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RESEARCH ARTICLE

Advanced Oxidation Using TiO_2 Environmental Detoxification Strategy: Photodegradation of Ethidium Bromide

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Abstract: Ethidium bromide dye is extensively used in biotechnology laboratories. Being a hazardous dye, laboratory effluent severely affects environment and requires safe disposal. Present paper investigates photocatalytic degradation of Ethidium bromide dye before it is released into environment. Environmental risk assessment reveals that TiO_2 assisted photocatalytic treatment of Ethidium bromide laboratory waste can be recommended as safe to environment but it needs to be accompanied with tertiary treatment before used widely.

Keywords: biosafety, Ethidium bromide dye, TiO_2, photocatalysis, environmental risk assessment

1 Introduction

Ethidium bromide dye is among hazardous effluent from analytical (biotechnology/ molecular diagnostic) laboratories and manufacturing units worldwide. It pollutes ground water resources and violets biosafety issues severely, while disposed in sink drain untreated. Disposal of Ethidium bromide into sanitary sewer or sink drain is not permitted over its hazardous concerns. Disposal of hazardous dye requires safe handling and effluent treatment to align with biosafety issues [1]. Before these degraded dye products are released into ecosystem, it must be confirmed whether water quality parameters lie in acceptable range. Earlier study investigates photocatalytic degradation of Ethidium bromide dye with TiO_2, but most of such studies remain silent over wide varieties of water quality parameters and environmental risk assessment [2]. Here investigation is carried out to photocatalytically degrade Ethidium bromide by TiO_2 nanoparticles to check the biosafety of hazardous waste [3]. To evaluate the efficacy of treatment, quality of hazardous waste water is examined and compared with standards for drinking water, World Health Organization. Antimicrobial studies are executed to observe any ill effect on aquatic organisms. Proposed investigation will redefine a road map if photocatalytic water treatment methodologies may be appropriate up to an extent?

2 Material and methods

2.1 Chemicals

TiO_2 nano particles were kindly supplied by Chem Life Enterprises [4]. 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 and methanol were purchased from Fisher Scientific. EDTA, $K_2Cr_2O_7$, $AgNO_3$, AgCl, Ag_2SO_4, $FeCl_3$, $MgSO_4$, HCl, NaOH were purchased from Central Drug House.

2.2 Photocatalysis of Ethidium bromide dye

A 100 ml solution (25 ppm) of Ethidium bromide dye was prepared in tap water at different pH (2, 4, 6, 8, 10, and 12) to screen optimum pH for photocatalysis. Absorbances of these solutions were measured at λmax 284.3 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) for 4 h with suspended TiO_2nano particles (1000 ppm; without stirring) for photocatalytic degradation with control [5]. 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} at 25°C temperature to assess photocatalytic degradation of Ethidium bromide dye at different pH (2, 4, 6, 8, 10, and 12). The pH, at which maximum % change in absorbance observed, was considered as optimum pH for all assigned experiments. Photocatalytic efficiency % was derived as 100(A-B)/A, where A is Pretreatment OD and B, Posttreatment OD.

2.3 Quantitative analysis of samples

The parameters (alkalinity, turbidity, hardness, nitrate, calcium ion concentration, magnesium ion concentration, COD, BOD (BOD incubator: Kumar Systems), sulfate ion concentration) of photocatalytically treated and pretreatment samples were analyzed at optimum pH. Estimation of nitrate, hardness, alkalinity, magnesium, COD, BOD, DO and sulfate were assessed by standard method of titration [6]. These results were compared with standards for drinking water, World Health Organization [7].

2.4 Environmental risk assessment

Antimicrobial assay was performed to assess risk upon exposure of Ethidium bromide dye (before and after photocatalytic treatment) to environment. This investigation evaluates the feasibility of photocatalytic degradation of Ethidium bromide dye. Thus disposal of degraded dye sample may be decided accordingly. *E. coli* VSBT.T.12.06 was used as model organisms for the study.

2.4.1 Antimicrobial assay

Antimicrobial assay was performed to assess effect of photocatalytically disposed dye 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. Agar well diffusion method was executed to determine the antibacterial activity of un-treated dye sample (3.33 ppm) and photocatalytically degraded dye samples (3.33 ppm dye + 1000 ppm TiO_2). Photocatalytically treated dye samples were centrifuged at 4000g for 5 minutes and supernatant was used for the experiment. Double sterilized (alcohol + flame) 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, 1×10^5 cfu/ml) was seeded by a sterile spreader (alcohol + flame sterilized) on nutrient agar (Hi media) poured petri plate. 40 μl samples were poured into agar wells by using sterile micropipette. Tetracyclin (30 μg/ml) was used as positive control. Zone of inhibition around the well was considered as antibacterial activity. Experiments were done in triplicate for 24 h incubation at 37°C [8].

