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RESEARCH ARTICLE

Photocatalysis of Giemsa Dye: An Approach towards Biotechnology Laboratory Effluent Treatment

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Abstract: Present investigation analyzes various water quality parameters after TiO_2 assisted photocatalytic degradation of Giemsa Dye in aqueous suspension. Significant changes were noted in alkalinity, turbidity, hardness, nitrate, calcium ion concentration, chloride ion concentration, magnesium ion concentration, COD, BOD, sulfate ion concentration, temperature and pH. At pH 2 investigated parameters were found within the WHO standards of drinking water. Environmental risk assessment reveals that beside photocatalytic treatment, waste disposal methodology still needs to be accompanied with secondary treatment of water.

Keywords: Giemsa Dye, photocatalytical degradation, environmental risk assessment, biosafety

1 Introduction

Giemsa dye is extensively used to stain DNA molecules as a non radioactive marker in biotechnology laboratories. Giemsa dye polluted water (after treatment with DNA) is discarded into sink in various biochemical laboratories. Disposal of Giemsa dye into sanitary sewer or sink drain is not permitted over its hazardous concerns. Although it is an effective tool to visualize nucleic acid, but its hazardous properties require safe handling and disposal procedures. It pollutes ground water resources and poses a threat to environment while released from manufacturing units or laboratories. Several attempts have been made to degrade dyes by semiconductors and their conjugated nanoparticles but it is also notable to observe if any change occurred in water quality after photocatalytic treatment. Most of such photocatalytic treatment methodologies deal with semiconductors, their conjugates, initial dye concentration, catalyst doses, reaction temperature and light illumination/ exposure time, but remain silent over wide varieties of water quality parameters [1–3]. Here a first ever attempt has been made up to photocatalytically degrade Giemsa dye by using Tio_2 semiconductor. Photocatalytic degradation of Giemsa dye was evaluated on the basis of change in optical density at concerned λ_{max}. Quality of photocatalytically degraded Giemsa dye polluted water was evaluated by analyzing alkalinity, turbidity, hardness, nitrate, calcium ion concentration, magnesium ion concentration, COD, BOD, sulfate ion concentration, temperature and pH. Proposed investigation is carried out to analyze various water quality parameters of Tio_2assisted photocatalytic degradation of Giemsa dye. Such studies can be helpful in deciding whether photocatalytic water treatment methodologies may be appropriate up to an extent? These studies will redefine a road map that how such dyes polluted water and their photocatalytically degraded products should be disposed off/ affects environment.

2 Material and Method

2.1 Chemicals

Anatase Tio_2nano particles were kindly supplied by Chem Life Enterprises. Giemsa dye was purchased from Sigma Aldrich. Sodium fluoride, zirconium reagent, sodium arsenite, sodium azide, sodium iodide, $Na_2S_2O_3$, eriochrome black T indicator, di phenyl amine indicator were purchased from Qualigens; murexide indicator, starch indicator, ortho phosphoric acid, ferrous ammonium sulphate, methanol were from Fisher Scientific; EDTA, $K_2Cr_2O_7$, $AgNO_3$, AgCl, Ag_2SO_4, $FeCl_3$, $MgSO_4$, HCl, NaOH were from Central Drug House.

2.2 Photocatalysis of Giemsa Dye

A 100 ml solution of Giemsa dye (25 ppm) was prepared in tap water at different pH (2, 4, 6, 8, 10, and 12) to screen optimum pH for photocatalysis. Absorbance of these solutions was measured at λ_{max} 660.7 nm at 25^{o}C by using UV-Vis spectrophotometer (Jasco-630). The solutions were maintained in glass bottles (without lid) under sun light exposure (3000 Lux/h) for 4 h with suspended Tio_2nanoparticles (1000 ppm; without stirring) for photocatalytic degradation with control. Control was used to check solar assisted dye degradation for comparative analysis. Photocatalytically treated samples were centrifuged at 4000g for 5 minutes (REMI cooling centrifuge C 24). The supernatant was analyzed for optical density at λ_{max} 660.7 nm at 25^{o}C temperature to assess photocatalytic degradation of Giemsa dye at different pH (2, 4, 6, 8, 10, and 12). The pH at which maximum % change in absorbance was noticed after photocatalysis was considered as optimum pH.

