## 6.1 INTRODUCTION:

Changing fashion trends and through away attitude as resulted into drastic increase in the use of azo dyes due to their bright shades and lasting fastness property. More than ten thousand sets of azo dyes are currently being used amounting to total production of one million tons per annum [Anjaneya *et al*. (2011)]. The industries which are major consumers of synthetic azo dyes are various industries like textile, carpet, lather, printing, food, cosmetics, pharmaceuticals, paints etc [Franciscon *et al*. (2009a); Franciscon *et al*. (2009b); Saratale *et al*. (2013)]. Due to the presence of conjugated bond and its aromatic structures, azo dyes are very stable and high resistant to light, washing and microbial attack [Linda *et al*. (2009); O'Neill *et al*. (2000); Sanjay *et al*. 2014)]. Synthetic dyes are classified according to chemical structure, molecular structure and their application [Saratale *et al*. (2013)]. The effluents being generated from a dying plant is generally large in amount and complex in nature as it is the sum of dying and washing processes. The contaminated present into these effluents are dyes, their degraded products and other left over chemicals being used in dying and washing process such as surfactant, salt and other toxic products **[**Sen *et al*. (2003)]. The dyes and other intermediate products coming from these industries causes harmful hazardous effect on the environment, carcinogenic and mutagenic to human being and also toxic effect on aquatic system like decreases oxygen concentration and reduces the light penetration [Rathod and Archana, (2013); Venkata Mohan *et al*. (2012, 2013b); Liu and Tay, (2007); Wijetunga *et al*. (2010); Jonstrup *et al*. (2011); Meng *et al*. (2012)]. The environmental problems created by dye wastewater have received increased attention for several

decades because these industries utilize large quantity of water in dying processes. Dyes are the first industrial contaminant to be recognized in wastewater due to their high visibility at very low concentration, but they can be resistant to biological treatment because of their synthetic in nature and complex aromatic structure. Various conventional treatments to reduce the wastewater stream include physical and chemical methods such as ion exchange, filtration, precipitation, electrochemical, chemical reduction, adsorption and membranes technology [Singh *et al.* (2011); Toor *et al*. (2012); Liao *et al*. (2012)]. The effluents exhibit very slow degradation kinetics and resistance to conventional biological treatment processes and conventional physico-chemical treatment has not been proved as an effective method for decolorization and organic matter removal. The physico-chemicals methods have their limitation such as energy consumption, supplemented chemicals, generated secondary product and highly costly [Pandey *et al*. (2007); Forgacs *et al*. (2004); Bafana *et al*. (2008)]. Biodegradation using organisms is gaining importance as it is cost effective, environment friendly and produces less sludge. Dyes and intermediate products produced in effluent they severely damage the surroundings ecosystem like aquatic and terrestrial. Biodegradation methods for azo dye degradation using aerobic, aerobic/anaerobic and anaerobic treatment have remarkable advantages over physico-chemical methods. The anaerobic condition for the cleavage of azo bond generates aromatic amines which are toxic, mutagenic and carcinogenic in nature [Isik *et al*. (2004); Libra *et al*. (2004)]. The aerobic condition is needed for the degradation of aromatic amines [Zimmermama *et al*. (1982); Brown *et al.* (1987)]. Several studies have been reported for degradation of azo dye by pure and mixed

consortia isolated from contaminated sites. The pure culture has narrow range for substrate [Tan *et al*. (2005)]. Therefore there is need for cheaper biological that can be readily adapted for textile effluent treatment. However anaerobic azo dye reduction is time consuming process which is reflected by the requirement of plentiful microorganism and long reduction rate. Hence aerobic treatment is preferred as safe methods for biodegradation of azo dyes. However aerobic consortium has been demonstrated to degrade azo dye.

The conventional biological processes do not always provide satisfactory results especially for industrial wastewater treatment. To improve the effluent quality, addition of physical and/or chemical treatments is necessary. In most of researchers used advanced oxidation treatment as pre-treatment before biological process. The effluent discharge after treatment contains toxic compounds which are toxic for the microorganisms that perform the biological treatment. Thus, the pretreatment aims at the improving the biodegradability of the organic compounds in wastewater. Thus chemical oxidation processes as pretreatment require high amounts of energy, excessive chemicals and costs. So the post treatment of chemical oxidation process is required.

