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 TiO2 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, Ca2+, Mg2+, NO3, SO42-, 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 TiO2 nanoparticles under constant conditions. Quality of treated water was evaluated by analyzing alkalinity, BOD, COD, Ca2+, Mg2+, NO3, SO42-, hardness, temperature, turbidity and pH; and compared with WHO standards for drinking water. 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.

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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 TiO₂ mediated photocatalytic treatment may be appropriate option to degrade Giemsa dye.

Material and Method

Chemicals

Anatase TiO₂ 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, Na₂SO₄, 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, K₂Cr₂O₇, AgNO₃, AgCl, Ag₂SO₄, FeCl₃, MgSO₄, 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 λₘₚₐₐₓ 660.7 nm at 25°C by using UV-Vis spectrophotometer (Jasco-630). The solutions were maintained in glass bottles (without lid) under sunlight exposure (3000 Lux/h, Metek digital lux meter 101 b, terrestrial sunlight intensity). The solutions were stirred magnetically with magnetic stirrer for 4h with suspended nanoparticles of TiO₂. Supernatant was dried on the TLC plate heater at 60°C for 10 minutes. Chromatographic chamber was pre-saturated with standards for drinking water, World Health Organization [32].

Results and Discussion

Photocatalysis

Maximum 73.43% photocatalytic degradation could be achieved at pH 2 by suspended nanoparticles of TiO₂ (Table 1). At other experimented pH, interaction between TiO₂ 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 TiO₂ 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 Rf 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...
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 (1µWcm$^{-2}$) 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 VSB.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.

### Table 1: TiO$_2$ assisted photocatalytic degradation of Giemsa dye at different pH.

<table>
<thead>
<tr>
<th>pH</th>
<th>OD Untreated water</th>
<th>% Photocatalytic efficiency</th>
<th>%Photolytic efficiency (control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0.64</td>
<td>73.43</td>
<td>8.40 %</td>
</tr>
<tr>
<td>4</td>
<td>0.32</td>
<td>44.56</td>
<td>9.96 %</td>
</tr>
<tr>
<td>6</td>
<td>0.25</td>
<td>29.68</td>
<td>0.43 %</td>
</tr>
<tr>
<td>8</td>
<td>0.34</td>
<td>62.59</td>
<td>9.56 %</td>
</tr>
<tr>
<td>10</td>
<td>0.30</td>
<td>46.13</td>
<td>2.47 %</td>
</tr>
<tr>
<td>12</td>
<td>0.15</td>
<td>24.38</td>
<td>5.81 %</td>
</tr>
</tbody>
</table>

### Table 2: Quantitative analysis of water quality.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Untreated water</th>
<th>Treated water</th>
<th>WHO Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkalinity</td>
<td>266.5 ppm</td>
<td>116.6 ppm</td>
<td>200 ppm</td>
</tr>
<tr>
<td>BOD</td>
<td>4.3 ppm</td>
<td>1.8 ppm</td>
<td>6 ppm</td>
</tr>
<tr>
<td>Ca$^{2+}$</td>
<td>28.0 ppm</td>
<td>20.0 ppm</td>
<td>75 ppm</td>
</tr>
<tr>
<td>Cl$^{-}$</td>
<td>213 ppm</td>
<td>165.6 ppm</td>
<td>200 ppm</td>
</tr>
<tr>
<td>COD</td>
<td>20.2 ppm</td>
<td>9.3 ppm</td>
<td>10 ppm</td>
</tr>
<tr>
<td>Hardness</td>
<td>381.3 ppm</td>
<td>296 ppm</td>
<td>100 ppm</td>
</tr>
<tr>
<td>Mg$^{2+}$</td>
<td>5.06 ppm</td>
<td>2.02 ppm</td>
<td>30 ppm</td>
</tr>
<tr>
<td>NO$_3^-$</td>
<td>2.33 ppm</td>
<td>1.55 ppm</td>
<td>50-100 ppm</td>
</tr>
<tr>
<td>SO$_4^{2-}$</td>
<td>38.73 ppm</td>
<td>22.70 ppm</td>
<td>200 ppm</td>
</tr>
<tr>
<td>TDS</td>
<td>111 ppm</td>
<td>99 ppm</td>
<td>500 ppm</td>
</tr>
<tr>
<td>pH</td>
<td>2</td>
<td>1.9</td>
<td>6.5-8.5</td>
</tr>
<tr>
<td>Temperature °C</td>
<td>34.6</td>
<td>41.1</td>
<td>Not available</td>
</tr>
</tbody>
</table>

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

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

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

### Figure 1b: HPTLC Densitogram at 450 nm for 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.

References