2.3 Quantitative Analysis of Samples

The parameters (alkalinity, turbidity, hardness, nitrate, calcium ion concentration, magnesium ion concentration, COD, BOD (BOD incubator: Kumar Systems), DO, sulfate ion concentration) of photocatalytically treated and pretreatment samples were analysed at optimum pH. Estimation of nitrate; hardness, alkalinity, magnesium, COD, BOD, DO and sulfate were assessed by standard method of titration [4]. The obtained results were compared with standards for drinking water, World Health Organization [5].

2.4 HPTLC of Samples

Giemsa dye samples were analysed before and after photocatalytic treatment by High Performance Thin Layer Chromatography (CAMAG).

2.4.1 Chromatogram Layer

HPTLC Silica gel 60 F_{254} Aluminium sheet (*Merck*) 10x20 cm was prewashed by immersing in methanol and then dried overnight in an oven at 60^{o}C temperature. This chromatogram was used for chromatography.

2.4.2 Chromatography

Vertical Chromatography was executed with methanol (Fisher Scientific, 99.5% purity) as mobile phase on silica gel plate. Chamber was presaturated for 30 minutes with solvent. After attaining 80 % of migration distance, TLC plate was dried on the TLC plate heater at 60^{o}C for 10 minutes.

2.4.3 Scanning and Densitometry of Chromatogram

CAMAG TLC Scanner 3 with winCATS software was used for scanning the chromatogram developed on TLC sheet. The parameters were set as slit dimension 6 mm x 0.45 mm, scanning speed 20 mm/s and data resolution 100 micrometer/step.

2.5 Environmental Risk Assessment

Antimicrobial assay and seed germination tests were performed to assess risk upon exposure of Giemsa dye (before and after photocatalytic treatment) to environment. to environment. This investigation indicates the feasibility of photocatalytic degradation of Giemsa dye. Thus the end use/ disposal of degraded dye sample may be decided accordingly. *E. coli* VSBT.T.12.06 and *Vigna radiata* (L.) R. Wilczek were used as model organisms for the study.

2.5.1 Antimicrobial Assay

Antimicrobial assay was performed to assess impact on aquatic creatures. *E. coli* VSBT.T.12.06 bacteria was considered as model organism for antimicrobial assay.

Agar well diffusion assay was performed to assess hazardous risks associated with photocatalytically treated dye sample. Microbial culture of *E. coli* VSBT.T.12.06 was procured from Microbial Culture Collection (MCC), School of Biotechnology, Baramati, Pune, Maharashtra, India. Bacterial growth curve of *E. coli* VSBT.T.12.06 was determined with the aid of optical density at 600 nm (*Jasco 630* Spectrophotometer) to pick up log phase bacteria for antimicrobial assay. To determine the antibacterial activity of un-treated dye sample (10 ppm dye) and photocatalytically degraded dye samples (10 ppm dye + 1000 ppm Tio_2), qualitative investigation was executed with agar well diffusion method. Photocatalytically treated samples were centrifuged

at 4000g for five minutes and supernatant was used for the experiment. Sterile (alcohol + flame sterilized) cork borer was used to make wells (4 mm diameter) on nutrient agar poured petri plate. 100 μl inoculum of *E. coli* VSBT.T.12.06 (log phase, 1x10^5 cfu/ml) was seeded by a sterile spreader (alcohol + flame sterilized) on nutrient agar (*Hi media*) petri plate. 40 micro liter samples were poured into agar wells by using sterile micropipette. Tetracyclin was used as positive control. Zone of inhibitions around the well were considered as antibacterial activity. Experiments were done in triplicate for 24 hour incubation at 37^{o}C temperature.

2.5.2 Seed Germination

80 seeds of *Vigna radiata* (L.) R. Wilczek were allowed to germinate for two days in control (water), Giemsa dye polluted water (10 ppm, pH 2), photocatalytically degraded Giemsa dye (pH 2) and photocatalytically degraded Giemsa dye water adjusted at pH 7 to observe the impact on irrigation. Observed results were categorized as germinated seeds, ungerminated seeds and 'partial/ inhibited' germination.

3 Results and Discussion

3.1 Analysis of Water Quality

λ_{max} 660.7 nm for Giemsa dye was determined by using UV-Vis spectrophotometer (Jasco-630) at 25^{o}C. Among experimented pH (2, 4, 6, 8, 10, 12), maximum photocatalytic degradation (73.43%) of Giemsa dye was observed at pH 2 (Table 1). Photocatalytic efficiency has been determined based up on % decrease in Optical density. Various water quality parameters were studied in photocatalyzed dye sample and compared with pretreatment dye samples (Table 2). A significant change was noticed in each studied parameter. These parameters are compared with World Health Organization standards for drinking water (Table 2) [5].