In Uttar Pradesh, Bhadohi, Carpet Industries is one of the most representative industries, in terms of turnover, number of firms and workers, generating large volumes of polluted water. Several recent studies have shown that microorganisms demonstrate an ability to adapt to higher concentrations of dye. For this region, microorganisms isolated from local contaminated sites have been used in treatability studies. The

immobilization of bacteria on waste material like klinkers has shown its potential for improving biodegradation efficiency in terms of sustainability as compared to free cells.

The present study investigate the idea of combine alternating aerobic modified Sequential Batch Reactor (SBR) followed by enhanced $TiO_2$ immobilized on klinkers for the decolorization and mineralization of azo dye Scarlet 4BS. In the case of azo dyes, the literature is limited to studies accomplished using only biological or chemical oxidation processes [Barbusinski *et al*. (2003); Hosseini Koupaie *et al*. (2012); Hosseini Koupaie *et al*. (2013a); Hosseini Koupaie *et al*. (2013b)]. In present investigation, a mixed culture BC1 has been enriched from dye contaminated soil samples. The effectiveness of the $TiO_2$ process was also enhanced. The klinkers used as packing material for immobilization of bacteria. In this novel reactor set up, klinkers are used as bioflim in aerobic chambers. Considering the purpose of this study, decolorization and COD removal efficiency were monitored in different steps of biological aerobic and $TiO_2$ process. The effect of external feeding on dye color removal, COD removal efficiency as well as biomass concentration was studied. Mineralization of the dye intermediates was also studied using UV-Visible spectrophotometer and HPLC analysis. In this study, the integrated aerobic- reactor was employed to evaluate the biodegradation of azo dye

**6.2 Result and Discussion:**

As in previous section simulated wastewater having azo dyes (scarlet 4BS) was treated in a novel sequential batch reactor using attached culture. The treated effluent of this reactor was then sent to another reactor of $TiO_2$ coated clinkers for adsorptive

treatment. The integrated treatment render approximately 96% removal of dye from waste water.

### *6.2.1. Cultivation, Enrichment and Analysis microbial Consortium*

The microbial consortia was cultivated and enriched as per protocol discussed in the material and methods. The prominent bacterial species thus obtained was identified using 16S rRNA gene sequence analysis. These were *Arthrobacter* sp. BHUAS X16 (KM199789), *Exiguobacterium* sp. IITES X12 (KM199786)**,** *Micrococcus* sp. BHUMC X14 (KM199787), *Pseudomonas putida* strain BHUPP X10 (KJ740223) and *Plannococcus* sp. BHUP X11 (KM199788) representing three bacterial phylum respectively. (*Figure. 5.1*) shows the phylogenetic relationship among these species. The sequences of 16S rRNA gene of the isolated bacterial strains are available under the Gene Bank with the accession number in (*Table. 6.1*). Results of the sequential analysis by strains matched with previously described NCBI database proved 98-99% similarity with the previously identified strains of similar family.

Batch investigation was made to study efficacy of these isolated bacterial strain to degrade scarlet 4BS (100-1000mg/l). It was observed that not a single bacterial strain was effective enough to get considerable degradation individually even at 400 mg/l concentration. All though the mixed consortia could degrade upto 90% within 20h under aerobic conditions and was effective upto 500 mg/l. Thus it was decided to use mixed consortia for degradation of dye using attached culture growth reactor.

**Table 6.1. Acession numbers of 16S rRNA gene sequence of four isolated bacterial strains submitted to NCBI database.**

| Bacterial Species | Strain | Accession Numbers | Phylum |
|---|---|---|---|
| *Arthrobacter* sp. | BHUAS X16 | KM199789 | Actinobacteria |
| *Exiguobacterium* sp. | IITES X12 | KM199786 | Firmicutes |
| *Micrococcus* sp. | BHUMC X14 | KM199787 | Actinobacteria |
| *Pseudomonas putida* strain | BHUPP X10 | KJ740223 | Proteobacteria |
| *Plannococcus* sp. | BHUP X11 | KM199788 | Firmicutes |

### *6.2.2. Morphology of Biofilm grown on klinkers*

The surface morphology of barren klinkers was compared with klinkers having 120 days old bioflim through SEM as shown in (*Figure. 6.1a-b*). It can be seen that all the pores of the klinkers (as seen in *Figure. 6.1a*) is completely covered by a layer of bioflim grown. SEM also reviled that the dominant species present in bioflim were spherical and coccoi shaped bacteria. It can also be seen that the grown bioflim is having fluffy structure and bacterial cells were even trapped in the pores of klinkers.

