Journal of
Environmental & Analytical Toxicology

Bharadwaj et al. J Environment Analytic Toxicol 2011, 1:4
DOI: 10.4172/2161-0525.1000113

Research Article Open Access

TiO2 Mediated Photocatalysis of Giemsa Dye: An Approach towards Biotechnology Laboratory Effluent Treatment

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Abstract

Photocatalysis offers low cost and durable treatment to dyes polluted effluent. But sometimes photocatalyzed dye intermediates appear to be more toxic than parent dye molecule. Present investigation uses suspended TiO_2 nanoparticles and sunlight irradiation for photocatalytic treatment of Giemsa dye at optimum pH. Giemsa dye has wide applications in molecular biology, cytology and histology laboratories across the world. Surplus and used dye is discharged in sink drain which is likely to cause hazardous concerns to environment. Paper analyzes toxicity concerns of treated and untreated Giemsa dye. Bioassays incorporating agar well diffusion assay and seed germination were employed to check the toxicity of water. Significant changes were noted in alkalinity, BOD, COD, Ca^{2+}, Mg^{2+}, NO_3^-, SO_4^{2-} ion concentration, hardness, temperature, turbidity and pH; and compared with standards for drinking water, World Health Organization. HPTLC of treated waste water confirmed that photocatalyzed dye intermediates exhibited increased absorbance in visible range of spectrum. At pH 2 various investigated parameters were found to match WHO standards for drinking water. Environmental risk assessment reveals that treated water possessed toxicity at pH 7 and was not found suitable for irrigation and potable purposes. 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; HPTLC

Introduction

Synthetic dyes have diverse applications in modern life, including but not limited to agriculture [1], food processing [2], hair colorings [3], leather tanning [4], medical [5], paper and pulp [6], research [7], textile industry [8] and many more. Synthetic dyes have such a great contribution to sophisticate human life that even we cannot imagine routine life without them. Roughly it is estimated that over 10, 000 tons per annum dye is produced globally [9]. If their production and application could discharge up to 10% dyes in environment, still it will cause hazardous concerns of environmental pollution [10] due to their stability, toxicity [11] and /or fast reactivity. Much hyped commercial dyes have sufficiently attracted scientist across the world for pollution, degradation and toxicity investigations [9]. Dyes, used in research and medical sector have been paid poor attention in this context, compared to much used industrialized dyes. Although net consumption of dyes used in research and medical sector is comparatively lesser than textile, leather and paper industries but these dyes may even pose superior risk of toxicity, mutation [12] and teretogenesis. Biosafety levels [13] and material safety data sheets [14, 15] have been designed and followed to deal with hazardous dyes in laboratories. Since these synthetic dyes are quite stable and toxic, disposal of hazardous laboratory effluent and dye wastage carries a challenging task. Present communication discusses about treatment and toxicity concerns of Giemsa dye. It is one of the most used dyes in biotechnology research laboratories. Giemsa dye exhibit specificity to bind with nucleotides [16]. Giemsa dye is extensively used to stain nucleic acid as a non radioactive marker in biotechnology, cancer, cytogenetics, histology and pathology laboratories [17-19]. Although it is used as gold standard in various research and diagnostic applications [19, 20] but due to its hazardous properties it requires safe handling and disposal procedures. Giemsa dye polluted water is discarded into sink in various biochemical laboratories. Disposal of hazardous dyes into sanitary sewer or sink drain should not be permitted over their hazardous concerns. It is likely to pollute ground water resources and pose a threat to environment while released from manufacturing units or laboratories. Several attempts have been made to degrade synthetic dyes by adsorption, coagulation [21], sedimentation, filtration, membrane technology [22], chlorination [23], AOPs [24], and biodegradation [25] but these have been diagnosed with high operational cost, toxic secondary pollutants, large amount of water and incomplete degradation [26]. Semiconductors and their conjugated nanoparticles are preferred over low operating cost and effective treatment [27] but it is also notable to observe if any changes 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, [28-30] but remain silent over wide varieties of water quality parameters and toxicity concerns. Photocatalytic treatment of dyes can generate even more toxic intermediates, so we recommend that design of experiment should always be enabled with bioassays [31] to check the efficacy of treatment. We have made a first ever attempt here to assess the risk of degraded Giemsa dye by bioassays and water quality analysis. Giemsa dye is photocatalytically degraded under sun light by TiO_2 nanoparticles under constant conditions. Quality of treated water was evaluated by analyzing alkalinity, BOD, COD, Ca^{2+}, Mg^{2+}, NO_3^-, SO_4^{2-}, hardness, temperature, turbidity and pH; and compared with