3.2 Analysis of Photocatalytic Degradation

A change in molecular signature (absorbance spectra) was noted in before (Figure 1) and after (Figure 2) photocatalytic treatment of Giemsa dye at multi wavelength light exposure (200 nm -800 nm) indicating the degradation of Giemsa dye. Comparative analysis of HPTLC densitogram reveals that numbers of peaks are increasing upon addition of TiO_2nano particles in Giemsa dye water sample (Figure 3 and 4) . After photocatalytic degradation of Giemsa dye numbers of peaks in HPTLC densitogram are decreasing indicating that few degraded dye products might have escaped/ evaporated from treated Giemsa dye solution (Figure 5). Numbers of peaks in densitogram (Figure 5) illustrate the reason behind the decrease in turbidity of photocatalytically treated water sample compared to untreated Giemsa dye sample (Figure 3). Comparative analysis of HPTLC results show that photocatalytically degraded dye products exhibit the increasing absorbance in visible range of electromagnetic spectrum (Figure 6, 7 and 8). Further research may be focused on these degraded dye products to decolorize waste water disposal. Most of the compounds in all reported chromatograms possess low R_f values in methanol as mobile stream.

3.3 Environmental Risk Assessment

No antibacterial activity (zone of inhibition) against *E.coli* VSBT.T.12.06 could be noticed by agar well diffusion method at experimented dye concentration (10 ppm; before and after photocatalytic treatment of dye samples). It indicates that reference strain of bacteria (model aquatic organism) is quite tolerable to Giemsa dye and its photocatalyzed products at 10 ppm concentration. Higher doses should also be checked for further evaluation since concentration of Giemsa dye in laboratory disposed waste may even be higher. Bioaccumulation of photocatalyzed Giemsa dye products may enter in food chain and affect human health.

Compared to control (Figure 9), delayed germination was noticed in *Vigna radiata* (L.) R. Wilczek seeds exposed with dye samples (Figure 10, 11, and 12). Photocatalytic treatment of dye improved the seed germination chances but deformity in seedlings was observed (Figure 11). It indicates that photocatalyzed products are being assimilated by seeds and these products are interfering in normal development of seedlings. Photocatalytically degraded dye products seem to possess toxicity/ hazardous nature at pH 7 resulting in less germination of seeds (Table 3) (Figure 12). These findings confirm that these photocatalyzed Giemsa dye products have rigorously interfered normal development of seed. Delayed germination in seeds is evidenced from Figure 12. Deformity in seedlings could not be reported at this early developmental stage. Although 10 % increased seed germination (Table 3) is noted in photocatalyzed Giemsa

dye sample (Figure 12) compared to untreated Giemsa dye sample (Figure 10). But due to bioaccumulation of photocatalyzed Giemsa dye sample in seedlings (causing delayed seed germination and deformity in seedlings) such treatment methodology for waste disposal can not be recommended for irrigation purpose at this stage. These seedlings may further be analysed for bioaccumulation of photocatalyzed Giemsa dye sample which is likely to enter in food chain affecting human health.

Table 1 TiO_2 assisted photocatalytic degradation of Giemsa dye at different pH

pH	Pretreatment ODat λmax 660.7 nm	Posttreatment OD at λmax 660.7 nm	Photocatalytic efficiency %	Control (Solar treatment)	% Photocatalytic efficiency in control
2	0.64	0.17	73.43	0.5993	6.40 %
4	0.32	0.18	44.58	0.3176	0.96 %
6	0.25	0.18	29.68	0.2509	0.43 %
8	0.34	0.13	62.59	0.3148	9.56 %
10	0.30	0.16	46.13	0.2991	2.47 %
12	0.15	0.11	24.38	0.1410	5.81 %

Table 2 TiO_2 assisted photocatalytic degradation of Giemsa dye at pH 2

Parameters	Pretreatment	Posttreatment	WHO Standard (permissive)
Alkalinity	266.5 ppm	116.6 ppm	200 ppm
BOD	4.3 ppm	1.8 ppm	6 ppm
Ca^{2+}	28.0 ppm	20.0 ppm	75 ppm
Cl^{-}	213 ppm	165.6 ppm	200 ppm
COD	20.2 ppm	9.3 ppm	10 ppm
Hardness	381.3 ppm	296 ppm	100 ppm
Mg^{2+}	5.06 ppm	2.02 ppm	30 ppm
$NO_3{}^{-}$	2.33 ppm	1.55 ppm	50-100 ppm
$SO_4{}^{-2}$	38.73 ppm	22.70 ppm	200 ppm
TDS	111 ppm	99 ppm	500 ppm
pH	2	1.9	6.5-8.5
^{o}C	34.6	41.1	