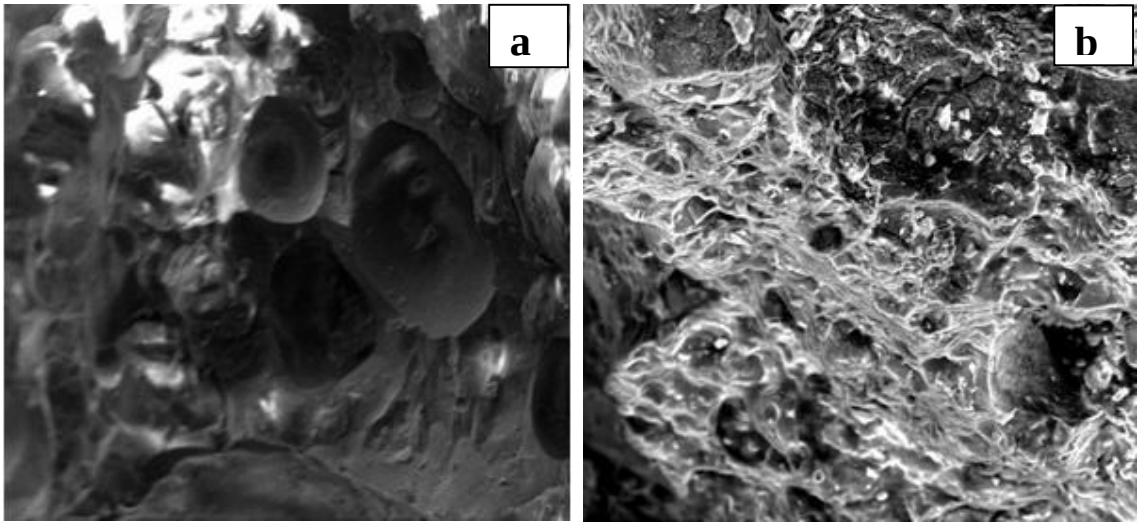


**Figure 6.1:** Scanning Electron Micrographs of support material clinkers immobilized bacterial strain (a) Control Clinkers (Without Bioflim) (b) Bioflim formation on klinkers.

### *6.2.3. Morphology of $TiO_2$ coated klinkers by using SEM analysis*

The scheme of the nanostructured material after deposition of the $TiO_2$ layer is shown in (*Figure 6.2a-c*)**.** Morphological studies by SEM of $TiO_2$ coated on klinkers before and after treatment, reported in *Figure 6.2a-c* show that, the non-regular surface with the presence of more regular and uniform layer after this process. The surface presents very small $TiO_2$ particles with a homogenous structure and surface that show instead large particles of agglomerated nanoparticles. The morphology of $TiO_2$ coated on klinkers was studied by SEM analysis. The $TiO_2$ coated on clinkers shows uniform distribution of $TiO_2$ with a magnification of 8K. The average particle size of the $TiO_2$ coated was in the range of 20 nm.