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Received October 19, 2011; **Accepted** December 07, 2011; **Published** December 09, 2011

Citation: Bharadwaj L, Bhardwaj M, Sharma MK (2011) TiO2 Mediated Photocatalysis of Giemsa Dye: An Approach towards Biotechnology Laboratory Effluent Treatment. J Environment Analytic Toxicol 1:113. doi:10.4172/2161-0525.1000113

standards for drinking water, World Health Organization [32]. Agar well diffusion assay has been performed to assess toxicity of treated and untreated water against *E. coli*. Since *E. coli* belongs to lower trophic level, it can profoundly influence energy pyramid and food chain in ecosystem. Seeds of *Vigna radiata* (L.) R. Wilczek were germinated in treated and untreated water to assess the probable hazardous risks to agriculture sector. The paper investigates whether TiO_2 mediated photocatalytic treatment may be appropriate option to degrade Giemsa dye.

Material and Method

Chemicals

Anatase TiO_2 nano 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.

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˚C by using UV-Vis spectrophotometer (Jasco-630). The solutions were maintained in glass bottles (without lid) under sun light exposure (3000 Lux/h, Metek digital lux meter 101 b, terrestrial coordinates: 18.15˚N 74.58˚E) for 4 h with suspended TiO_2 nanoparticles (1000 ppm; without stirring) for photocatalytic degradation with control. Control was used to check solar assisted dye degradation for comparative analysis. Treated water was centrifuged to sediment the mineralization at 4000g for 5 minutes (REMI cooling centrifuge C 24). Supernatant was analyzed for optical density at λ_{max} 660.7 nm at 25˚C temperature to assess photocatalytic degradation of Giemsa dye at different pH (2, 4, 6, 8, 10, and 12). The pH at which maximum % photocatalytic efficiency recorded, was considered as optimum pH. % photocatalytic efficiency was derived as 100(A-B)/A, where A and B were OD for untreated and treated water.

Photocatalytically treated and untreated water samples were analyzed at optimum pH for alkalinity, BOD, COD, Ca^{2+}, Mg^{2+}, NO_3^-, SO_4^{2-}, hardness, temperature, turbidity and pH [33]; and compared with standards for drinking water, World Health Organization [32].

HPTLC

Treated and untreated water samples were analyzed by High Performance Thin Layer Chromatography (CAMAG). Prewashed (methanol, dried overnight at 60˚C) silica gel plate (HPTLC Silica gel 60 F_{254} aluminium sheet; *Merck*) was used for chromatography. Vertical Chromatography was executed with methanol (Fisher Scientific, 99.5% purity) as mobile phase. Chromatographic chamber was pre-saturated for 0.5 h with solvent. After attaining 80% of migration distance, TLC plate was dried on the TLC plate heater at 60˚C for 10 minutes. CAMAG TLC Scanner 3 (winCATS software) was used for scanning the chromatogram. The parameters were set as slit dimension 6 mm x 0.45 mm, scanning speed 20 mm/s and data resolution 100 µm/step.