Table 3 Seed germination in *Vigna radiata* (L.) R. Wilczek after exposure of two days in samples

Sample	pH	Germinated seeds	Un-germinated seeds	Partial/inhibited germinated Seeds
Tap Water (Control)	7	95%	3.75 %	1.25 %
Giemsa dye (10 ppm, pretreated)	2	0 %	60 %	40 %
Giemsa dye (10 ppm, photocatalytically degraded)	1.9	85 %	15 %	0 %
Giemsa dye (10 ppm photocatalytically degraded)	7	0 %	50 %	50 %

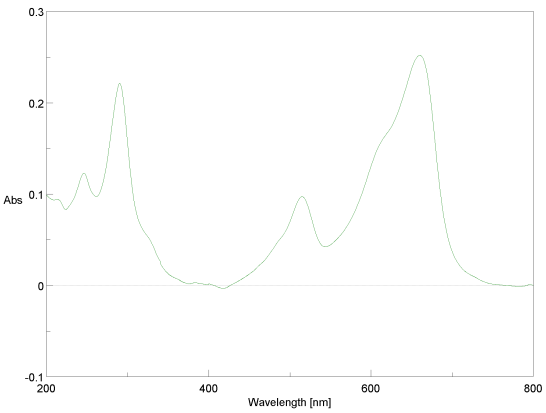

Figure 1 Absorbance spectra (molecular signature) exhibited by Giemsa dye on multi wavelength light exposure: Before photocatalytic treatment

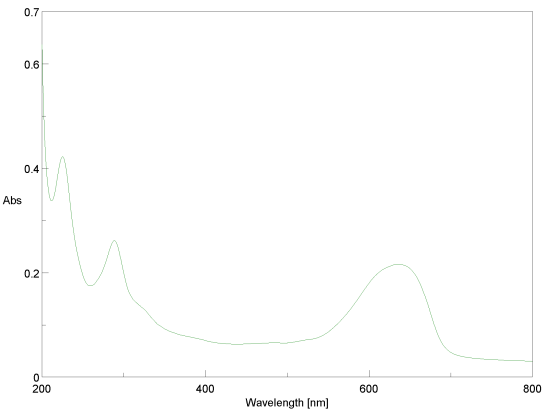

Figure 2 Absorbance spectra (molecular signature) exhibited by Giemsa dye on multi wavelength light exposure: After photocatalytic treatment

