter	55.8 mm
Inner Diameter	50.8 mm
Height	800 mm
Total Volume	6l
Working Volume	4.5l
Packing Material	Clinkers
No. of Pieces	45-50
Height occupied	400 mm
Weight of Packing material	500 gm

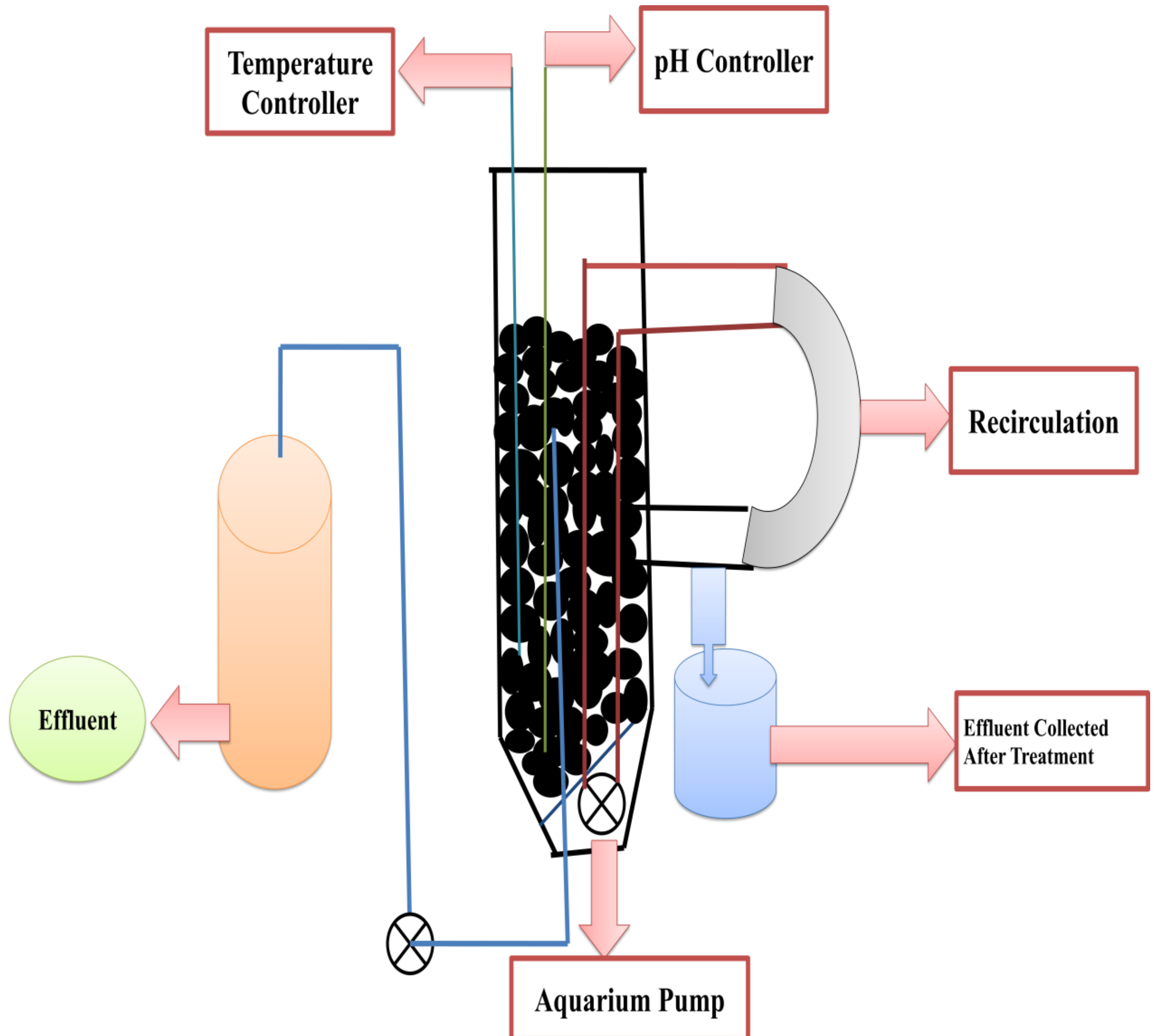


Figure 2.4: Schematics Diagram of Attached culture Fixed bed Reactor (Bioflim Packed Bed Reactor).