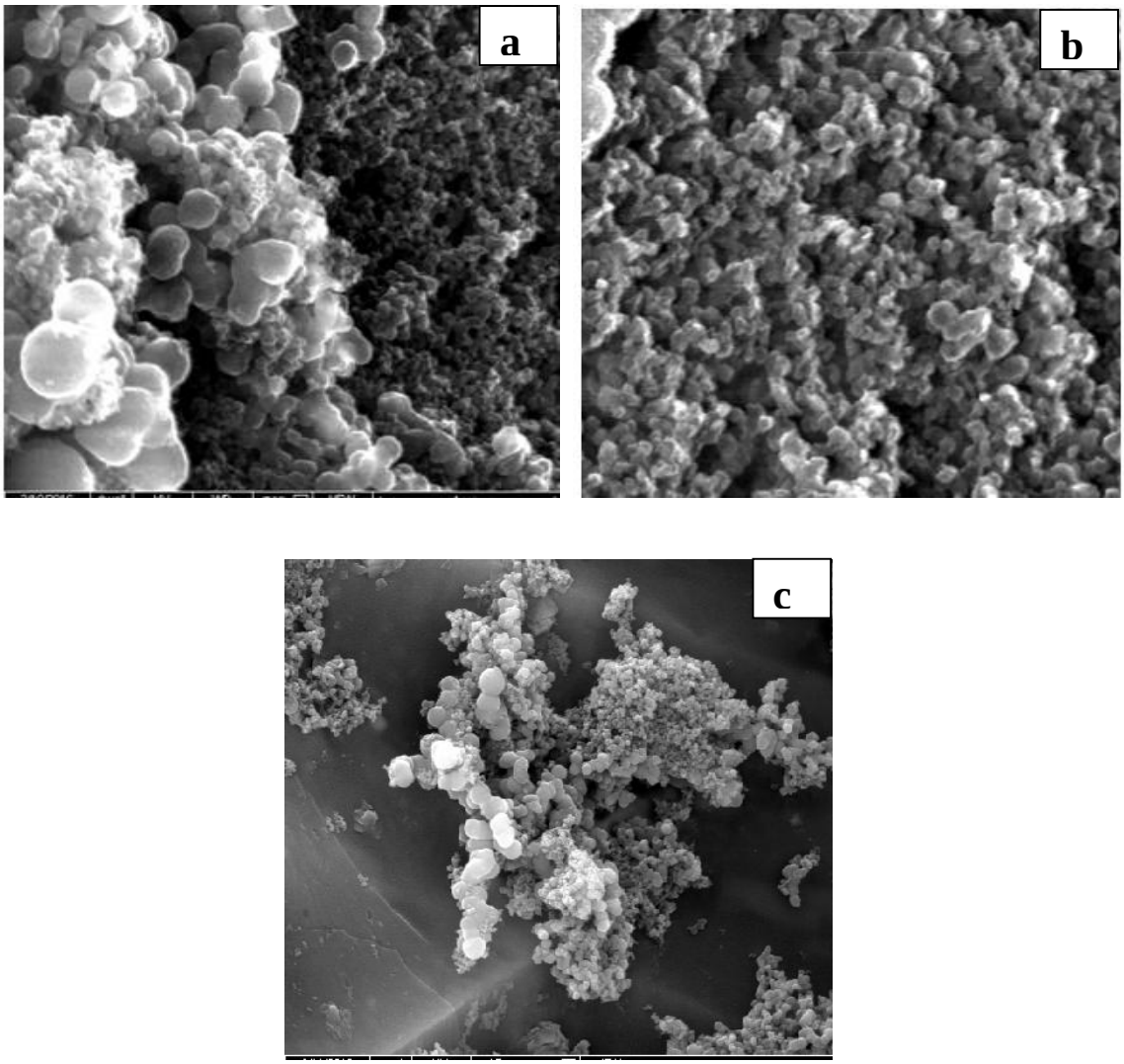


**Figure 6.2:** Scanning Electron Micrographs of Support material klinkers coated $TiO_2$ (a, b) before degradation (c) After Degradation.

### *6.2.4 Performance of Aerobic Packed Bed Reactor for Dye Decolorization*

Nutrient media and isolated microbial consortia were placed in the specially design and fabricated sequential batch reactor as shown in (*Figure 2.4)* chapter 2 and kept for 120 days for the growth of desired bioflim. It was ensured that packing material (klinkers) was submerged in nutrient media. After 120 days of the reactor was used for biodegradation of simulated azo dye wastewater (Scarlet 4BS) for four days with sample withdrawal after every 12h cycle for the characterization to measure the degree of treatment. The concentrations of dye sample used in treatment were in the range of 50-500 mg/l. The result thus obtained are shown in *Figure 6.3* it can clearly be seen that maximum decolorization of 72% could be achieved after 84h for 50 mg/l sample. The decolorization efficiency reduced with increase effluent concentration. Only 42% decolorization could be achieved at 500 mg/l integrating increased toxicity inhibiting microbial action.

