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Cytotoxicity and mutagenicity evaluation of gamma radiation and hydrogen peroxide treated textile effluents using bioassays

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ABSTRACT

Cytotoxicity and mutagenicity of textile effluents, treated by gamma radiation in combination hydrogen peroxide was investigated. The *Allium cepa*, heamolytic, brine shrimp bioassays were used for cytotoxicity evaluation, whereas mutagenicity was tested using Ames tests. Textile effluents were irradiated to the gamma radiation absorbed doses of 5 kGy, 10 kGy and 15 kGy in combination with 20 mM hydrogen peroxide. Before treatment, textile effluents showed a significant cytotoxicity and mutagenicity signs and reduced significantly after treatment. *A. cepa* showed reduction in cytotoxicity 50%, whereas 56–59% in case of heamolytic test and up 93% reduction in cytotoxicity was recorded by brine shrimp assays. The mutagenicity of gamma radiation treated effluents reduced up to 59% and 54% in case of TA98 and TA100, respectively. Results revealed that the gamma radiation in combination with hydrogen peroxide can be implemented for the detoxification of textile effluents.

Introduction

Textile industries produce huge volumes of water from wet processing units and resultantly, dyes along with other chemicals are discharged into water sheds. The presence of even very low amount of dyes in the effluent is highly visible and undesirable. Million ton of dye-stuffs of more than 100,000 types are produced and consumed annually [1]. Degradation of textile dye effluent does not occur when treated aerobically and over 90% of 4000 dyes tested in the Ecological and Toxicological Association of Dyes and Organic Pigments Manufacturers (ETAD) survey showed LD_{50} values greater than 2000 mg/kg [2]. There is evidence that some reactive azo dyes cause contact dermatitis, allergic conjunctivitis, rhinitis, occupational asthma or other allergic reactions in workers [1]. Many dyes are difficult to decolorize due to their complex structure and synthetic origin. Various methods have been applied for decolorization of textile dyes [3–7] and the conventional treatment systems and biological treatment were found to be inefficient in the removal of these dyes and others environmentally problematic [2,8,9]. Hence, advanced oxidation processes (AOPs) are needed for dyes efficient removal [10,11]. In advanced oxidation treatment, strong oxidizing specie like hydroxyl radical ($^{\bullet}OH$ radical) is produced in situ, which break down the complex organic molecule into harmless substances such as CO_2, H_2O and inorganic ions through a chain reactions [7,12]. The $^{\bullet}OH$ can be generated by radiation alone/ in combination with O_3, H_2O_2/electron beam irradiation/or Fenton's reagent [13]. The treatment of wastewater effluents by radiation has advantages such as it degrade non biodegradable organic substances without causing secondary pollution and are considered eco-friendly [7,14].

Bioassays have been used to evaluate toxicity levels of target contaminants and complex aqueous matrices. Generally, the organism used includes microorganisms, plants and algae, invertebrates and fishes [7,15,16]. Among higher plants, *Allium cepa*, *Vicia faba*, *Zea mays*, *Tradescantia*, *Nicotiana tabacum*, *Crepis capillaris* and *Hordeum vulgare* species are regarded as more favorable to assess toxicity [16,17]. Similarly, brine shrimp and heamolytic are suitable bioassays for cytotoxicity evaluation [10]. The Ames test, which measures the reversion of the bacterial mutants, is a reference test in chemical mutagenicity testing and was extensively validated and serves as a quick and convenient way to estimate the mutagenic potential of a target compound [18]. Unfortunately, the partial oxidation of organic contaminants produce more toxic intermediates than parent compounds and monitoring of treated effluents using toxicity assays is helpful in understanding the biological efficiency of treatment method. Literature showed that AOPs such as O_3, TiO_2/UV, sunlight irradiation, electro-Fenton, wet-air oxidation, UV/electro-Fenton, Photo-Fenton, O_3/UV, TiO_2 based photocatalysis, H_2O_2/UV and TiO_2/H_2O_2/UV (Table 1) [15] showed considerable toxicity reduction. To best of our knowledge, the textile wastewater toxicity, treated by gamma radiation has not been reported previously using *A. cepa*, heamolytic, brine shrimp and Ames test. Therefore, it

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Table 1
Reported bioassay used in evaluating toxicity of wastewater/simulated solutions treated by AOPs. Q8