8.2.2 TiO_2 coated clinkers for adsorption

The effluent from bioreactor was fed into a Cylindrical Reactor packed with TiO_2 coated clinkers as shown in *Figure 2.5 and 2.6* (Schematics Diagram and Photograph). The column was packed with TiO_2 coated clinkers (4-6mm size). Coating was done by TTIP solution followed by calcinating at 500 °C. Experiments were conducted for fixed bed height of 400 mm and constant flow rate of 10 l/hr. The initial dye concentrations were varied between 50-500 ppm. The UV-Vis Spectrophotometer (Double beam spectrophotometer 2202) was utilizing for decolorization measurement.

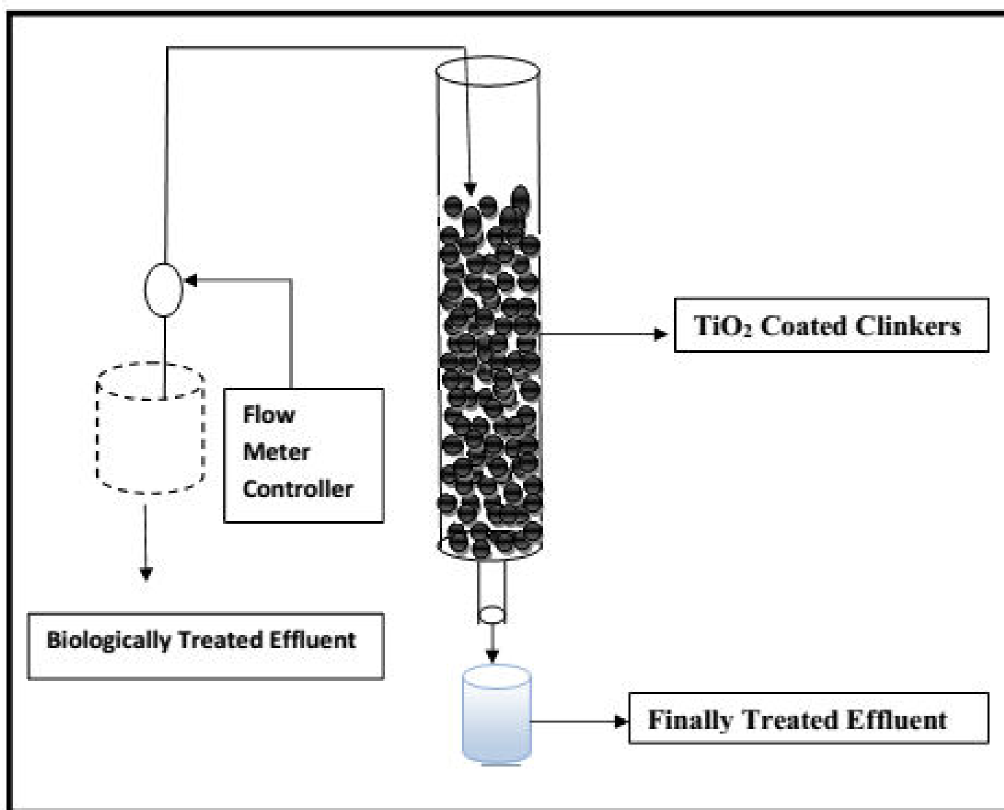


Figure 2.5: Schematics Diagram Fixed Bed TiO_2 Coated Reactor.

