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COPPER OXIDE NANOPARTICLES AND FARMING SQUANDERS UTILIZED IN IMPROVING THE BIOREMEDIATION AND EXPLANATION STRATEGIES IN MATERIAL EMANATING TREATMENTS

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Abstract:

Textile dyeing industry is considered as one of the largest generators of wastewater in India, particularly Tiruppur. Dyes released by the textile industries cause a major threat to environmental safety. Apart from physical and chemical method, dye decolourisation through biological means can have a wide range. The present study focused on the screening of microbial isolates from the effluent with respect to the synthetic dye decolourisation. Three bacterial namely Bacillus sp., Pseudomonas sp., Acinetobacter sp. and two laccase producing fungal species, identified as Aspergillus niger and Aspergillus fumigatus, were isolated that were efficient in decolorizing the synthetic dyes. These isolated colonies were used as free cells, microbial consortium and immobilized microbial consortium having the copper oxide nanoparticles, for effluent treatment. Treatment by microbial consortium and immobilized consortium showed better efficiency when compared to individual microbes, but the sludge settlement was again a threat. Effluent treatment by the immobilized microbial consortium with peanut husk and Copper oxide nanoparticles showed promising decolorization and efficient sludge removal during the treatment. By using magnetically induced biological waste the removal of dyes and separation of sludge contents can be achieved with low cost. The process could very well be used in localized companies since it is cost effective and thus aid in the eradication of the scar caused through pollution thus far.

Keywords: *Effluent, consortium, immobilization, absorbent, nanoparticles, decolorization.*

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INTRODUCTION:

Textile industrial effluent associated with water pollution is a matter of great concern over decades. In textile industries, numerous number of chemicals are used for dying variety of fabric materials. On commercial basis, among different dyes, azo dyes are used in most of the dyeing units in Tiruppur district due to their colour intensity and cheap cost. These azo dyes have poor exhaustion properties that end up as the effluent of the dyeing industries(Puvaneshwari*et al*., 2006).Azo dyes are considered to be biorecalcitrant under aerobic conditions that are cleaved under oxygen limiting conditions leading to the formation of toxic aromatic amines. These aromatic amines could not be further reduced under limited oxygen but are auto-oxidizable or could be mineralized by microbes at a faster rate under aerobic condition. This treatment of effluents under anaerobic conditions is time consuming and commercially demanding, making it unfeasible. The disposal of synthetic dyes into the environment causes serious damage as they intensely affect the photosynthetic activity of hydrophytes by limiting the light penetration and their breakdown products may be toxic to some aquatic organisms(Senan and Abraham, 2004).It also affects water bodies, ecosystem integrity, soil fertility and plant growth. The azo dyes are becoming a great concern due to their visible colour, biorecalcitrance and toxicity to animals and humans (Darwesh*et al*., 2008).

Sludge, a toxic and hazardous remains produced during textile processing and after textile wastewater treatment. Thus, colored effluent and sludge problems are one of the the major concerns of the textile industry management and development of appropriate treatment system is desired.Remotion is a new technique that enables effluent treatment by utilizating both biological and chemical technologies (Sathyabama*et al*., 2013). Biological approach involves the microbial degradation mediated by enzyme. Many research study reported that the enzymatic approach was attractive with regard to decolourisation and degradation of azo dyes in wastewater (Kirby *et al*., 2000). Among different enzymes, laccase have been extensively studied for their degradation of azo dyes (Novotny *et al*., 2004). These enzymes are multicopper phenol oxidases that decolourise azo dyes through a highly nonspecific free radical mechanism forming phenolic compounds, thereby avoiding the formation of toxic aromatic amines (Peralta-Zamora *et al*., 2003).

The present work was focused on the bioremediation not only of a specific group of xenobiotic textile dyes, but alsovariousclasses of dyes.Remotion technique was carried using immobilized microbial consortium, adsorbent being peanut husk and Copper oxide nanoparticles which effectively eliminates the dyes and escaping to the environment. This enables a clear separation of the sludge generated during the effluent treatment. By subjecting the magnetically induced adsorbent to a magnetic field, the sludge component would be separated out more effectively enhancing the efficiency of the treatment process manifolds. The production rate of textile sludge-based adsorbent depends on the generation rate of sludge from the textile wastewater treatment processes.