*Figure 6.4* Explain the percentage of COD removal with time at different dye concentration (50-500 mg/l). The trend was similar as to percentage of dye decolorization and the maximum COD removal could be achieving at lowest concentration 50 mg/l. The reduced COD removal efficiency at higher dye concentration can again be attributed due to increase toxicity at higher concentration.

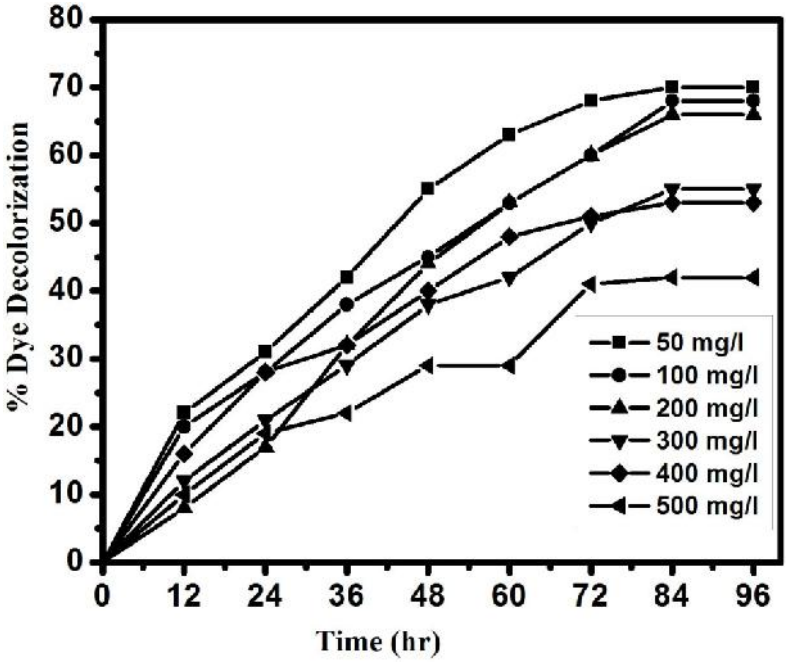


**Figure 6.3:** Performance of Packed Bed Bioflim Bioreactor operations with the function of different concentration of dye.