Bioassays

Agar well diffusion assay was performed to assess toxicity associated with treated (10 ppm dye + 1000 ppm TiO_2) and untreated water (10 ppm dye). Microbial culture of *E. coli* VSBT.T.12.06 was procured from Microbial Culture Collection (MCC), V.P. 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 assay. Treated water was centrifuged at 4000g for 5 minutes and supernatant was used for the experiment with tetracyclin as positive control. Sterile (alcohol + flame) cork borer and spreader were used for experiments. 100 µl inoculum of *E. coli* VSBT.T.12.06 (log phase, $1x10^5$ cfu/ml) was seeded on to nutrient agar (Hi media) poured petri plate. 40 µl samples were poured into agar wells; experiments were done in triplicate for 24 h incubation in dark at 37˚C temperature. Zone of inhibition around the well was considered as antibacterial activity [34].

80 seeds of *Vigna radiata* (L.) R. Wilczek were allowed to germinate for 2 days in control (water), untreated (10 ppm, pH 2), treated (pH 1.9) and treated water adjusted at pH 7 to observe the impact upon irrigation. Observed results were categorized as germinated seeds, un-germinated seeds and 'inhibited (partial)' germination.

Results and Discussion

Photocatalysis

Maximum 73.43% photocatalytic degradation could be achieved at pH 2 by suspended nanoparticles of TiO_2 (Table 1). At other experimented pH, interaction between TiO_2 and dye seems to be minimal due to the absence of adequate electrostatic forces. Maximum 9.56% photolytic efficiency was observed in negative control at pH 8. Position of conduction and valence bands are influenced by pH. pH influences surface charge on catalyst hence, size of catalyst aggregates and isoelectric pH are affected [35]. Thus photocatalytic degradation efficiency is evidenced to be affected by pH. Incomplete degradation at different pH can yield diverse intermediates. Photocatalyzed intermediates seems to have slightly different functional groups [36] compared to parent dye, hence a minor decrease in pH is noted along with altered cationic and anionic concentration (Table 2). A change in molecular signature (absorbance spectra) is noted after photocatalytic treatment indicating the degradation of Giemsa dye. Comparative analysis of HPTLC densitogram reveals that numbers of peaks are increasing upon addition of TiO_2 nanoparticles in Giemsa dye water sample (Figure 1a and 1b). After photocatalytic degradation of Giemsa dye numbers of peaks in HPTLC densitogram are decreasing indicating that few degraded dye products might have sedimented or evaporated from treated water (Figure 1c). Numbers of peaks in densitogram (Figure 1c) illustrate the reason behind the decrease in turbidity of photocatalytically treated water (Figure 1c). Comparative analysis of HPTLC results show that photocatalytically degraded dye products exhibit the increased absorbance in visible range of electromagnetic spectrum (Figure 2a, 2b and 2c). Further research may be focused on these degraded dye products to decolorize treated waste water. Most of the compounds in all reported chromatograms possess low R_f values in methanol as mobile stream. Water quality is compared with standards for drinking water, World Health Organization (Table 2). At high temperature charged chemical species may be recombined and adsorption of dye intermediates may be inhibited on catalyst [37]. Optimum reaction temperature for photomineralization is reported to be in the range of 20-80˚C [38]. Dissolved oxygen [39] and stirring affects the photocatalytic efficiency. Oxygen can serve as electron sink to trap the excited conduction-band electron from reactive oxygen species. Dissolved oxygen is proposed to cleave aromatic ring [40] of

pH	OD Untreated water	% Photocatalytic efficiency	%Photolytic efficiency (control)
2	0.64	73.43	6.40 %
4	0.32	44.58	0.96 %
6	0.25	29.68	0.43 %
8	0.34	62.59	9.56 %
10	0.30	46.13	2.47 %
12	0.15	24.38	5.81 %

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

Parameters	Untreated water	Treated water	WHO Standard
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
Temperature ˚C	34.6	41.1	Not available

Table 2: Quantitative analysis of water quality.