MATERIALS AND METHODS:

Dyes and Chemicals

Dyes used in the study (Indigo blue, Remazol brilliant violet 5R, Reactive Black 5, Reactive red 120 and Reactive Orange 16) were obtained from Sigma Aldrich a part of Merck. Peanut husk was procured from local stores, Coimbatore. Hi-Media chemicals and media were used for the work.

Collection of effluent sample

Sample collection location sites: Textile dying industry outlet, Tirupur District, Tamil Nadu, India. Samples were collected in polyethylene cans, previously rinsed with 6M HNO_3 and distilled water.The samples were subjected to on-site analysis,recommended by APHA (1995), which involved recording of parameters such as temperature, pH and color.The sampleswere then shifted to the laboratory as soon as possible for the analysis of various physico-chemical parameters, followed by preservation at 4˚C for further analysis.

Characterization of the effluent using physico-chemical parameters

Collected raw effluent was characterized by analysing variousphysico-chemical parameters including the measurement of Colour, Turbidity, Chemical Oxygen Demand (COD), Total Solids (TS), Total Suspended Solids (TSS), Total Dissolved Solids (TDS), pH, and Hardness, chosen in accordance with the regulations of Tamil Nadu Pollution Control Board (TNPCB).

Preliminary screening and isolation of dye decolourising microbial strain

The screening of bacterial and fungal isolates from textile effluent was based on synthetic dye decolourisation ability using plate assay method. About five different synthetic dyes (Indigo blue, Remazol brilliant violet 5R, Reactive Black 5, Reactive red 120, Reactive Orange 16) were 0.01% amended in solid media individually. Microbial strain isolations were carried out by serially diluting textile effluent and subsequent plating onto the solid media supplemented with dye. The plates were incubated at 37˚C (24 hour) for bacteria and 48 hours at 28˚C for fungi (Anamika and Sarabjeet, 2013). After incubation, the morphologically distinct microbial isolates showing clear zone around their colonies due to decolourisation of dye were selected for further studies. The pure cultures were stored at 4˚C for further analysis.

Secondary screening of selected bacterial isolates
Selected bacterial strains were subjected to secondary screening using decolourisation assay. The bacterial strains were inoculated into the nutrient broth containing 0.01% of five different dyes individually. After incubation, the samples (5 ml) were centrifuged at 10,000 rpm for 15 minutes and its absorbance was measured at λ_{max} of the particular dye. The dye free uninoculated medium was used as blank (control). All assays were performed in triplicate and compared with control. The decolourisation efficiency of bacterial isolates was expressed in terms of percentage by using the following equation (Khelifi*etal*., 2009).

Screening of laccase producing fungal isolates
Selected dye degrading fungal isolates were inoculated in the plate containing 15ml of potato dextrose agar amended with 0.01% of Guaiacol. The plates were incubated at 30˚C for 1 – 3 days. The presence of brick red colour around the mycelium was considered as guaiacol oxidizing extracellular laccase secreting organism (Kiiskinen*et al*., 2004).

Identification of selected microbial isolates
Bacterial isolates were identified using standard biochemical and microscopic (Gram's staining) techniques. The selected efficient laccase producing fungal strains were subjected to microscopic identification using Lactophenol cotton blue technique (Cappuccino and Sherman, 1996).

Compatibility analysis of selected microorganisms
To prepare the microbial consortium, the microbial cultures must be compatible with each other. The selected dye degrading organisms were swabbed on the Luria Bertani (LB) agar plates individually in each plate. After inoculation, wells were cut by using gel puncture. In each of these wells about 10µl of the culture supernatant were added expect the culture of organisms swabbed in the plate (Rajendran *et al*., 2011). The plates was incubated for 48 hours at room temperature and observed for the formation of zone of clearance around the wells. The presence or absence of zone around the well is an indication of compatibility of the organism. The microbial cultures when compatible with decolourisation capabilities were chosen to construct the microbial consortia.