3 Results and Discussion

3.1 Analysis of water quality

λ_{max} 284.3 nm was determined for Ethidium bromide dye at 25°C temperature. λ_{max} of a molecule is sensitive to change in pH and temperature. Among experimented pH (2, 4, 6, 8, 10, and 12) maximum photocatalytic degradation (50.14%) of dye was observed at pH 10, hence regarded as optimum pH for photocatalysis (Table 1) [9, 10]. Negative control was used to check solar assisted dye degradation for comparative analysis. Maximum (7.53%) photodegradation of dye was noted at pH 6 in negative control. Results for negative control were found unsatisfactory ($<$ 10% at all experimented pH). Photocatalytic efficiency has been determined based upon % decrease in Optical density. Photocatalysis, being an exothermic oxidation process leads minor increase in temperature of dye treated water which may affect susceptible organisms if exposed directly into ecosystem. Various water quality parameters were studied in photocatalyzed dye sample and compared with pretreatment dye samples (Table 2). Significant changes were noticed in studied parameters. These parameters are compared with standards for drinking water, World Health Organization (Table 2) [6]. Only hardness of solution was found higher than WHO standards for drinking water at tested pH 9.2. Unless tertiary treatment is done to reduce hardness of photocatalyzed dye polluted water, it can not be recommended yet as potable and usable for irrigation even after neutralization of pH.

Table 1 TiO_2 assisted photocatalytic degradation of Ethidium bromide dye at different pH

pH	Pre-treatment OD	Post-treatment OD	Photocatalytic efficiency %	Negative Control (Solar treatment)	Photocatalytic efficiency % in negative control
2	0.34	0.18	47.24	0.33	2.85
4	0.33	0.19	41.02	0.32	2.20
6	0.32	0.16	49.75	0.30	7.53
8	0.32	0.17	47.73	0.31	3.50
10	0.32	0.16	50.14	0.31	3.08
12	0.33	0.17	48.85	0.33	0.12

Table 2 TiO_2 assisted photocatalytic degradation of Ethidium bromide dye at pH 10

Parameters	Pretreatment	Posttreatment	Permissive WHO standard
Alkalinity	300 ppm	150 ppm	200 ppm
BOD	4.5 ppm	2.1 ppm	6 ppm
Ca^{2+}	24.0 ppm	16.0 ppm	75 ppm
Cl^{-}	213 ppm	142 ppm	142 ppm
COD	21.7 ppm	6.1 ppm	10 ppm
Hardness	386.6 ppm	298.6 ppm	100 ppm
Mg^{2+}	5.22 ppm	1.74 ppm	30 ppm
$NO_3{}^{-}$	2.4 ppm	2.08 ppm	50-100 ppm
$SO_4{}^{-2}$	39.0 ppm	25.91 ppm	200 ppm
TDS	160 ppm	110 ppm	500 ppm
pH	10	9.2	6.5-8.5
Temperature °C	32.2	32.9	

3.2 Analysis of photocatalytic degradation

Table 1 displays % photocatalytic efficiencies at various experimented pH. Photocatalysis is susceptible to pH variation [11]. pH affects the surface charge of semiconductors and can also lead to aggregation of dye molecules in diluted solutions [12]. It is quite obvious to observe a decrease in OD after photocatalysis since these products have settled down or may have evaporated depending upon their boiling point [13]. Remaining degraded dye products in solution may be evaluated further for tertiary treatment and effect upon ecosystem. Photocatalyzed products are subject to change in catalysts. Further research may be focused on these degraded dye products to decolorize waste water dispose [14].

3.3 Environmental risk assessment

Antibacterial activity (zone of inhibition) against *E.coli* VSBT.T.12.06 could be noticed by agar well diffusion method at experimented dye concentration (3.33 ppm, before and after photocatalytic treatment of dye samples). Zone of inhibition, corresponding to photocatalyzed dye sample is found to be reduced significantly (Table 3). It indicates that photocatalyzed dye products may not be harmful to reference strain of bacteria (model aquatic organism). Resultant zone of inhibition in photocatalyzed dye sample may have appeared due to remaining parent dye molecules. Results confirm that sink drain of Ethidium bromide dye is hazardous to aquatic life even at low concentration (3.33 ppm). Concentration of Ethidium bromide dye in laboratory disposed wastage/ sink drain may even be higher across the globe. These results confirm that dye sample is being assimilated in test organism and these products have interfered normal development of bacteria/ aquatic creatures. Although, photocatalysis could decline the hazardous concerns of Ethidium bromide dye, significantly but it is also more important to check mutagenic activities of degraded dye products before they are released into environment [15].