Culture medium	pH	Germinated	Un-germinated	Inhibited (partial) germination
Tap Water (Control)	7	95%	3.75 %	1.25 %
Giemsa dye (10 ppm, untreated)	2	0 %	60 %	40 %
Giemsa dye(10 ppm, photocatalytically treated)	1.9	85 %	15 %	0 %
Giemsa dye(10 ppm, photocatalytically treated)	7	0 %	50 %	50 %

Table 3: Seed germination in *Vigna radiata* (L.) R. Wilczek after 2 days.

dye molecules. Solar irradiation consisting UV-A (315 to 400 nm; 3.10-3.94 eV) and UV-B (280 to 315 nm; 3.94-4.43 eV) provide sufficient photons for the activation of TiO_2 catalyst [41]. UV-C (100 to 280 nm) is absorbed by earth's atmosphere and does not reach to earth surface however; UV lamp may be employed for UV-C irradiation. Fujishima and associates claimed that initiation of TiO_2 photocatalytic reaction rate is not critically dependent on light intensity, since a few photons of energy ($1\mu Wcm^{-1}$) can sufficiently induce the surface reaction [42]. Doped TiO_2 have been investigated for effective utilization of photoillumination [43-45]. Doping introduces impurity in the band gap of TiO_2 and thus, reduces the photonic energy requirements. We investigated with justified ratio of Giemsa dye and catalyst to prohibit photocatalyst deactivation [46]. Oxidants and immobilization techniques have been investigated for photocatalytic treatment efficiency [47-50]. Photocatalyzed dye intermediates can cause turbidity in reactor leading to decreased photocatalytic efficiency [51]. Moreover, inorganic ions can also influence photocatalytic efficiency [52-54]. Salt formation in reactor diminishes colloidal stability resulting decreased photocatalysis [55].

Environmental risk assessment

Agar well diffusion assay confirms no antibacterial activity of treated and untreated water (10 ppm) against *E.coli* VSBT.T.12.06. It indicates that reference strain of bacteria (model aquatic organism) is quite tolerable to Giemsa dye and its photocatalyzed products at 10 ppm concentration. Reactive oxygen species and oxidative/ toxic intermediates in treated water can damage bacterial cell leading to lipid peroxidation and homeostasis impairment [56]. Due to fast repair mechanism bacteria could better adapt themselves in changed chemical climate. TiO_2 photocatalyst have earlier been reported to inactivate *E.coli* [57, 58] and cause ecotoxicity [59] but here bacterial incubation was carried out in dark (absence of photoillumination) so there were no chances of photocatalytic disinfection. Cytochrome P450s genes have earlier been reported to metabolize xenobiotics [60]. Higher doses should also be checked for further evaluation since concentration of Giemsa dye in laboratory disposed waste may even be higher. Ethyl red, methyl red, reactive red and other azo dyes have earlier been reported to be degraded by *Escherichia coli* and other microbes [61-63]. Bioaccumulation of photocatalyzed Giemsa dye products must be investigated further because these may enter in food chain and affect human health.

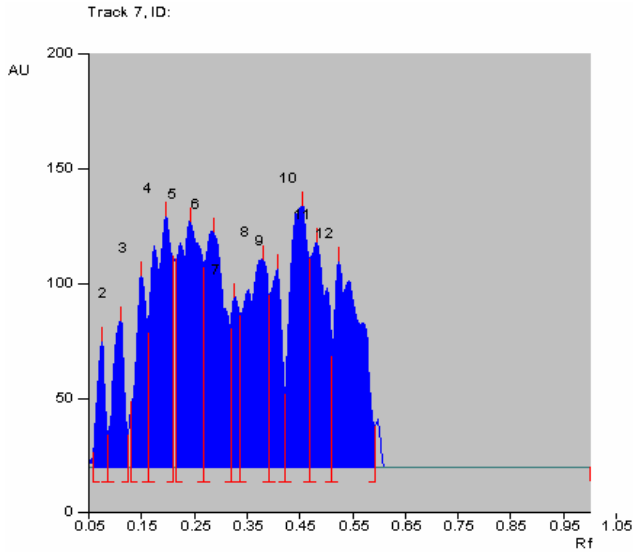

Figure 1a: HPTLC Densitogram at 450 nm for Giemsa dye.