Synthesis of Copper oxide nanoparticles
Magnetically responsive nanocomposite materials were prepared by modification of diamagnetic materials with magnetic fluids. The Copper oxide nanoparticles were synthesized using the co-precipitation method. Cupric chloride ($CuCl_3$) solution (3.7g/500ml distilled water) and copper sulphate ($CuSO_4$) solution (9.4g/500ml distilled water) were prepared individually (Safarik *et al*.,2007). Both the solutions were mixed in the ratio of 1:1 and stirred at 80˚C for 10 minutes. Once the Copper compounds was completely dissolved, 25 % of the ammonia solution was added drop-wise to the reaction solution and continuous stirring was resumed until the complete black magnetite precipitation was achieved (pH 9-11). After the ammonia reaction, the reaction solution forms a black precipitate in the bottom of the reaction flask (Mutasim, 2015) and the precipitates were separated by a permanent magnet and washed several times with distilled water until pH neutralize followed by acetone wash twice. Finally, the precipitate was dried in oven at 60 - 70˚C to obtain the Copper oxide nanoparticles (Poedji *et al*., 2013).

Characterization of the synthesized nanoparticles
Synthesised Copper oxide nanoparticles were characterized using Ultra Violet – Visible (UV - Vis) Spectrophotometer and Dynamic Light Scattering (DLS) analysis**.** The synthesised Copper oxide nanoparticles, after dilution with distilledwater, was monitored for reduction of pure Fe3+ by measuring of the UV – Vis spectrum at the wavelength of 330 – 450 nm. The technique of DLS has been widely employed for sizing magnetic nanoparticles in liquid phase. The average size of a magnetic Copper oxide nanoparticles was determined by DLS and the results were recorded using Malvern zetasizer version 2.2. There were 100 scans for each sample and the mean was taken as the average particle size and zeta potential of the sample.

Immobilized beads for the effluent treatment
Immobilized microbial consortia have a broad spectrum of applications in fields like industrial or environmental process, especially in the field of waste water treatment. The remediation efficiency of free cell was enhanced by immobilizing the microbes in a matrix of sodium alginate. About 3.6 % of Sodium alginate was prepared with 5% of microbial free cells. The solution was taken in a pipette/beaker and was administered dropwise into the beaker containing 2% Calcium Chloride solution it was collected in the form of beads (Wang *et al*., 2003).

The Immobilization of the microbial consortium containing magnetically induced adsorbent with Copper oxide nanoparticles were also prepared using similar procedure. the Sodium alginate was mixed with 1% of magnetically induced agricultural waste (peanut husk) and the synthesized Copper oxide nanoparticles along with the microbial free cell.

Treatment of effluent

Effluent sample was treated using three different modes of microbial samples. First set was treated using microbial consortium containing all the cultures in liquid form, the 2nd sample was inoculated with immobilized microbial consortia, the 3rd sample was inoculated with immobilized microbial consortia with Copper oxide nanoparticles amended in peanut husk. The samples were incubated in a metabolic shaker (120 rpm) for 15 days at room temperature. The samples were retrieved from the flasks intermittently; every 48 hr and the physico-chemical parameters were analyzed.

RESULTS AND DISCUSSION:

Physico chemical characterisation of the collected effluent sample

Physico-chemical characterizations of the collected effluent sample were analyzed based on TNCPB. The colour of the effluent sample, which typically depends upon the different industrial processes, was observed to be black. The measurement and removal of colour is an essential part of the treatment process. The samples had a fishy odour, a potential indicator of the presence of decaying vegetation, inorganic and organic constituents. The physico-chemical analysis results of the raw effluent sample were observed and represented in table 2. In the present study, the physico-chemical values of the collected samples were higher than the permissible limit of Tamil Nadu Pollution Control Board (TNPCB) for the collected effluent sample. It confirms that the collected effluent sample may contain heavy organic and formation of toxic aromatic amines, will have detrimental effect on the water body when released in to the environment. In order to overcome this problem, the textile effluent should treated.

Preliminary screening and isolation of dye decolourising microbial strain

Dye decolourising by microbial colonies were screened based on the formation of visual zone of clearance observed on the plates containing synthetic dye after incubation. Among them 15 predominant microbial cultures (10 bacterial and 5 fungal cultures) were isolated.The decolorisationof the dye could be due to any of the three: the ability of the organisms to breakdown the chromophorefacilitated by enzyme production**,** the adsorption to microbial cells or biodegradation (Khadijah *et al*., 2009).

