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Crepe Bandages Effluent Treatment Using *C.Vulgaris* and Nanomaterial

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Increased anthropogenic activities had led to greater contamination of water bodies which had resulted in paucity of water and depletion of water resources. Production in industries such as crepe cotton bandage processing factories has tremendously increased following COVID19 pandemic due to an unprecedented use of personal protective equipment's and surgical cottons and bandages. The effluent from such industries releases Chemical Oxygen Demand in range of 10 -15 g/L, Ammonia of 150 mg/L and pH > 10. Treatment of such effluents in an effective and economical way reduces water demand on natural sources and water reuse. In this study, photocatalysts such as TiO_2 , ZnO, $ACTiO_2$, AgZnO were found to be effective in Chemical Oxygen Demand reduction of real effluent by 37.8 ± 2.41%, 41.79 ± 2.44%, 39.92 ± 2.4%, and 68.94 ± 2.19% under visible light set up within 4hrs of incubation period. Biological systems, on the other hand consumes organic compounds as a source of carbon and energy. Microalgae, *Chlorella vulgaris* was found to remove 92.94% of Chemical Oxygen Demand within 5 days with simultaneous reduction in ammonia, nitrogen, and phosphate. The results show the efficiency of the photocatalysis and microalgae-based system to remove organic and inorganic pollutants from wastewater. A combined photocatalytic pre or post treatment to a biological system will allow to meet regulatory discharge standards.

Keywords: Biodegradation, Crepe bandage effluent, Microalgae, Nanoparticles, Photocatalysis

1 Introduction

With a market value of $15 billion, India is currently the third-largest cotton exporter in the world, after China and the European Union, and is anticipated to expand quickly[1]. Almost 81 percent of the country's entire textile industries are in Tamil Nadu, Gujarat, and Punjab. CPCB (Central Pollution Control Board), 2007 reported that Tamil Nadu ranked first with 741 textile mills. Mostly the textile wastewater (TWW) comprises of heavy metal, dyes, salts, highly loaded with organic, and inorganic pollutants[2,3]. There have been several procedures examined, including activated carbon adsorption, ozonation, ion exchange, flocculation, irradiation,[4-7] However, no single method can completely treat textile wastewater. The majority of these methods are expensive, inefficient, energy consuming, and also produce sludge[8,9]. Bioremediation of textile dyes has been investigated using a variety of microorganisms such as bacteria, yeast, fungi, and algae.[3,12] Microalgae, on the other hand, is more promising than other microorganisms because it can accumulate lipids that can be trans-esterified into biodiesel in addition with treating textile effluent by up taking nutrients and dyes as well[10,11].

Many crepe cotton bandages units and surgical cotton industries can be found in Tamil Nadu's southern region, and the state exports a huge amount of cotton products. Crepe cotton fabrics are extensively used in pharmaceuticals and healthcare facilities in a range of medical situations to provide insulation, warmth, and support around the world. Desizing, kiering, bleaching, mercerizing are the principal processing steps in the cotton industry, in which a lot of water is being used, and these are the major sources of suspended particles, chemical oxygen demand (COD), dissolved solids, biochemical oxygen demand (BOD), and inorganic nutrients such as nitrogen, phosphate, and sulphate. Along with this the cotton processing wastewater is highly loaded with total organic carbon where n-hexadecenoic acid, hydroxy cannabinol, and octadecenoic acid were detected as the potent organic compound[12]. Many textile wastewater treatment plants fail to fulfil IS discharge limitations, according to a study by[13].

Microalgae are photosynthetic microorganisms that absorb CO_2 and produce oxygen as a byproduct.