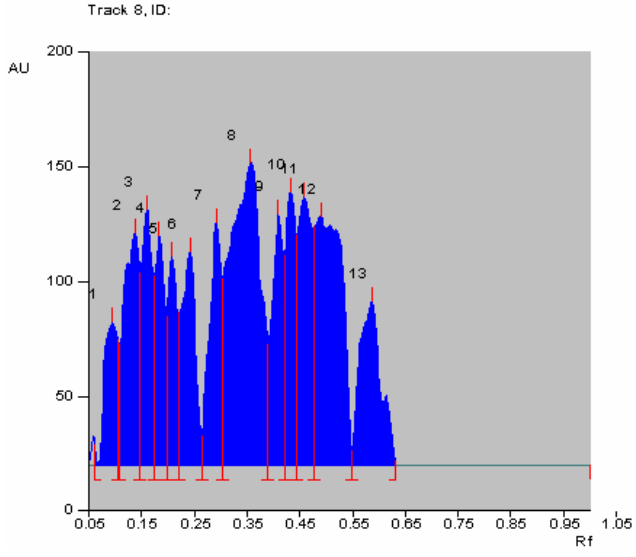

Figure 1b: HPTLC Densitogram at 450 nm for Giemsa dye + TiO_2.

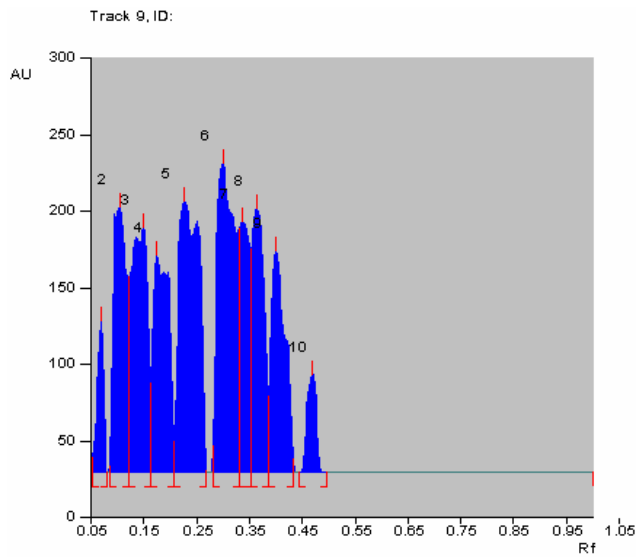

Figure 1c: HPTLC Densitogram at 450 nm for photocatalyzed Giemsa dye.

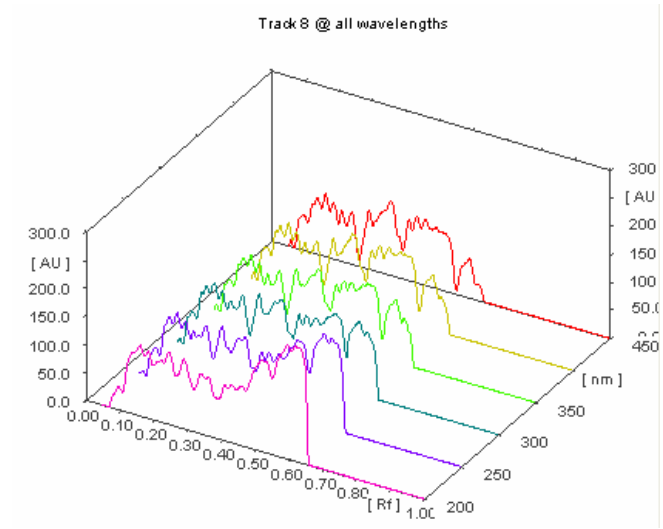

Figure 2b: HPTLC absorption spectra at multi wavelength for Giemsa dye + TiO_2 (without photocatalysis).