Secondary screening of selected bacterial isolates

Initially screened bacterial isolates were subjected to quantitative dye decolourisation by broth assay method. The percentage of dye decolourisation was calculated and represented in table 3. The results show that all the selected bacterial strain was able to decolourise the five different dyes. Whereas the bacterial isolates 1, 2 and 3 showed 66-95% decolourisation in the aerobic shaking condition after 24 hr and thus it was selected for further studies.

Screening of laccase producing fungi

Secondary screening of fungal isolates was based on their ability to produce laccase enzyme. Two fungal sp. showed positive results for the laccase production. They were identified by the development of dark brown zone around and below the colonies. The formation of the brown colour and the incubation period required for the proper growth vari**e**s with individual organism. Laccases catalyze the oxidation of both phenolic and non-phenolic compoundsand thus can decolourise a wide range of synthetic dyes in the effluent without the formation of toxic aromatic amines (Abedin *et al*., 2008).

Identification of selected microbial isolates

Selected bacterial isolates 1, 2 and 3 were tentatively identified as *Pseudomonas*sp, *Bacillus*sp, and *Acinetobacter*sp respectively. The isolated extra cellular enzyme laccase secreting two fungal strains were tentatively identified as *A. niger*(A) and *A. fumigatus* (B).

Compatibility analysis of selected microorganisms

Efficient dye decolourising bacterial isolate (1, 2 and 3) and laccase producing fungal isolate (A & B) were tested for compatibility analysis and it was found out that there was no zone of clearance observed in any of the plates swabbed with all the selected cultures individually, indicating compatibility with eachother. This may be due to the fact that autochthones were isolated, that would have co-existed earlier in the same environment. These microorganisms when used as a consortium it could be used in the decolourisation of dye as well as the degradation of complex organic compounds that are present in the effluent (Rajendran *et al*., 2011).

Characterization of the synthesized nanoparticles

The Copper oxide nanoparticles (Fe_3O_4) synthesized by co-precipitation of ferric and ferrous chloride was validated by UV-Visible spectroscopic analysis and their scanning absorbance vs wave length (λ) has been established. The characteristics peaks of ion nanoparticles were observed at 585 nm. Dynamic light scattering was processed to determine a hydrodynamic size of synthesized nanoparticles. Particle size distribution of chemically synthesized Copper oxide nanoparticles are shown in figure 1. The average sizes of Copper oxide nanoparticles were found to be 153.8 nm.

Treatment of the collected effluent sample

Treatment of the collected textile effluent sample was carried out using microbial free cells, immobilized microbial consortia and the immobilized magnetically induced peanut husk with Copper oxide nanoparticles.

Treatment of effluent with microbial free cells

Effluent sample treated with the free cells of microbial consortia under aerobic condition was withdrawn at an interval of every 48 hours to analyse the physico-chemical characters. The uninoculated textile effluent serves as the control (Usha *et al*., 2010). A pattern of reduction in parameters was observed up to 15 days. The physico–chemical characters showed a continuous reduction from the first day and the maximum reduction were observed at the 11th day of incubation. The physico–chemical characters like COD - 66.04%, Hardness - 51.95%, TS - 62.70%, TSS - 60.93%, TDS - 55.02%, pH - 25.92%, Turbidity - 79.86%, colour - 65.09% was observed as the maximum reduction in the effluent treated by the free microbial cell. There was only slight change occurred in few parameters after 11th day to 15th day (Table 4).A bacterium offers a cheaper and environment friendlier alternative for colour removal in textile effluents. Microbial consortia free cell treatment in effluent showed a moderate reduction of physico-chemical characters by lower operating cost, but the main drawback behind the treatment was the formation of sludge during the treatment.

Treatment of effluent with immobilized microbial consortia

The collected textile effluent was treated with microbial consortium immobilized in a matrix of sodium alginate. The percentage reduction was gradually increased from 1 to 11th day after which there is only slight increase in few characters (Table 5). The COD -73.22%**,** Hardness - 79.60%, TS - 83.14%, TSS - 80.6%, TDS - 85.63%, pH - 33.33%, Turbidity - 88.27%, colour - 84.51%, are recorded at the end of 15thday.Once the microbial cells were immobilized, the cell viability must be concomitantly sustained over a long period of time. Thus, Immobilization is advantageous for sustaining solely growing cells. The dye and the organic molecules would be adsorbed on the surface of the immobilized cells and these adsorbed molecules acts as the energy substrate for the microbes present in the core of the matrix (Bulut*et al*., 2007). The results indicated that sodium alginate immobilized with microbial consortium can be used as an efficient and eco-friendly adsorbent for the removal of synthetic dyes (Wang *et al*., 2008). Even though treatment attains almost 80% of the physico-chemical reduction, sludge settlement pose a severe problem in this criteria to be focused. In order to overcome the problem of sludge formation, an adsorbent with magnetic property could be used.