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Based on recent research it is shown that microalgae had shown a significant improvement in the treatment of wastewater. In the degradation studies of many pollutants from various wastewaters, several pure cultures or mixed microalgae culture have been examined. Numerous species of microalgae, including Chlorella sp., Scenedesmus sp., Nostoc sp., and Anabena sp., have been thoroughly investigated in the reduction of different contaminants[14]. Microalgae have been reported to have potential to degrade textile, municipal, palm oil, and even petroleum wastewater in current studies[15]. Along with this microalgae-based wastewater treatment are well investigated in case of microplastics, dyes, pesticides, heavy metals removal, pharmaceutical compound, and personal care products (PPCPs) removal from contaminated water[16-18]. Besides pollutant removal, microalgal biomass obtained from industrial wastewater has been utilized to make renewable fuels such as bioethanol, biobutanol, biodiesel, biohydrogen, and syngas[19]. Researchers have demonstrated the ability of microalgae to have metabolic diversity able to convert organic contaminants to beneficial molecules[20].

Apart from microalgae-based remediation, recent study shows that Nanotechnologies for environmental remediation identifies photocatalysis as the main technology breakthrough for water treatment. Photocatalysis is the advanced oxidation process which is used in current research as a propitious wastewater treatment technology for the complete disintegration, and mineralization of a broad array of organic contaminants including pesticides, different chemical dyes, phenolic and medicinal compounds, at ambient temperature and pressure. Different catalysts like titanium dioxide (TiO_2), zinc sulphide (ZnS), zinc oxide (ZnO), molybdenum trioxide (MoO_3), magnetite (Fe_3O), are used for the complete breakdown of organic pollutants from wastewater into a toxic inorganic compounds like H_2O, and CO_2[21]. One of the most significant features of TiO_2, and ZnO nanoparticles is 'photocatalysis'. The process by which the action of a substance is activated by absorbing a photon, is called photocatalysis[22]. Photocatalysis comprises of two crucial steps, the first requires activating the substrate by transferring energy in the form of a photon to it, and the second involves oxidizing the substrate, which is made simpler by the substrate's active state[23]. A degradation study of activated sludge supernatant from a wastewater treatment plant was done by using PVP-ZnO NPs (zinc oxide-polyvinylpyrrolidone nanoparticles), a modified ZnO Nanoparticle, where highest 95%reduction of COD, and 97% phosphate removal efficiency were achieved[24]. Photocatalytic degradation of aniline from aqueous solution was done by using Cr: ZnO NPs (chromium doped zinc oxide nanoparticles) under sunlight, and 95% of degradation of aniline was found within 6 hrs. of retention time[25]. The filtration and treatment of wastewater using nanoparticles has several advantages over reverse osmosis[26].

Only a few research works have been done on degradation of pollutants present in cotton processing wastewater, such as Electrocoagulation (EC) and electrooxidation (EO). Iridium dioxide-coated titanium (IrO2/Ti) and aluminum (Al/Al) electrodes were chosen as the anode and cathode for the EC-EO and EC tests, respectively. At the optimum operating conditions where EC process obtained- 76% COD removal, and EC-EO process achieved 97% COD removal rate[27]. Cotton dying wastewater was treated with carbonized titanium dioxide coated melamine foam, and resulted 90% degradation of dyes under the exposure of simulated sunlight within 120 minutes[28]. Surgical cotton industry effluent was treated by operating continuous fed up flow anaerobic microbial fuel cell at different Organic loading rate and maximum removal efficiency of 78.8% of total COD and 69% of soluble COD was obtained at an optimum organic loading rate of 1.9 g COD/l per day[29].

The objective of this study is to investigated the treatment of crepe bandage effluent using both photocatalysis and microalgae-based system to remove organic and inorganic pollutants from wastewater.

2 Materials and methods

2.1 Collection of Wastewater and Characterization

Crepe cotton processing industries are situated close to Rajapalayam town in southern region of Tamil Nadu. An adequate amount of wastewater samples was collected from the cotton processing industry. Before the effluent is discharged, a variety of dangerous chemicals such as peroxide, caustic soda as bleaching agents, and chlorine, hypochlorite is employed to mercerize the crepe cotton bandages[30]. Crepe bandage effluent was collected through a sterile 10L cane and was stored in refrigerator at -4°C to

avoid any further contamination. The physiochemical parameters such as conductivity and pH were tested every day. Phosphate, nitrate, ammonia, COD were tested once in five days by following the standard methods described in APHA[31].