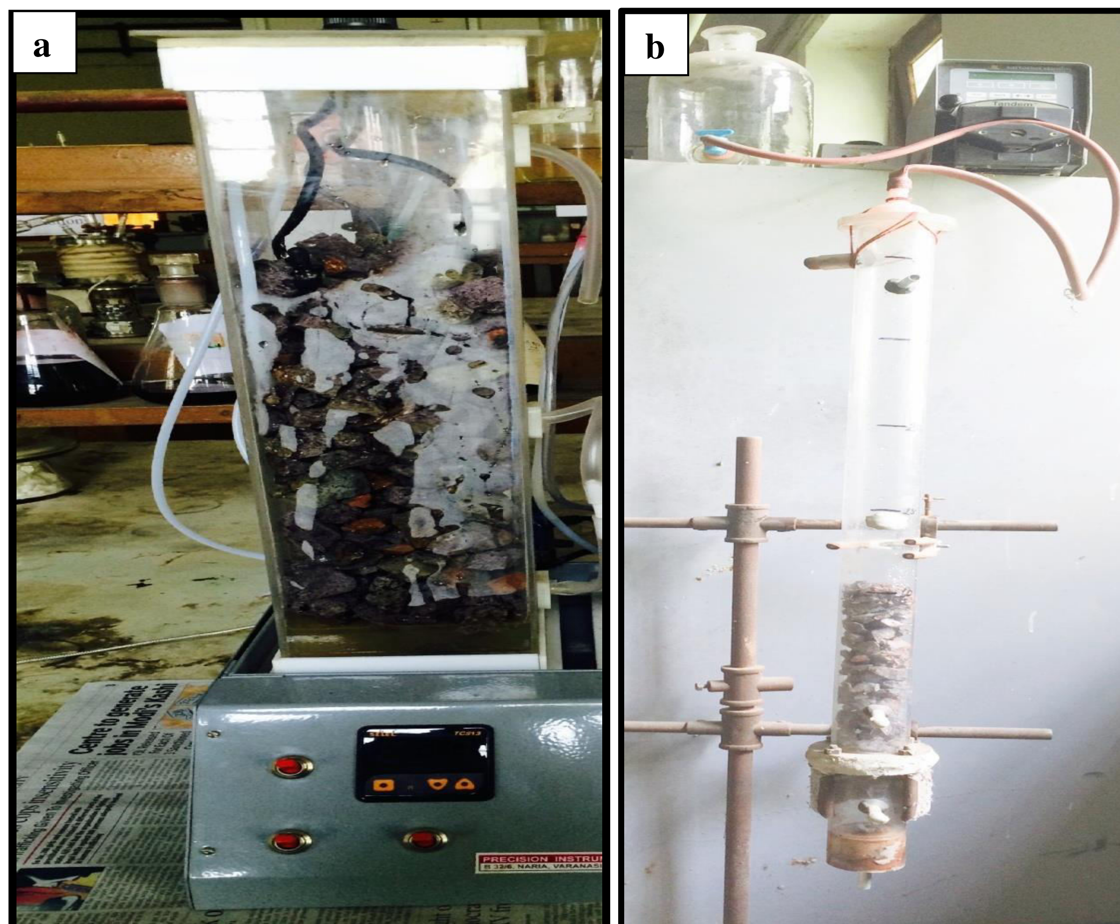


Figure 2.6. Photograph of Reactor (a) Attached culture fixed bed Reactor (b) TiO_2 coated clinkers packing for chemical oxidation.

2.8.2.3 Packing Material

The cheap and waste product is used for packing material for bacterial bioflim formation was collected from Brick klin Vishnupura village of Cheirai-Gaon block of Varanasi district were used as packing material for generation of desired bioflim Varanasi, India. The characteristic and property are given in Table 3.2. The packing material were washed with tap water for five times and put in distilled water for 48h. The

packing material is autoclaved at 120 °C for 15 min for the removal of microorganism and kept in hot air oven at 100 °C for 24h for proper dry.

2.9 Analytical Technique Used

The effluent eliminating out of reactor daily was centrifuged for 15 min at 7000 rpm. The supernatant thus obtained was analyzed for color, COD, pH, total suspended solids (TSS), and TDS. The pH of the samples was monitored by digital pH-meter (ELICO-) whereas DO meter () was used to measure dissolved oxygen level. A Conductivity meter was used to measure the electrical conductivity of treated dye sample whereas turbidity was estimated by Turbidity meter (Eutech, TN-100), similarly standard protocol was used for the measurement of TDS and TSS. Standard colorimetric method was used for COD. The color of dye sample pre and post treatment was estimated using UV-VIS double beam spectrophotometer (Systronic 2202). The sample was scanned for 200-800 nm wavelengths resulting into two characteristics peaks. The characteristic peak at 254nm signifies the peak of aromatic compound whereas 500nm represent the azo group.

The extends of degradation of dye was measured using HPLC (water, Model No. 501), for this reactor effluents after centrifugation was filtered 0.25µm membrane filter (Millipore). The filtrates were then extracted three times with diethyl ether and flash evaporated in a rotary vacuum evaporator at 45-50 °C in water bath. The residues thus obtained were dissolved in 2 ml methanol. This Extracted samples was then analyzed by HPLC using a mobile phase of methanol: water in C-18 column (void volume 2.9 ml, length 250 mm and internal diameter 4.6 mm) with flow rate of 0.8 ml/min, chart speed of 1cm/min and UV detector at 280 nm.