Chem Rep, 2025, 5(1): 291-297
DOI: 10.25082/CR.2024.01.004

RESEARCH ARTICLE

An Analysis of Tio_2 Assisted Photocatalytic Degradation of Nigrosine Dye

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Received: February 15, 2025;
Accepted: May 21, 2025;
Published: May 27, 2025.

Citation: Bharadwaj L. An Analysis of Tio_2 Assisted Photocatalytic Degradation of Nigrosine Dye. *Chem Rep*, 2025, **5**(1): 291-297.
https://doi.org/10.25082/CR.2024.01.004

Abstract: This study examined the photocatalytic degradation of Nigrosine Dye (25 ppm) in aqueous solution using TiO_2 nanoparticles under sunlight irradiation. The optimal degradation efficiency of 70.23% was achieved at pH 6 after 4 hours of treatment. Comprehensive water quality analysis revealed significant alterations in parameters including alkalinity, turbidity, hardness, nitrate, calcium, magnesium, COD, BOD, sulfate, temperature and pH, with water hardness exceeding WHO drinking water standards. HPTLC analysis demonstrated successful dye degradation through reduced peak numbers in densitograms. Environmental risk assessment showed: 1) no antibacterial activity against E. coli at 10 ppm concentration; 2) improved seed germination rates (85% at pH 5.4 and 80% at pH 7) compared to untreated dye (10%), though slightly lower than control (96.25%). The results indicate TiO_2 photocatalysis effectively degrades Nigrosine Dye but requires further optimization to address residual hardness and minor phytotoxicity observed at neutral pH conditions.

Keywords: Nigrosine Dye, Tio_2, environmental risk assessment

1 Introduction

Nigrosine Dye is extensively used to stain biological specimens in microscopy and biotechnology laboratories. Nigrosine Dye polluted water (after treatment with specimen) is discarded into sink in various laboratories. Disposal of Nigrosine Dye into the sanitary sewer or sink drain is not permitted over its hazardous concerns. Although it is an effective tool to visualize biological specimens, 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 has been made to degrade dyes by semiconductor/ nano particles/ or their conjugates 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 attempt has been made up to photo catalytically degrade Nigrosine Dye by using Tio_2 semiconductor. Degradation of Nigrosine Dye was evaluated on the basis of change in optical density at concerned λ_{max}. Quality of photo catalytically degraded Nigrosine Dye polluted water was evaluated by analyzing alkalinity, turbidity, hardness, nitrate, calcium ion concentration, magnesium ion concentration, COD, BOD, sulfate ion concentration, temperature, pH and Environmental risk assessment. 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 photo catalytically degraded products should be disposed off/ affects environment.

2 Materials and methods

2.1 Chemicals

Anatase Tio_2nano particles were kindly supplied by Chem Life Enterprises. Nigrosine Dye was purchased from Sigma Aldrich. Sodium Fluoride, Zirconium reagent, Sodium arsenite, Sodium azide, Sodium iodide, $Na_2S_2O_3$, Erichrome 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, $AgSO_4$, $FeCl_3$, $MgSO_4$, HCl, NaOH were from Central Drug House.

2.2 Photo catalysis of Nigrosine Dye

A 100 ml solution of Nigrosine Dye (25 ppm) was prepared in tap water at different pH (2, 4, 6, 8, 10, and 12) to screen optimum pH for photo catalysis. Absorbance of these solutions was measured at λ_{max} 522.3 nm at 25°C by using UV-Visual spectrophotometer (Jasco-630). The solutions were maintained in glass bottles (without lid) under sun light exposure (3000 Lux/hr) for 4 hours with suspended Tio_2 nano particles (1000 ppm; without stirring) for photocatalytic degradation. Photo catalytically treated samples were centrifuged at 4000g for 5 minutes (Remi cooling centrifuge C 24). The supernatant was analyzed for optical density at λ_{max} 522.3 nm at 25°C to assess photocatalytic degradation of Nigrosine Dye at different pH (2, 4, 6, 8, 10, and 12). The pH at which maximum % change in absorbance was noticed after photo catalysis was regarded as optimum pH for all assigned experiments.

2.3 Qualitative 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 photo catalytically 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 [4, 5]. The obtained results were compared with standards for drinking water, World Health Organization [6].