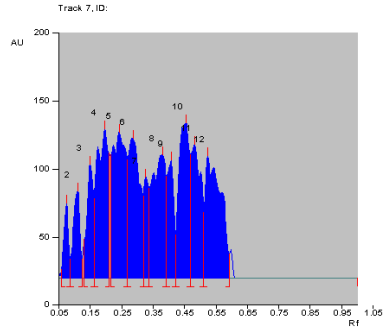

Figure 3 HPTLC Densitogram at 450 nm for Giemsa dye

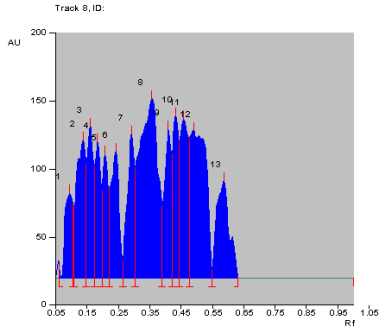

Figure 4 HPTLC Densitogram at 450 nm for Giemsa dye + TiO_2

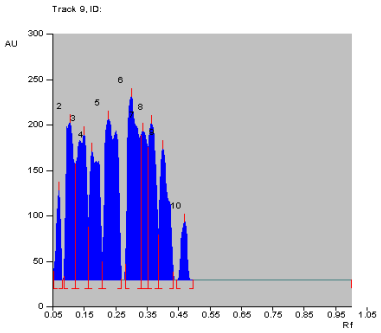

Figure 5 HPTLC Densitogram at 450 nm for photocatalyzed Giemsa dye + TiO_2

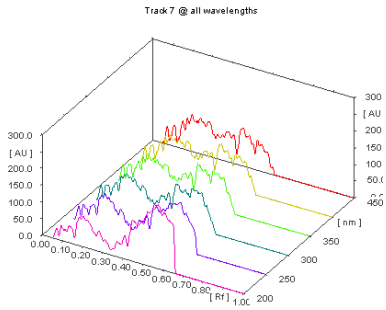

Figure 6 HPTLC: absorption spectra at multi wavelength for Giemsa dye

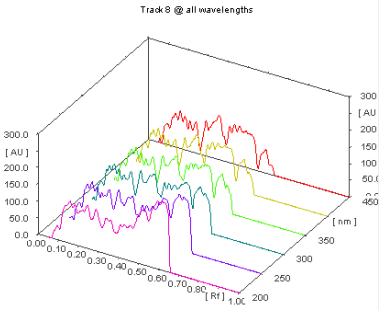

Figure 7 HPTLC: absorption spectra at multi wavelength for Giemsa dye + TiO_2 (without photocatalysis)

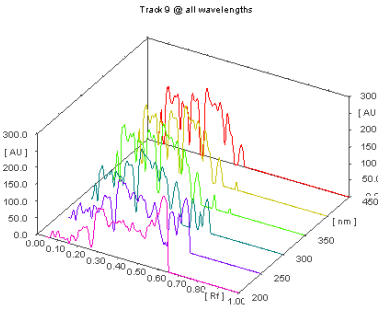

Figure 8 HPTLC: absorption spectra at multi wavelength for photocatalyzed Giemsa dye + TiO_2

Figure 9 Seed germination of *Vigna radiata* (L.) R. Wilczek in water at pH 7; after two days (control)

Figure 10 Seed germination of *Vigna radiata* (L.) R. Wilczek in water + Giemsa dye at pH 2; after two days

Figure 11 Seed germination of *Vigna radiata* (L.) R. Wilczek in water + photocatalyzed Giemsa dye at pH 1.9; after two days

Figure 12 Seed germination of *Vigna radiata* (L.) R. Wilczek in water + photocatalyzed Giemsa dye at pH 7; after two days

4 Conclusion

The use of nanocrystalline TiO_2 in the photocatalytic oxidation of organic molecules represents a promising remediation strategy for wastewater systems [6]. Researchers investigated photocatalytic efficiency of immobilized TiO_2 and in suspension [7–10]. Piscopo et al. investigated the effect of pH on photocatalytic degradation of organic compounds in aqueous solution of TiO_2 [11]. It is evident from Table 1 that Tio_2 exhibits maximum photocatalytic degradation of Giemsa dye at pH 2. Absorbance at λ_{max} 660.7 tends to decrease with reference to increase in pH. Due to exothermic oxidation process increase in temperature was noted in photocatalytic treatment of Giemsa dye at pH 2.

Wang et al. investigated TiO_2 assisted photocatalysis under visible light [12]. The use of high energy UV light is not only costly, but also can be hazardous [13]. Proposed investigation is first report on photocatalytic degradation of Giemsa dye by using TiO_2nano particles. The addition of transition metal ions like Au [14] and Ag [15] into the TiO_2 lattice increases the rate of photocatalytic oxidation in sunlight. Due to exothermic photo oxidation reaction a hike in water temperature (6.5^{o}C) was noticed. It is evident from comparative analysis of absorbption spectra (Figure 1 and 2) that after photocatalytic treatment, chemical environment/ chemical entities of solution have changed slightly. Photocatalytically degraded products of Giemsa dye must be analyzed further for their toxic, mutagenic, xenobiotic and carcinogenic nature to environment. Giemsa dye and its photocatalyzed products were not found toxic to *E.coli* VSBT.T.12.06 at 10 ppm concentration. Investigated parameters *i.e.* alkalinity, turbidity, hardness, nitrate, calcium ion concentration, magnesium ion concentration, COD, BOD, sulfate ion concentration were found to match WHO standards for drinking water after TiO_2 assisted photocatalytic treatment of aqueous Giemsa dye at pH 2. Immobilization techniques using quartz [16], silica, activated carbon [17], fiberglass, cloth, zeolites [18] and stainless steel can further nourish total dissolved solids in water. Experiments were carried out at optimum pH 2 to obtain maximum photocatalysis which can be tuned to pH 7 by secondary treatment of water. Investigation reflects that Tio_2nanoparticles may be used for degradation of Giemsa dye in research laboratories which pose a continual threat to environment but still it needs secondary treatment before it is disposed off into environment.

Conflicts of Interest

The author declares no conflict of interest.

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