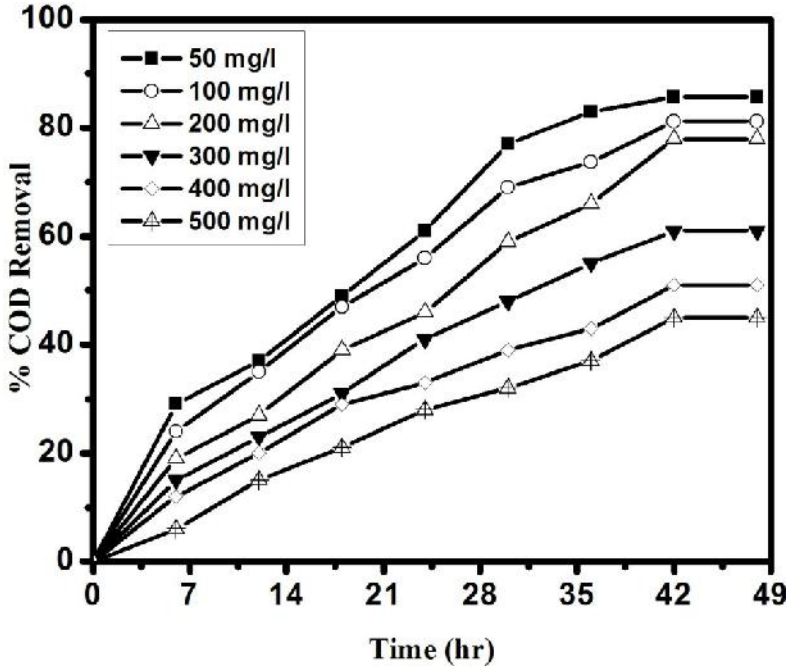


**Figure 6.4:** Performance of biofilm Packed Bed Bioreactor operations with the function of COD removal.

### *6.2.4. Adsorption of Biologically Treated Dye Effluent*

The maximum decolorizattion of 72% was achieved using mixed microbial consortia in attached culture bioflim reactor that to at lower concentration of 50 mg/l whereas most of the surface discharge from the carpet and other textile contains dye concentration more than 100 mg/l and some time even ISI 700- 800 mg/l whereas surface discharge limit is to achieved COD below 250 mg/l for any effluent stream. To treat azo dye effluent to this limit, it was decided to integrate adsorption with bioremediation. Thus effluent of microbial sequential batch reactor was used as influent (feed) to the packed bed reactor having $TiO_2$ coated klinkers as adsorbent. COD removal upto 98% and decolorizartion efficiency 96% could be achieved at initial dye concentration 100 mg/l as can be seen from the *figure 6.5*. When untreated simulated dye wastewater at 100 mg/l was exposed to adsorptive treatment by $TiO_2$ coated klinkers, only 62% reduction in COD and 52% decrease in color could be achieved. Impaling that integrated treatment is vial solution for the treatment of azo dyes (scarlet 4BS). The complete decolorization cannot be achieved economically with individual treatment of bioremediation and adsorption. In the present scheme bioremediation was done prior to adsorptive treatment to avoid increased toxicity due to $TiO_2$ coated**.**

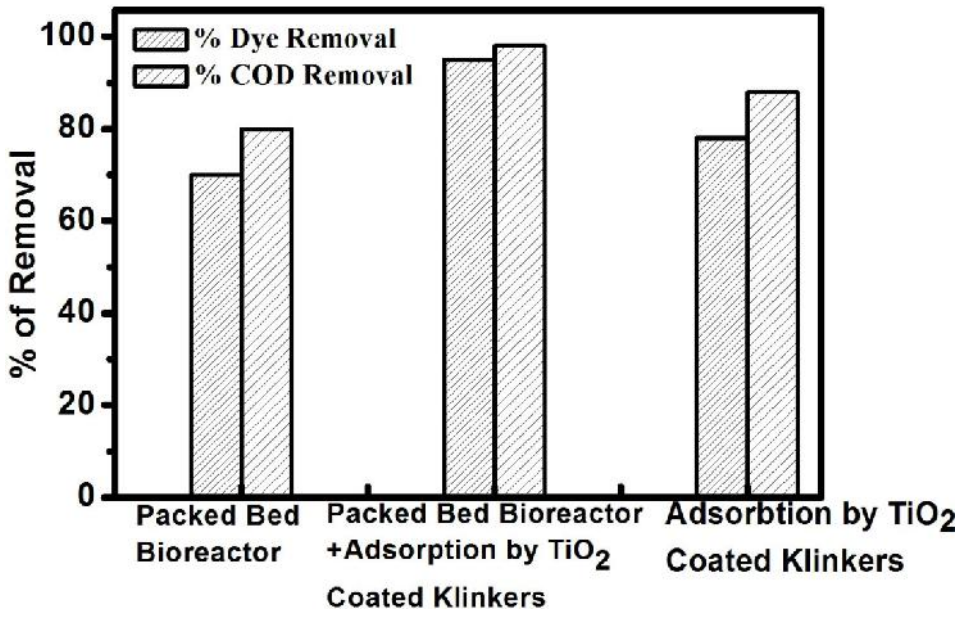


**Figure 6.5:** Combined treatment of dye wastewater using (Packed Bed Bioflim Bioractor + $TiO_2$ Coated Chemical Oxidation Reactor).

### 6.3 Analytical Investigation

The UV-Visible spectra of control, microbially treated wastewater at 24h and 49h and finally treated wastewater using adsorption are presented in (*Figure. 6.6*). There are three characteristics peaks as we can seen clearly from spectra of control samples out of which to ultraviolet range (260-380 nm), whereas third one is in visible range (514 nm). The peak in Ultra-violet range signifies the presence of aromatic group and third, which is in visible range are characteristics peaks of azo compounds. It is evident from the (*Figure. 6.6*). That intensity of this peak reduced with increase exposes signifying degradation of azo group and others compounds. The fourth spectrum representing integrated treatment don't have single peak, as nearly complete degradation has been achieved.