2.4 HPTLC of samples

High Performance Thin Layer Chromatography (CAMAG) was performed to analyze pre and post treated Nigrosine Dye samples (10 PPM).

2.4.1 Chromatogram Layer

HPTLC Silica gel 60 F_{254} Aluminum sheet (*Merck*) 10x20 cm was pre washed by immersing in methanol and then dried overnight in an oven at 60°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 pre saturated for 30 minutes with solvent. After attaining 80 % of migration distance, TLC plate was dried on the TLC plate heater at 60°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 pre and post photo catalytically treated Nigrosine Dye (10 PPM) to environment. This investigation indicates the feasibility of photocatalytic degradation of Nigrosine 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 wells diffusion assay was performed to assess hazardous risks associated with photo catalytically 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 photo catalytically degraded dye samples (10 ppm dye + 1000 ppm Tio_2), qualitative investigation was executed with Agar well diffusion method. Photo catalytically treated samples were centrifuged at 4000g for 5 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 micro

liter inoculum of *E. coli* VSBT.T.12.06 (log phase, 1x10^5 cfu/ml) was spreaded 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°C temperature.

2.5.2 Seed germination

20 seeds of *Vigna radiata* (L.) R. Wilczek were allowed to germinate for two days in control (water), Nigrosine Dye polluted water (10 ppm, P^H 6), photo catalytically degraded Nigrosine Dye (P^H 5.4) and photo catalytically degraded Nigrosine Dye water adjusted at P^H 7 to observe the impact on irrigation. Four sets of experiments were executed and observed results were categorized as germinated seeds, un- germinated seeds and 'partial/ inhibited' germination.

3 Results and Discussion

3.1 Analysis of water quality

λ_{max} 522.3 nm for Nigrosine Dye dye was determined by using UV-Visual spectrophotometer (Jasco-630) at 25°C. Among experimented pH (2, 4, 6, 8, 10, 12), maximum photocatalytic degradation (70.23 %) of Nigrosine Dye was observed at pH 6 (Table 1). Photocatalytic efficiency has been determined based up on % decrease in Optical density. Various water quality parameters were studied in photo catalyzed 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) [6]. Hardness of solution was found higher than WHO standards for drinking water.

Table 1 TiO_2 assisted photocatalytic degradation of Nigrosine Dye at different pH

pH	Pre-treatment OD at λmax 522.3 nm	Post-treatment OD at λmax 522.3 nm	Photocatalytic efficiency%	Control (Solar treatment)	% Photocatalytic efficiency in control
2	0.4219	0.1807	57.16	0.4196	0.54%
4	0.5136	0.1952	61.99	0.4712	8.25%
6	0.4795	0.1427	70.23	0.4008	16.41%
8	0.5006	0.1638	67.27	0.4283	14.44%
10	0.5188	0.1937	62.66	0.3986	23.16%
12	0.5731	0.2030	64.57	0.5469	4.57%

Table 2 TiO_2 assisted photocatalytic degradation of Nigrosine dye at pH 6

Parameters	Pre-treatment	Post-treatment	WHO (Standard) permissive	Excessive
Alkalinity	200 ppm	50 ppm	200 ppm	600 ppm
BOD	4.2 ppm	2.3 ppm	6 ppm	
Ca^{2+}	32.0 ppm	20.0 ppm	75 ppm	200 ppm
Cl^-	284 ppm	142 ppm	142 ppm	600 ppm
COD	10.5 ppm	2.4 ppm	10 ppm	
Hardness	381.3 ppm	296 ppm	100 ppm	500 ppm
Mg^{2+}	5.63 ppm	2.47 ppm	30 ppm	150 ppm
$NO_3{}^-$	3.35 ppm	1.45 ppm	50-100 ppm	
$SO_4{}^{2-}$	36.60 ppm	27.78 ppm	200 ppm	400 ppm
TDS	105 ppm	70 ppm	500 ppm	1000 ppm
pH	6	5.4	6.5-8.5	6.5 -8.5
^{o}c	38.4	39.9		

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

Sample	pH	Germinated seeds	Un-germinated seeds	'partial/inhibited' germination in Seeds
Tap Water (Control)	7	95 %	3.75 %	1.25 %
Nigrosine Dye (10 ppm, pre treated)	6	10 %	90 %	0 %
Nigrosine Dye (10 ppm, photocatalytically degraded)	5.4	85 %	15 %	0 %
Nigrosine Dye (10 ppm photocatalytically degraded)	7	80 %	20 %	0 %