Table 3 Antimicrobial activity (zone of inhibition in mm ± SD) of dye samples

Ethidium bromide dye	Photocatalyzed Ethidium bromide dye	Tetracyclin
18 ± 1.33	5 ± 1	21±1.5

TiO_2 catalyzed photodegradation of Ethidium bromide need tertiary treatment, before it is recommended for irrigation purpose. The test organism should further be analyzed for bioaccumulation of photocatalyzed Ethidium bromide dye which are likely to enter in food chain, affecting ecosystem.

4 Conclusion

Metals are detoxified by phytochelatins in plants. Excess production of phytochelatins can even detoxify essential metals in plants as an adaptive response to environmental stress. Keeping this in view, immobilization of photocatalyst (on glass, quartz, silica, activated carbon, fiberglass cloth, zeolites) can be recommended as a suitable approach, other hand it may nourish TDS also [16]. Transition metal ions may be added into TiO_2 lattice to boost photocatalysis [17–19]. Most of the investigated parameters i.e. alkalinity, turbidity, hardness, nitrate, calcium ion concentration, magnesium ion concentration, COD, BOD, sulfate ion concentration were found to match within WHO standards for drinking water after TiO_2 assisted photocatalysis of Ethidium bromide dye at pH 9.2. Investigation claims that sink drain of Ethidium bromide is hazardous to ecosystem. Laboratory effluent polluted with Ethidium bromide dye should not be allowed to mix with irrigating water canals or potable water resources. TiO_2 nanoparticles may be recommended for degradation of Ethidium bromide dye in analytical laboratories which pose a continual threat to environment [20–22]. But still it needs tertiary treatment before it is disposed off into environment. Sodium hypochlorite has also been used for removal of Ethidium bromide dye [23, 24]. To execute maximum photocatalysis, experiments were carried out at optimum pH 10 which can be tuned to pH 7 by optimized treatment of water. Before the technique is used widely, photocatalytically degraded products of Ethidium bromide dye must be analyzed further for their mutagenic, xenobiotic and carcinogenic nature to environment [11,25].

Conflicts of Interest

The authors declare that they have no conflict of interest.

References

[1] Krakowiak R, Musial J, Bakun P, et al. Titanium Dioxide-Based Photocatalysts for Degradation of Emerging Contaminants including Pharmaceutical Pollutants. Applied Sciences. 2021, 11(18): 8674. https://doi.org/10.3390/app11188674

[2] Adán C, Martínez-Arias A, Fernández-García M, et al. Photocatalytic degradation of ethidium bromide over titania in aqueous solutions. Applied Catalysis B: Environmental. 2007, 76(3-4): 395-402.
https://doi.org/10.1016/j.apcatb.2007.06.013

[3] Mao T, Zha J, Hu Y, et al. Research Progress of TiO2 Modification and Photodegradation of Organic Pollutants. Inorganics. 2024, 12(7): 178.
https://doi.org/10.3390/inorganics12070178

[4] Bashar MA, Ibrahim ABM, Hasan MK, et al. Bio-dye-sensitized TiO_2 nanophotocatalysts for visible-light-driven sustainable wastewater treatment. RSC advances, 2025, 15(49): 41710-41723.

[5] Youssef A, El-Ella Hussein Gad A, Ghannam H, et al. Synthesis of high efficient CS/PVDC/TiO2-Au nanocomposites for photocatalytic degradation of carcinogenic ethidium bromide in sunlight. Egyptian Journal of Chemistry. 2020, 63(5): 5-9.
https://doi.org/10.21608/ejchem.2020.21987.2313

[6] International standards for drinking-water. World Health Organization, 1958.

[7] WHO. Guidelines for drinking quality recommendations, World Health Organization, 1984.

[8] NCCLS. Methods for determining bactericidal activity of antimicrobial agents. Approved guideline M26-A. NCCLS, Wayne, Pa., 1999.