2.2 Cultivation of Microalgae

The pure microalgae culture of C. Vulgaris were purchased from Phycospectrum, an Algal Research Institute, Chennai. They were grown in BBM (Bold Basal Medium). Media was prepared based on the composition of reagent listed in Table1. Culture was maintained in 25±2°C with 12:12 h light and dark condition. The Trace elements should be autoclaved to dissolve the reagents completely, and pH should be maintained at 6.8.

2.3 Photocatalysts used

Photocatalysis was carried out in three ways: under the exposure of solar light, visible light (145.6 mW/cm^2) and UV light (764mW/cm^2) and the reduction level in COD and pH was observed. Four different nanoparticles were selected based on photoreaction efficiency, and potential for economically efficient contaminant degradation i.e., Titanium dioxide (TiO_2), Zinc oxide (ZnO), Activated carbon titanium dioxide ($ACTiO_2$), and silver zinc oxide (AgZnO). Ag-ZnO nanorods were synthesized using hydrothermal process. A clear solution was prepared by dissolving 11.9 g of Zn $(NO_3)_2.6H_2O$ (0.4 M) and 0.128 g of AgNO3 (0.3 wt.% of Ag) in 100 ml of deionized water with stirring. To ensure complete precipitation, oxalic acid in distilled water (0.6M) solution was added dropwise with continuous stirring of 8 hours at 95°C. Silver ions and zinc oxalate were precipitated, and the mixture was then filtered before being rinsed with distilled water and ethanol and dried for 12 hours in the air at 90°C. To obtain Ag-ZnO nanoparticles, the precipitate was calcined at 450°C for 12 hours in a muffle furnace[32]. The Ag-ZnO nanoparticle was tested using Scanning Electron Microscope (SEM). 0.2g of each nanoparticle was added per litre of crepe bandage effluent separately in four different beakers for four hours to study the degradation potential of each photocatalyst. and calcined at 450°C for 12 hours to purify it. The Ag-ZnO nanoparticle was tested using Scanning Electron Microscope (SEM).

Table 1 — Composition of BBM Medium

Chemicals	Stock solution	Quantity
Macronutrients	(1L d.H_2O)	
KH_2PO_4	1.25	10 ml
K_2HPO_4	0.5	10 ml
$MgSO_4.7H_2O$	0.42	10 ml
$CaCl_2$ $2H_2O$	1.9	10 ml
$NaNO_3$	1.5	10 ml
NaCl	0.15	10 ml
EDTA solution		1 ml
EDTA	4.5	
KOH	3.1	
Acidified Iron Solution		1 ml
$FeSO_4.7H_2O$	0.0498	
H_2SO_4(ml)	0.05	
Boron solution		1 ml
H_3BO_3	1.15	
Trace metals solution		1 ml
$ZnSO_4.7H_2O$	0.89	
$MnCl_2.4H_2O$	0.144	
MoO3	0.071	
$CuSO_4.5H_2O$	0.16	
$Co(NO_3)_2.6H_2O$	0.05	
pH	6.9±0.1	

2.4 Experimental setup

Batch study was done using pure culture of *Chlorella Vulgaris.* The initial pH was maintained at 7.2±1 for each batch. To minimize the toxicity level, and eliminate the suspended particles, wastewater was filtered prior to the experiment. Four batches were prepared by different concentrations of wastewater, and BBM medium using 250 ml Erlenmeyer flask. Along with it one control was prepared by using pure wastewater shown in Table 2. Each sample and blank were tested and reduced the level of toxicity to observe the reduction efficiency of COD, phosphorus, ammonia, and nitrate. The batch study was performed in triplicates in a closed shaker at room temperature with proper exposure to light. On other hand four different types of nanoparticles like Titanium dioxide, zinc oxide, activated carbon TiO_2, activated carbon ZnO were used in three different light setups like solar light, UV light and Visible light. The changes in COD, and pH were determined in each hour interval from 1st to 4th hour.