3.2 Analysis of photocatalytic degradation

Numbers of peaks in HPTLC densitogram were found increasing upon addition of Tio_2 nano particles into Nigrosine Dye sample (Figure 1 and 2). After photo catalytic degradation of Nigrosine Dye numbers of peaks in HPTLC densitogram are decreasing indicating that few degraded dye products might have escaped/ evaporated from treated Nigrosine Dye solution (Figure 2 and 3). Peaks show retardation of absorption upon addition of Tio_2 nano particles (Figure 1, 2 and 3) indicating the degradation of dye sample. Decreased counts of peaks in densitogram (Figure 3) illustrate the reason behind the decrease in turbidity of photo catalytically treated dye sample (70 ppm TDS) compared to untreated Nigrosine Dye sample (Figure 1, 105 ppm TDS). Comparative analysis of HPTLC results at multiwavelength show that photo catalytically degraded dye products exhibit slight variation in absorbance between 200 nm and 450 nm (Figure 5 and 6).

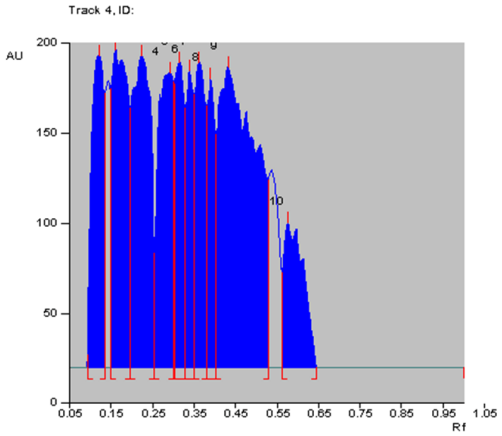

Figure 1 HPTLC Densitogram at 450 nm for Nigrosine Dye

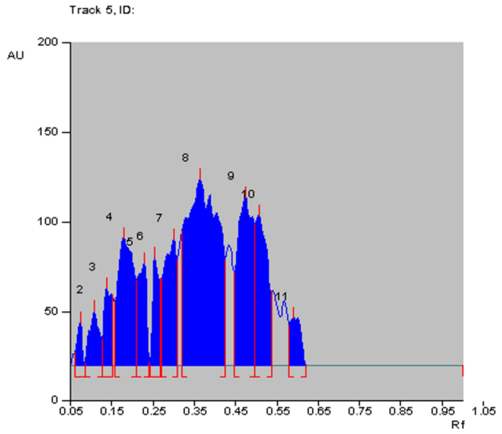

Figure 2 HPTLC Densitogram at 450 nm for Nigrosine Dye + TiO_2

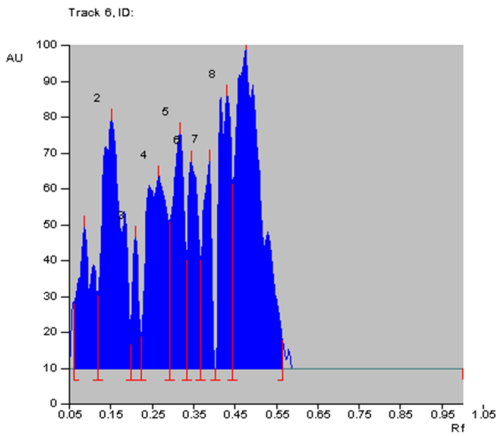

Figure 3 HPTLC Densitogram at 450 nm for Photo catalyzed Nigrosine Dye + TiO_2

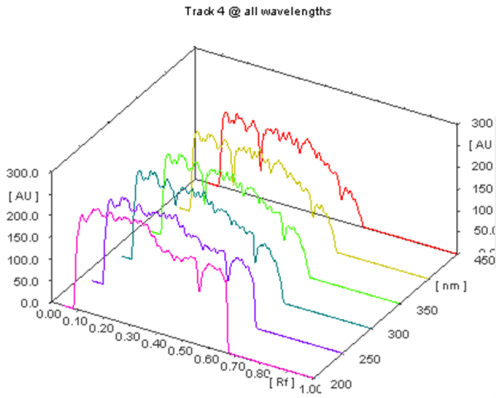

Figure 4 HPTLC: Absorption spectra at multi wavelength for Nigrosine Dye

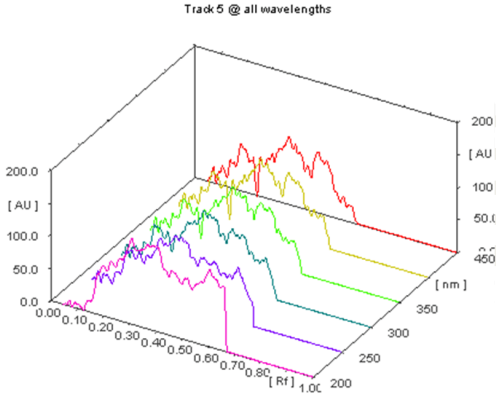

Figure 5 HPTLC: Absorption spectra at multi wavelength for Nigrosine + TiO_2 (without photo catalysis)

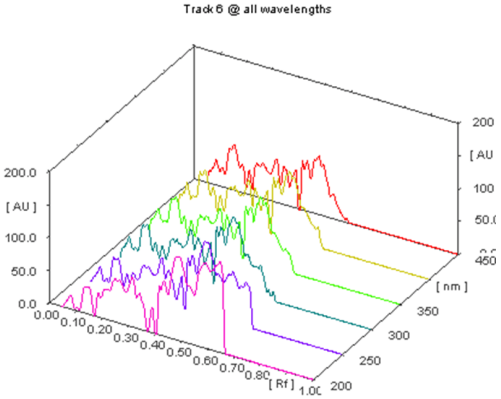

Figure 6 HPTLC: Absorption spectra at multi wavelength for Photo catalyzed Nigrosine + TiO_2

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) in pre and post photo catalytically treated dye samples. It indicates that reference strain of bacteria (model aquatic organism) is quite tolerable to Nigrosine Dye and its photo catalyzed products at 10 ppm concentration. Higher doses should also be checked for further evaluation since concentration of Nigrosine Dye in laboratory disposed wastage may even be higher. Bioaccumulation of photo catalyzed Nigrosine Dye products may enter in food chain and affect human health. These photo catalyzed products must also be checked further for their carcinogenic/ mutagenic properties.