Treatment of effluent with immobilized microbial consortia, peanut husk and Copper oxide nanoparticles

Percentage reduction of the textile dye decolourisation was calculated and graphically represented in table 6. The maximum percentage reduction was observed at the end of 15th day COD - 83.33%**,** Hardness - 95.85%, TS - 98.01%, TSS - 86.04%, TDS - 98.73%, pH - 33.33%, Turbidity - 95.10%, colour - 95.22%. The treatment process effectively degrades the effluent since both the absorbents (Copper oxide nanoparticles and peanut husk) and the microbes act on the dyes. Results indicated that peanut husk showed maximum biosorption capacity in removal of synthetic dyes present in the textile effluent (Wang *et al*., 2008).Immobilized Copper oxide nanoparticles trapped with the organic matters in the effluent are removed through the external magnetic force. Therefore, the treatment was found to effective when compared with the free cells and immobilized cells because of the sludge removal. Since it is cost effective and also colour removal were almost more the 90%.

Peanut husk with Copper oxide nanoparticles and microbial consortium effectively replaces the physical and chemical treatment. Major advantages of this treatment is sludge free process and the complete removal of colour from the effluent by breaking down the chromophore of the reactive dyes, adsorption on the cell wall of microorganisms and also adsorption by the pea nut husk adsorbents. This shows that the magnetically induced peanut husk with Copper oxide nanoparticles is more efficient for the treatment of effluent samples.

Upon analyzing the three different patterns of bioremediation it was found that the immobilized cell containing the additive in the form of peanut husk which is impregnated with Copper oxide nanoparticles was not only found to efficiently remediate the textile effluent but would also be involved in clarification process next to the remediaion process. The major reason that could have favoured the efficiency of the beads could be because of the adsorption of the components onto the peanut husk and the Copper oxide nanoparticle.

Separation of sludge

The separation sludge with the Copper oxide nanoparticles and adsorbents with microbial consortium in the treated effluent was facilitated by subjecting it a magnetic field. It was confirmed that the rapid settlement of the solid particles through the visual observation (Figure 2). The immobilized microbial cells with Copper oxide nanoparticles loaded absorbent in the treated effluent get aggregated. The sludge settlement was observed within 4-5 min. Remotion is a new technique that enables the utilization of both the biological and chemical technologies. Till date the effluent is treated only by a single mode i.e., either through physical, chemical or biological. The inclusion of physical, chemical and biological techniques aids in enhancing the overall potential of the developed technique (remotion). Through remotion the presence of an adsorbent (powdered agricultural waste particles peanut husk) in the immobilized bead adsorbs the dissolved dye component while the microbial system present at the core produces enzymes that would reduce the dye.

CONCLUSION:

Inclusion of physical, chemical and biological techniques aids in enhancing the overall potential of the developed technique (remotion). Through remotion the presence of an adsorbent (powdered agricultural waste particles peanut husk) in the immobilized bead adsorbs the dissolved dye component while the microbial system present at the core produces enzymes that would reduce the dye. From the result it was observed that peanut husk is a promising adsorbent for the removal of different types of xenobiotics dyes. Also, the microbial consortium immobilized with sodium alginate considered to be an efficient and eco-friendly adsorbent for the removal of synthetic dyes. Magnetic nanoparticles precipitated on the peanut husk surface both in the form of individual particles and agglomerates of particles. Magnetic modification of this material enables to use magnetic separation techniques for its rapid separation from complex samples containing different impurities, including suspended solids. The maximum adsorption capacities of tested dyes on this magnetically labeled material are relatively high. This kind of treatment has been effective in bioremediation of dumped dye stuffs and dye waste in the effluents with a lower operating cost than other remediation process. Itscrucialfor textile industry to overcome the negative impact on the environment especially in case of coloured wastewater and sludge.