Table 2 — Batch culture, and composition

Batch	Volume of crepe bandage effluent (ml)	Volume of BBM Medium (ml)	Microalgae culture
Blank (B1)	150	50	Absent
Sample 1 (S1)	20	180	Present
Sample 2 (S2)	13.33	186.7	Present
Sample 3 (S3)	10	190	Present
Sample 4 (S4)	8	192	Present

2.5 Analytical methods

For five days of the incubation period, the liquid test samples as well as the blank were analyzed at one-day intervals for microalgal batch study. For microalgal test samples, the initial pH was kept at 7.2, and further changes in pH in both test samples, and blank were examined for five days by using MK VI Digital pH Meter. Conductivity was measured by MK-509 Digital conductivity meter, Systronic India. The LABMAN Spectrophotometer (Model No. LMSP V325) was used to measure NO_3-N (Nitrate), NH_4-N (Ammonia), and PO_4^3—P (Phosphate) by following APHA standard procedures[31]. Centrifugation of microalgal sample Prior to wastewater analysis was done by REMI R-4C centrifuge. Total chlorophyll of *C. vulgaris* in each batch was estimated using the standard procedure for determining chlorophyll[33]. Along with-it reduction in COD, and pH of filtered raw wastewater were also observed by using four different nanoparticles within 4 hrs. of incubation period by following APHA standard methods[31].

3 Results and Discussions

3.1 Characteristics of real wastewater

Due to unrestricted use of caustic soda, peroxide, and hypochloride like harmful chemical during the manufacturing of crepe bandage, the pH of the crepe bandage effluent was found to be alkaline (9-11). Along with it near about 45000 m^3/ton, 25000 m^3/ton, and 32000 m^3/ton effluent has been generated from the kiering, bleaching and mercerizing process of crepe bandage industry. Physical and chemical metrics like BOD, COD, TDS were determined to be over the dischargeable limit and will cause ecological disruptions if released untreated or only partially treated. In this investigation, it was observed that crepe bandage effluent had a significant organic load, with 46 times greater COD, and having 10 times higher BOD than the textile wastewater discharge limit as per International Standard as seen in Table 3, inorganic contaminants like ammonia, nitrate, and phosphate were also observed to be rising.

Table 3 — Characteristics of crepe bandage effluent

Parameters	Range of values	I.S discharge limit of textile effluent
pH	9.5±0.8	5.5-9.0
Conductivity (ms/cm)	4.23±0.5	-
TDS (mg/l)	2200±50	500
COD (mg/l)	10520±288	250
BOD (mg/l)	305±5.59	30
Nitrate (mg/l)	42.193±2.185	10
Ammonia (mg/l)	115.59±2.516	5
Phosphate (mg/l)	62.67 ± 6.03	5

3.2 Microalgal batch study

No significant changes were observed in the pH value of Blank along with the test samples within five days as per the result shown in Table 4. Microalgae uses CO_2 as an inorganic carbon source and could change the pH of wastewater[34]. When CO_2 is absorbed into water it forms bicarbonate (HCO_3^-), and H^+ ions with the help of enzyme carbonic anhydrase. Now microalgae can easily consume this bicarbonate and utilize it for its growth[11,35]. Microalgae have inherent potency to assimilate the inorganic pollutants such as nitrate, phosphate, sulphate, and ammonia and utilize it for its growth[36]. The results obtained from the batch study showed almost 40-45% reduction in nitrate, 45-50 % removal in ammonia, and maximum reduction in phosphorus i.e., up to 20-25% was noticed in both Sample 1, and Sample 2 (Fig. 1). However, no variation in inorganic nutrient