[9] Saint UK, BARAL SC, Sasmal D, et al. Effect of Ph on Photocatalytic Degradation of Methylene Blue in Water by Facile Hydrothermally Grown Tio2 Nanoparticles Under Natural Sunlight. Published online 2024.
https://doi.org/10.2139/ssrn.5050108

[10] Tinoco Navarro LK, Jaroslav C. Enhancing Photocatalytic Properties of TiO2 Photocatalyst and Heterojunctions: A Comprehensive Review of the Impact of Biphasic Systems in Aerogels and Xerogels Synthesis, Methods, and Mechanisms for Environmental Applications. Gels. 2023, 9(12): 976.
https://doi.org/10.3390/gels9120976

[11] Gaya UI, Abdullah AH. Heterogeneous photocatalytic degradation of organic contaminants over titanium dioxide: a review of fundamentals, progress and problems. Journal of photochemistry and photobiology C: Photochemistry reviews. 2008, 9(1): 1-12.

[12] Hihara T. Azo-hydrazone tautomerism of phenylazonaphthol sulfonates and their analysis using the semiempirical molecular orbital PM5 method. Dyes and Pigments. 2003, 59(1): 25-41.
https://doi.org/10.1016/s0143-7208(03)00093-7

[13] Zhang Z, Huo K, Yan T, et al. Mechanistic insights into the selective photocatalytic degradation of dyes over TiO2/ZSM-11. Frontiers of Environmental Science & Engineering. 2023, 17(8). https://doi.org/10.1007/s11783-023-1701-5

[14] Ramesh K, Chellam PV, Sundaram B. Transport of layered and spherical microplastics in aqueous ecosystems: a review. Environmental Chemistry Letters. 2024, 22(3): 1221-1255. https://doi.org/10.1007/s10311-024-01730-6

[15] Simončič B, Glažar D. TiO2 and ZnO as Advanced Photocatalysts for Effective Dye Degradation in Textile Wastewater. Tekstilec. Published online August 31, 2023: 178-198. https://doi.org/10.14502/tekstilec.66.2023045

[16] Rojas-García E, García-Martínez DC, López-Medina R, et al. Photocatalytic Degradation of Dyes Using Titania Nanoparticles Supported in Metal-Organic Materials Based on Iron. Molecules. 2022, 27(20): 7078. https://doi.org/10.3390/molecules27207078

[17] Madduri SB, Kommalapati RR. Photocatalytic Degradation of Azo Dyes in Aqueous Solution Using TiO2 Doped with rGO/CdS under UV Irradiation. Processes. 2024, 12(7): 1455. https://doi.org/10.3390/pr12071455

[18] Sultana M, Mondal A, Islam S, et al. Strategic development of metal doped TiO2 photocatalysts for enhanced dye degradation activity under UV–Vis irradiation: A review. Current Research in Green and Sustainable Chemistry. 2023, 7: 100383. https://doi.org/10.1016/j.crgsc.2023.100383

[19] Photocatalytic Degradation of Organic Dyes Using Titanium Dioxide (TiO2) and Mg-TiO2 Nanoparticles. Journal of Nanotechnology and Nanomaterials. 2022, 3(2). https://doi.org/10.33696/nanotechnol.3.032

[20] El Sharkawy HM, Shawky AM, Elshypany R, et al. Efficient photocatalytic degradation of organic pollutants over TiO2 nanoparticles modified with nitrogen and MoS2 under visible light irradiation. Scientific Reports. 2023, 13(1). https://doi.org/10.1038/s41598-023-35265-7

[21] Abdullah TA, Al-Obaidi Q, Abdulla TA, et al. A Critical Review of the Photocatalytic Degradation of Pharmaceutical Residues by a TiO2-Based Photocatalyst. Hungarian Journal of Industry and Chemistry. 2023, 51(2): 65-75. https://doi.org/10.33927/hjic-2023-20

[22] Ajmal A, Majeed I, Malik RN, et al. Principles and mechanisms of photocatalytic dye degradation on TiO2based photocatalysts: a comparative overview. RSC Adv. 2014, 4(70): 37003-37026. https://doi.org/10.1039/c4ra06658h

[23] Armour MA. Hazardous laboratory chemicals disposal guide. CRC press, 2016.

[24] Helmy ET, El Nemr A, Mousa M, et al. Photocatalytic degradation of organic dyes pollutants in the industrial textile wastewater by using synthesized TiO2, C-doped TiO2, S-doped TiO2 and C, S co-doped TiO2 nanoparticles. Journal of Water and Environmental Nanotechnology. 2018, 3(2): 116-127.

[25] Ghamarpoor R, Fallah A, Jamshidi M. A Review of Synthesis Methods, Modifications, and Mechanisms of ZnO/TiO2-Based Photocatalysts for Photodegradation of Contaminants. ACS Omega. 2024, 9(24): 25457-25492. https://doi.org/10.1021/acsomega.3c08717