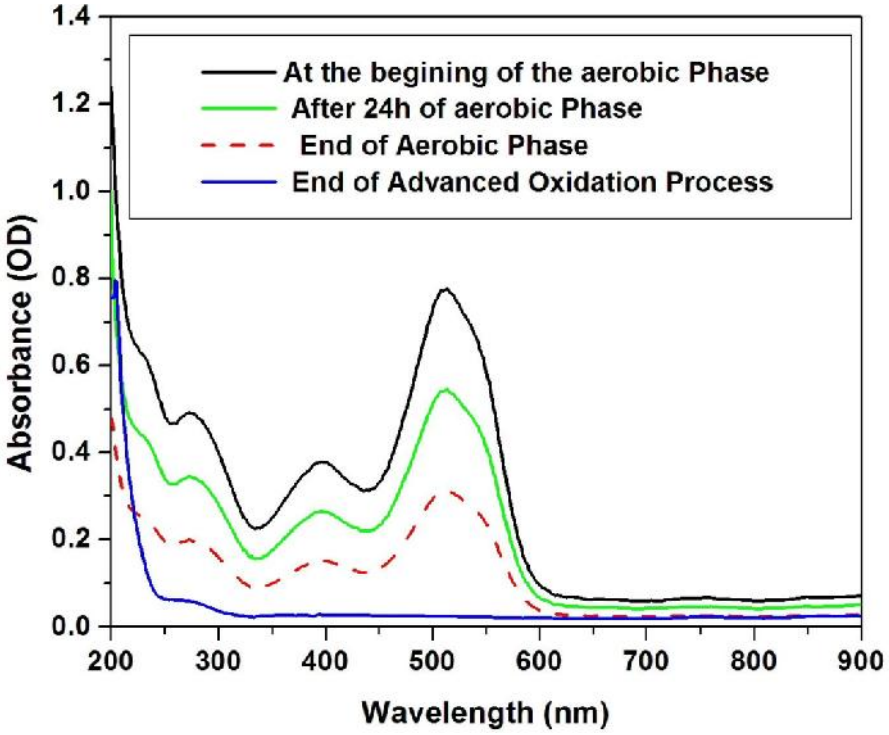


**Figure 6.6:** Variations in the UV-Visible spectra of inlet and outlet samples with the function of time in Packed Bed Bioflim Bioractor + $TiO_2$ Coated Chemical Oxidation Reactor.

### *6.4 Phytotoxicity investigation*

The results of phytotoxicity are presented in *Table 6.2*. For this investigation *P. mungo* were placed in distilled water, untreated azo dye sample, aerobically treated sample after 4 days and treated wastewater though combination of bioremediation and adsorptive treatment. It can be seen that germination was adversely impacted in control sampled as 26% germination take place. Whereas 66% germination is could be achieved in aerobically treated sample signifying reduced toxicity. 100% germination in the case of distilled water and finally treated sample represent complete elimination of toxicity though integrated treatment (Biodegradation + $TiO_2$ coated sample).

**Table 6.2: Phytotoxicity of textile industrial wastewater and its degraded products for *and Phaseolus mungo*.**

| Plants | Observation | Treatments Distilled water | | | |
|---|---|---|---|---|---|
| | | **Distilled Water** | **Untreated waste water** | **Aerobic effluent (84h)** | **Combined Treatment (20min)** |
| ***Sorghum vulgare*** | Germination (%) | **100** | **26** | **66** | **100** |
| | Plumule (cm) | **14.8 ± 2.2** | **2.5 ± 3.4** | **8.8 ± 3.6*** | **14.0 ± 3.2*** |
| | Radical (cm) | **7.6 ± 1.4** | **0.9 ± 1.3*** | **2.0 ± 2.8*** | **7.5 ± 1.9*** |

## 6.5 CONCLUSION:

The mixed consortia of microorganism isolated from soil sample of carpet effluent fared a lot better as compared to individual microorganism. Bioremediation experiment in indigenously design and fabricated bioreactor using klinkers as packing material resultant into resignedly good decolorization efficiency upto 72%. Further treat of the effluent and to achieve surface discharge condition are to attain the rechargeable water, the treated effluent was send for adsorptive treatment using packed bed reactor having $TiO_2$ coated klinkers as adsorbent. The decolorization upto 96% could be achieved with this integrated scheme of treatment. Phytotoxicity studies through germination of *P. mungo seeds* stabilized the comparability (100% germination) between distilled water and treated effluent. Whereas only 66% and 26% germination was observed in the case biologically treated and pure effluent respectively.