Bioassay	AOPs investigated	Tested system
V. fischeri	O_3 (1 mmol L^{-1}, 0.38 L min^{-1} flowrate)	Bezafibrate (0.2–0.5 mmol L^{-1})
Ankistrodesmus braunii and *S. capricornutum*	O_3 (2.0% per volume, 36 dm^3 h^{-1} flowrate).	Carbamazepine (3.3×10^{-6} mmol dm^{-3})
D. magna,*P. subcapitata* and *A. salina*	TiO_2/UV (0.2–1.6 g L^{-1})	Diclofenac (5–80 mg L^{-1})
P. subcapitata, *Cyclotella meneghiniana*, *Synechococcus leopoliensis*	Sunlight irradiation, UV/H_2O_2 (254 nm low-pressure lamp) and O_3	Lyncomicin (0.5 mM)
S. leopoliensis, *Brachyonus calyciflorus*	O_3 (0.42 mM, 36 dm^3 h^{-1} flowrate), H_2O_2/UV (5 and 10 mM of H_2O_2) and TiO_2/UV (suspended or immobilized TiO_2, 0.3 g L^{-1}, Degussa, 300 W sunlight simulator, up to 48 h contact time)	Mixture of six pharmaceuticals
P. putida	UV/TiO_2, electro-Fenton, wet-air oxidation, and UV/electro-Fenton	Reactive Red 120 (20–100 mg L^{-1})
V. fischeri, *D. magna* and *S. capricornotum*	Solar driven photo-Fenton and TiO_2 photocatalysis pilot plant	Methomyl (50 mg L^{-1})
D. magna and *Bacillus subtilis* sp.	Photo-Fenton (2 L reactor, three 6 W Philips black-light fluorescent lamps ($I = 5 \times 10^{-6}$ Einstein s^{-1}), controlled temperature (25 °C)	Imidacloprid (100 mg L^{-1})
D. magna, *Photobacterium phosphoreum*, umu (genotoxicity) test	O_3 and O_3/UV (40 mg L^{-1} O_3dosage, 20 and 40 min treatment), H_2O_2 and H_2O_2/UV (6 mL L^{-1} of 30% H_2O_2)	Mixture of municipal and industrial wastewater pre-treated by MBR
V. fischeri (toxicity) and*Salmonella typhimurium*(mutagenicity)	Ozonation (applied ozone 2.5–8.0 mg L^{-1}, 2–30 min treatment)	Effluent from secondary biological treatment
D. magna, *P. subcapitata*, *L. sativum*	TiO_2 photocatalysis, catalyst loading in the range of 0.2–0.8 g L^{-1}, 125 W black-light fluorescent lamp, 120 min maximum irradiation time	Effluent from secondary biological treatment
FELST with rainbow trout (*Oncorhynchus mykiss*)	Ozonation (maximum applied ozone concentration 1 mg O_3/mg DOC)	Effluent from secondary biological treatment plant
D. longispina.	Photo-Fenton process before and after biological treatment by three species of fungi	Diluted and undiluted wastewater samples from olive mill plant
P. subcapitata and phytotoxicity to seeds of*R. sativus*, *C. sativus*and *L. sativa*	Ozonation, solar photolysis, solar modified photo-Fenton, solar modified photo-Fenton–ozonation.	Centrifuged wastewater sample
A. salina	H_2O_2/UV, TiO_2/H_2O_2/UV and TiO_2/UV in a continuous operated annular reactor (15 W UV-lamp, 1 g TiO_2 L^{-1}, 1 h)	Coagulated/settled wastewater

was hypothesized that gamma radiation treated textile effluents toxicity can be evaluated using bioassays. The textile wastewaters collected from different industries were treated by gamma radiation in the combination with hydrogen peroxide and cytotoxicity and mutagenicity was evaluated using standard bioassays. Q3

Material and methods

Sample collection

Three textile industries were selected for sampling from the industrial city, Faisalabad, Pakistan and denoted as TIW I, II and III. The samples were collected using standard method (Eaton et al., 2005). Briefly, the plastic gallon were pre-cleaned by soaking in nitric acid 1% (v/v) for 24 h and rinsed with ultra pure water (Milli-Q system, Millipore). Triplicate samples were collected from each industry and stored at 4 °C until experimentation. Q4

Sample irradiation

Cesium-137 gamma radiation source was used for irradiation of wastewater samples at Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan. The dose rate at the time of sample irradiation was 1.25 kGy h^{-1} which was calibrated using Fricke dosimeter (Eq. (1)). The samples were irradiated to the absorbed doses of 5 kGy, 10 kGy and 15 kGy in combination with 20 mM hydrogen peroxide. Q5

$$D = \left[\frac{N \times \Delta A \times 100}{\varepsilon \times \rho \times G(\mathrm{Fe(III)})}\right] \quad (1)$$

where D is absorbed dose, ε is the molar extinction coefficient of ferric ion (0.2205 M^{-1} cm^{-1} at 304 and 25 °C, ΔA is representing absorbance difference of irradiated and un-irradiated samples, N is Avogadro's number (6.02×10^{23}), ρ is the density of Fricke solution (1.024 g/cm^3 for 0.4 M H_2SO_4 and G (Fe^{3+}) is the number of Fe^{3+} ions produced/100 ev of absorbed energy which is 15.6 for Fricke solution.

Bioassays

The bioassays such as *A. cepa* test (ACT), heamolytic, brine shrimp (cytotoxic tests) and Ames tests (mutagenic test) were performed precisely as reported previously [16]. Before toxicity evaluation, the un-reacted hydrogen peroxide was removed from treated samples by adding ~0.80 mg MnO_2/mL of solution [19], mixed for 1 h, filtered and subjected to the toxicity tests. All samples were run in triplicate except *A. cepa* test (5 repetitions) and data, thus obtained was averaged and results were reported as mean ± SD.

Results and discussion

Physicochemical characteristics of textile wastewater

Preliminary water quality assurance test performed, showed that the water quality parameters values were very high and beyond the permissible limits. The water quality parameter e.g. pH, COD, BOD, DO, TDS and TSS values were in the range of 9.17–11.8, 1766–2100 mg/L, 800–874 mg/L, 1.2–1.5 mg/L, 1530–1590 mg/L, 447–505 mg/L respectively. The λ_{max} was recorded to be 539 nm, 625 nm and 486.5 nm for TIW I, II and III, respectively (Table 2) and UV–vis spectra of treated and untreated textile effluents can be seen in Fig. 1A–C. The pH of textile wastewater was alkaline in nature. The bleaching agents and chemicals NaOCl, NaOH, surfactants and sodium phosphate used in the processes, might be the reasons for high alkalinity of wastewater [16,20]. A low DO in TIW indicated highly polluted nature of effluent contaminated with organic matter and previous finding also highlighted that DO might be very low of textile effluents due high organic load and [20] reported nil DO value. A slight change in pH values of TIW II and III might be due to biodegradation of organic matter present in wastewater. BOD is the most important parameter which in true sense determines the pollution load of an effluent and is expressed as a measure of the quantity of oxygen consumed by microorganisms in the degradation of organic matter. The BOD recorded values were higher than previously