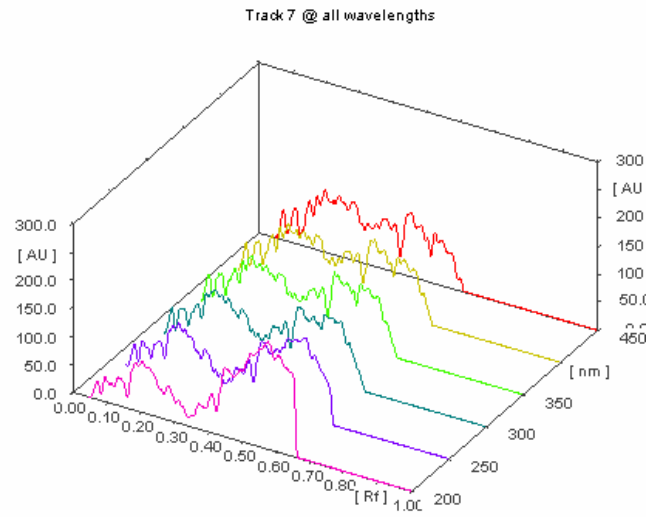

Figure 2a: HPTLC absorption spectra at multi wavelength for Giemsa dye.

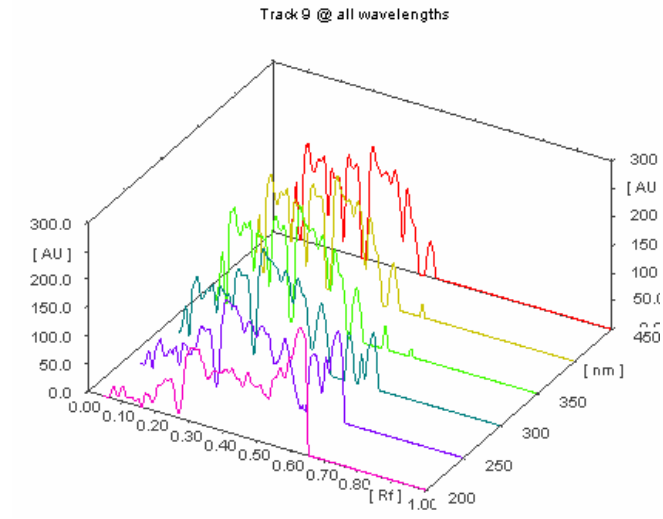

Figure 2c: HPTLC absorption spectra at multi wavelength for photocatalyzed Giemsa dye + TiO_2.

Seed germination assay confirmed delayed germination in *Vigna radiata* (L.) R. Wilczek seeds exposed with dye samples (Table 3). Photocatalytic treatment of dye improved the seed germination chances (45%) but at pH 7 degraded dye products seem to possess toxicity/ hazardous nature resulting in less germination of seeds. pH can influence permeability of molecules through cell membrane. It indicates that photocatalyzed products are being assimilated in seeds and these products are interfering in normal development of seedlings [64]. At pH 7 these degraded products may inhibit gene expression via DNA interaction and must be investigated for nucleotide binding specificity if any. Deformity in seedlings could not be reported at this early developmental stage. Despite, several water quality parameters are in accordance with World Health Organization's standards for drinking water but such treatment methodology cannot be recommended for irrigation purpose at this stage due to bioaccumulation of photocatalyzed Giemsa dye and delayed seed germination. These seedlings must further be analyzed for bioaccumulation of photocatalyzed Giemsa dye sample which is likely to enter in food chain affecting human health. We recommend that photocatalyzed dye intermediates should further be degraded by genetically modified organisms and ecotoxicity of nanoparticles should be evaluated before treated water is discharged from research laboratories.

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