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Table 1: Maximum wavelength scan for different dyes

Dyes	Type of dye	Maximum wavelength (nm)
Indigo blue	Reactive dye	602
Remazol brilliant violet 5R	Reactive dye	583
Reactive Black 5	Reactive dye	597
Reactive red 120	Reactive dye	538
Reactive Orange 16	Reactive dye	494

Table 2: Physico – chemical characterization of untreated effluent sample

Physico-chemical parameters	Observed values	TNPCB Permissible limits
COD (mg/L)	960	400
Hardness (mg/L)	820	600
TS (mg/L)	3970	2500
TSS (mg/L)	480	50
TDS (mg/L)	2050	1500
pH	10.8	6.5-9.0
Turbidity	1.5943	Not objectionable
Colour	1.9705	25HU

Table 3: Percentage reduction of dye decolourisation by bacterial strains

Isolated bacterial strain	% Decolourisation of synthetic textile dye				
	Indigo blue	**Remazol Brilliant Violet 5R**	**Reactive Black 5**	**Reactive Red 120**	**Alizarine Red 5R**
1	69.7	91.5	88.6	83.4	71.7
2	94.5	76.7	66.2	68.5	67.0
3	89.7	93.6	70.8	70.9	89.2
4	23.2	28.6	35.7	34.8	27.4
5	25.9	32.8	32.9	32.5	24.54
6	56.78	26.4	29.76	31.98	23.5

Table 4: Physico – chemical characterization of effluent samples treated with free microbial cells

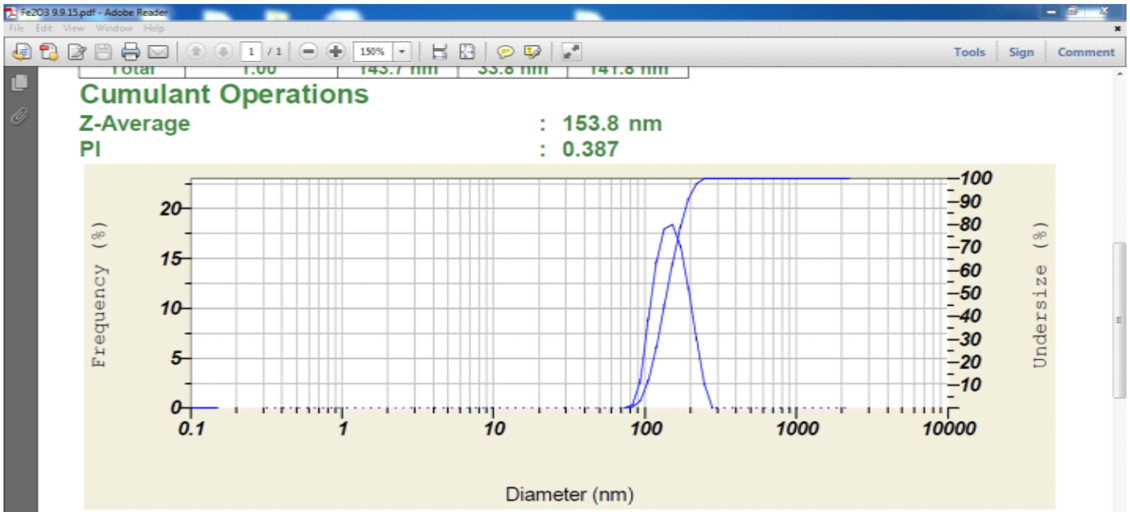
S. NO	Physico-chemical parameters	Percentage reduction of the textile dye decolourization (%) Number of days						
		3	**5**	**7**	**9**	**11**	**13**	**15**
1	COD (mg/L)	15.7	20.8	32.8	49.8	57.09	60.7	66.04
2	Hardness (mg/L)	9.8	15.5	20.64	39.5	49.55	50.87	51.95
3	TS (mg/L)	55.17	57.09	55.8	58.74	60.9	62.59	62.70
4	TSS(mg/L)	32.69	42.36	51.7	67.5	79.65	66.04	60.93
5	TDS(mg/L)	14.64	16.05	18.54	19.78	40.65	53.09	55.02
6	pH	16.66	23.14	25.92	25.92	25.92	25.92	25.92
7	Turbidity	7.43	20.84	29.64	47.1	68.6	76.54	79.86
8	Colour	18.91	43..58	60.43	63.4	63.5	65	65.09
9	Salinity(ppt)	0.56	0.53	0.50	0.46	28.57	28.57	28.57