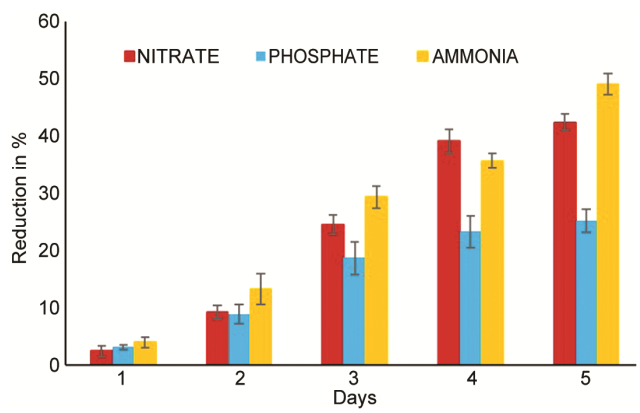

Fig. 1 — Reduction in nitrate, phosphate, and ammonia by *C.vulgaris*

Table 4 — Changes in pH by *C.vulgaris* during batch study

Sample	Day 0	Day 1	Day2	Day 3	Day4	Day 5
B1	9.53	9.5	9.51	9.49	9.45	9. 51
S1	7.02	7.26	7.56	7.87	7.91	8.36
S2	7.02	7.19	7.42	7.59	7.68	8.12
S3	7.02	7.12	7.39	7.68	7.93	8.02
S4	7.02	7.1	7.29	7.51	7.68	7.89

Table 5 — Microalgal species used for the remediation of textile effluent

Microalgae	COD reduction efficiency (%)	Time interval (days)	References
Chlorella vulgaris UMACC 001	38.3− 62.3	12	[38]
Chorella vulgaris	85.7	28	[39]
Chlorella sp.	89	5	[40]
mixture *of Synedra sp., Scenedesmus sp, Achnanthidium sp. and Chlorella sp.*	91.5	20	[41]
Chlorella sp G23	75 ±3	7	[42]
Chlorella sorokiniana	57.17	27	[43]
Chlorella vulgaris	92.94	5	Present study

concentration was detected in Blank(B1) within 5 days of batch study. Babu et al[30]., reported a 62% depletion in ammonia nitrogen, and 30% reduction in phosphate while cultivated *C. vulgaris* in surgical cotton industry wastewater for seven days. C. vulgaris strain was found to remove 94% nitrogen while cultivated in domestic wastewater for 12 days[37].

Although COD reduction by microalgae from various textile effluent has been reported for a longer time there is still no technical indication for crepe bandage effluent shown in Table 5. In crepe bandage effluent COD ranges between 10,000–11,000 mg/L (Table 3). Therefore, to reduce its turbidity, and suspended particles, this effluent was filtered prior to the batch experiment, so that the level of toxicity would be reduced to a certain level. Furthermore, in tannery, and textile effluents, microalgal culture of *Chlorella vulgaris* was found to remove 94% and 97% of COD, respectively[44]. For crepe bandages effluent, the highest efficiency of COD removal by *C. vulgaris* was noticed in S4 i.e. 92.94% as shown in Fig. 2 over a period of 5 days. This is most likely due to the presence of two organic contaminants named as octadecenoic acid, and hexadecenoic acids[30] seen to be degradable progressively[45]. These contaminants had a negative impact on biological systems and might be slowly reduced in diluted wastewaters. Control studies (i.e., wastewater samples without algal cells) showed no COD loss, indicating One or more biological mechanisms responsible for the dissipation of carbon from such effluent, rather than physical or chemical one[46].

Algal cells can convert inorganic carbon to organic forms in the photoautotrophic mode, whereas in the photoheterotrophic mode, assimilation of organic carbon takes place by microalgae in the Tricarboxylic Acid (TCA) cycle, which is driven by the Calvin cycle[47]. Different algal strains may remove organic materials from wastewater through different processes such as biodegradation, bioaccumulation, and biosorption[48]. The organic chemicals such as hydroxy