96.25 % germination of seeds was observed in control cases. Compared to control (Figure 7), only 10 % germination was observed in dye sample. 90 % seeds could not germinate in dye sample indicating hazardous risk associated with Nigrosine Dye (Figure 8). 85 % seeds could germinate in photo catalyzed dye sample (Figure 9). Remaining 15 % seeds could not germinate in photo catalyzed dye sample (Table 3) Further only 80 % seeds could germinate in photo catalyzed dye sample at pH 7. Compared to control cases, delayed and inferior growth

was observed in photo catalyzed dye sample at pH 7 (Figure 10). Although these seedlings (Figure 9, 10) can be discriminated from control (Figure 7) but at this seed developmental stage we could not further characterize the deformity in seedlings.

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

Figure 8 Seed germination of *Vigna radiata* (L.) R. Wilczek in water + Nigrosine dye at pH 6; after two days

Figure 9 Seed germination of *Vigna radiata* (L.) R. Wilczek in water + photocatalyzed Nigrosine dye at PH 5.4; after two days

Figure 10 Seed germination of *Vigna radiata* (L.) R. Wilczek in water + photocatalyzed Nigrosine dye at pH 7; after two days

4 Conclusion

Results confirm that dye sample is being assimilated by seeds and these products are interfering in normal development of seedlings. After photocatalytic treatment of dye sample delayed germination of seeds was observed with increased rate of seed germination. Photocatalytic treatment of dye improved the seed germination chances but slight deformity in seedlings was observed. It indicates that photo catalyzed products seem to possess slight toxicity/hazardous nature at pH 7. Hardness of water was also found high in photo catalyzed water. In lieu of bio safety Tio_2 nano particles mediated photo catalysis can be recommended for Nigrosine dye with further improvements. Such water remediation strategies must be aligned to consider metal toxicity to plants and animals. Metals are detoxified by chelation with phytochelatins. Excess production of phytochelatins in plants can even detoxify essential metals as an adaptive response to environmental stress. Keeping this in view immobilization of photocatalyst would appear as a suitable approach.

Conflicts of interest

The author declares no conflict of interest.

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