Table 5: Physico – chemical characterization of effluent samples treated with immobilized microbial consortium

S.NO	Physico-chemical parameters	Percentage (%) Reduction of textile effluent decolourization Number of days						
		3	**5**	**7**	**9**	**11**	**13**	**15**
1	COD (mg/L)	14.06	25	40	38.22	59.68	71.97	73.22
2	Hardness (mg/L)	1.21	8.04	39.87	65.24	76.34	79.51	79.60
3	TS (mg/L)	76.73	79.96	80.69	81.25	82.26	83.12	83.14
4	TSS(mg/L)	34.87	45.65	67.48	77.89	80.02	80.4	80.6
5	TDS(mg/L)	64.14	75.28	83.86	84.74	85.36	85.53	85.63
6	pH	25.92	31.48	33.33	33.33	33.33	33.33	33.33
7	Turbidity (620 nm)	13.31	27.3	59.65	81.59	82.70	84.10	88.27
8	Colour (490 nm)	44.12	62.48	70.21	73.59	76.54	80.76	84.51
9	Salinity(ppt)	1.78	8.92	10.71	10.71	28.57	28.57	28.57

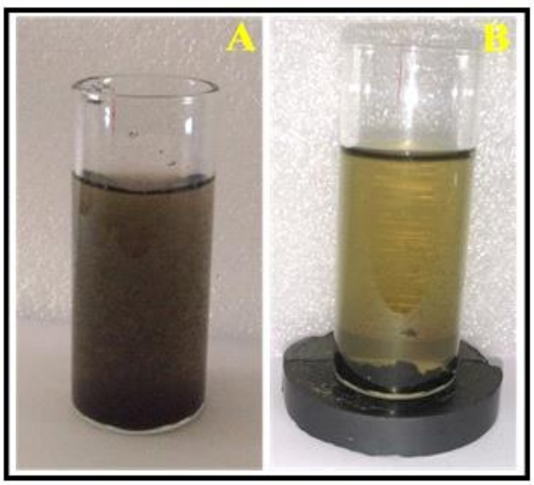
Table 6: Physico - chemical analysis of treated effluent sample with immobilized microbial consortia amended with Copper oxide nanoparticles on peanut husk

S. NO	Physico-chemical parameters	Percentage (%) Reduction of decolourization Number of days						
		3	**5**	**7**	**9**	**11**	**13**	**15**
1	COD (mg/L)	21.6	33.33	59.58	77.18	81.45	83.02	83.33
2	Hardness (mg/L)	23.65	26.82	81.7	93.90	95.60	95.60	95.85
3	TS (mg/L)	79.09	85.69	92.60	97.63	97.88	97.09	98.01
4	TSS(mg/L)	39.58	65.62	72.91	84.58	85.41	85.83	86.04
5	TDS(mg/L)	84.65	89.80	94.40	98.31	98.63	98.71	98.73
6	pH	16.66	24.07	33.33	33.33	33.33	33.33	33.33
7	Turbidity (620 nm)	37.02	61.11	74.22	93.35	94.54	94.98	95.10
8	Colour (490 nm)	44.73	69.85	93.91	94.62	95.02	95.12	95.22
9	Salinity(ppt)	1.78	3.57	28.57	28.57	28.57	28.57	28.57



No.	Diameter	Frequency	Cumulation	No.	Diameter	Frequency	Cumulation	No.	Diameter	Frequency	Cumulation
1	0.34	0.000	0.000	26	7.17	0.000	0.000	51	151.57	18.343	62.691
2	0.38	0.000	0.000	27	8.10	0.000	0.000	52	171.25	16.157	78.847

Figure 1: Particle size distribution of the Copper oxide nanoparticles



[A – Treated effluent with Copper oxide nanoparticles with absorbent; B– Seperation of sludge through permanent magnet in the treated effluent]

Figure 2: Sludge settlement seperation by permanent magnet