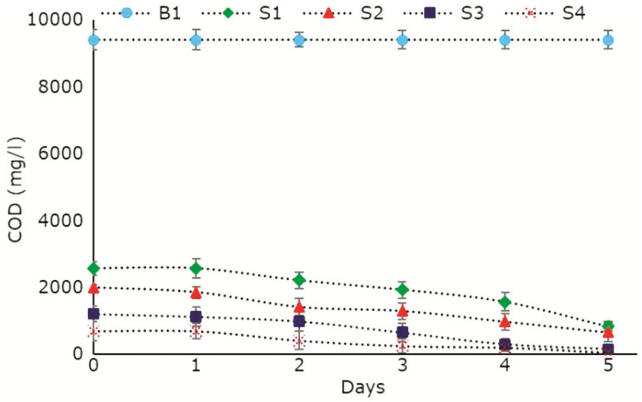

Fig. 2 — COD reduction in real effluent using *C.vulgaris*

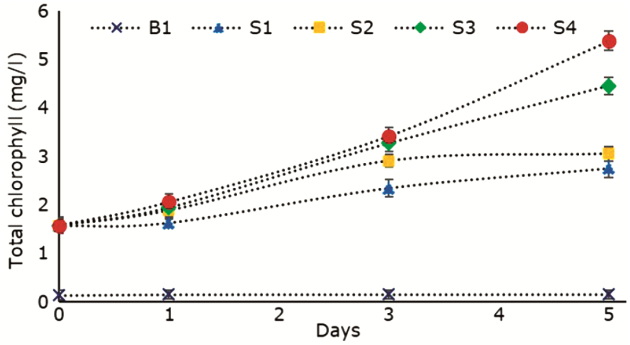

Fig. 3 — Total chlorophyll content of *C. vulgaris* in different batches

cannabinol, octadecanoic acid, and hexadecanoic acid, and present in wastewater was incorporated through the TCA cycle into the microalgae, as evidenced by the increase in total chlorophyll (chl. a, and chl. b) by shown in Fig. 3, increase in chlorophyll content in highest amount was observed in S4 (25 times diluted) test sample.

3.3 Photocatalytic treatment

In the present study four different types of nanoparticles such as Titanium dioxide (TiO_2), Zinc oxide (ZnO), activated carbon Titanium dioxide ($ACTiO_2$), and Silver Zinc oxide (AgZnO). Fig. 4 (A), (B), and (C) shows the Scanning Electron Microscope images of AgZnO, was synthesized by hydrothermal process.

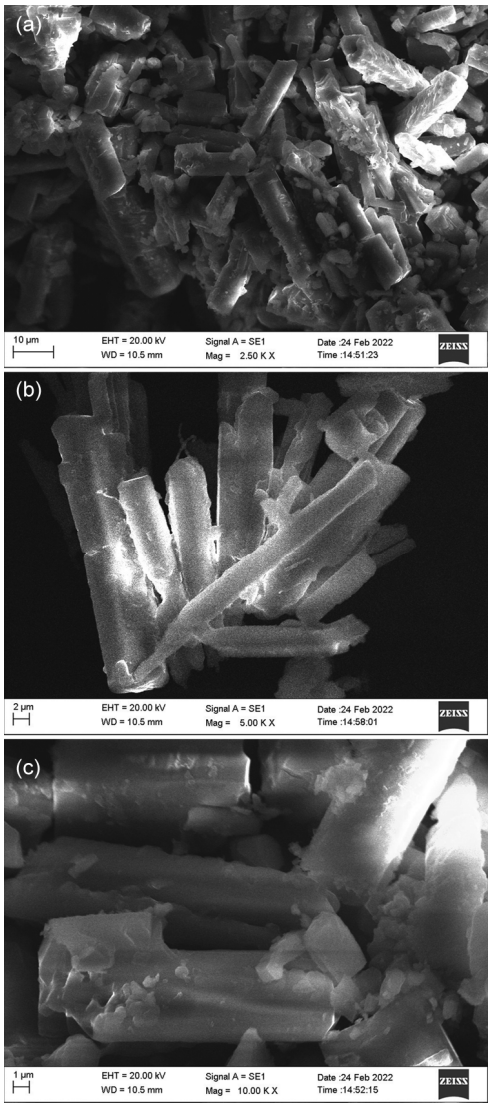

Fig. 4 — Scanning Electron Microscopic images of AgZnO at (a) Scanning Electron Microscopic image of AgZnO at 10 µm, (b) 2 µm, & (c) 1µm

All the four photocatalysts were used in three different light setups such as solar light, UV light and Visible light. The reduction level in COD, and pH for different catalysts were well studied within 4 hrs. of light exposure. Maximum removal of 71.48 ± 2.51% of COD was noticed when real effluent was treated by ZnO under UV light for 4 hrs. of retention period. Whereas lowest COD reduction was investigated by TiO_2, under the exposure of visible light i.e., 37.8 ± 2.41% shown in Fig. 5. The unique characteristics such as broad band gap, and less quantum yield of photo degradable semiconductors, restrict the use of photocatalysts in visible light[49].

However, $ACTiO_2$, and AgZnO showed a significant removal efficiency of COD in solar, visible, and UV light compared to TiO_2, and ZnO in Fig. 6. The removal efficiency of COD was shown by AgZnO in UV light i.e., 92.92 ± 2.39%, and in visible light AgZnO was also observed to reduce 68.94 ± 2.19% of COD. About 85% degradation of the dye methyl red in textile wastewater within 180 minutes under visible light by using Zinc nitrate doped TiO2 nanoparticle[50]. Apart from this the COD reduction efficiency of other reported photocatalyst w.r.t present study has been shown in Table 6.

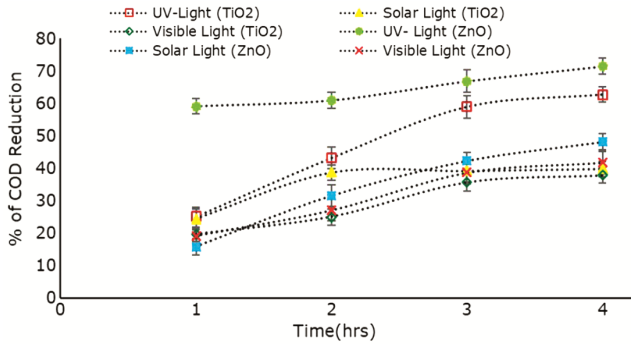

Fig. 5 — COD reduction in real effluent using TiO_2, and ZnO

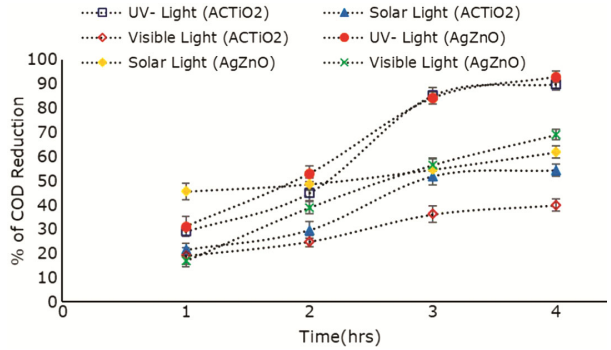

Fig. 6 — COD reduction in real effluent using $ACTiO_2$, and AgZnO

Table 6 — Nanoparticle used for the remediation of textile effluent

Nanoparticle	Light source	Reduction of COD (%)	Time intervals (hrs)	References
ZnO/persulfate	UV	96.62	5	[51]
Carbon black Nb_2O_5	UV	51.29	5	[52]
TiO_2	UVA	>90	3	[53]
ZnO/ZTO	Solar	>75	3	[54]
TiO_2	UVA	40	4	[55]
MgO	UVA	98.3	5	[56]
MgO	Solar	97.4	3	[57]
AgZnO	UV	92.92	4	Present study
AgZnO	Solar	61.81	4	Present study
AgZnO	Visible	68.94	4	Present study

4 Conclusion

The present study was aimed to learn about the capability microalgae *C. vulgaris* to degrade the organic and inorganic load of the crepe bandage effluent in different dilution rate. The results showed a positive impact on the microalgae capable to degrade the inorganic nutrients and reduce the chemical oxygen demand in the wastewater. On the other hand, the photocatalyst also showed some positive and effective results in different light sources and we could observe acceptable reduction in the COD levels by each of the nanoparticle. The study had given evidence that microalgae and photocatalysis can treat the wastewater efficiently.

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