

Short Communication

Gamma Radiation Treatment for Reducing Cytotoxicity and Mutagenicity in Industrial Wastewater

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Abstract

Our study evaluates the effect of gamma radiation treatment on detoxification of textile effluents. The effluents were tested for cytotoxicity and mutagenicity using *Allium cepa*, hemolytic, brine shrimp, and Ames tests before and after gamma radiation treatment. The collected wastewater samples were irradiated to the absorbed doses of 5kGy, 10kGy, and 15kGy, and subjected to toxicity tests. The gamma radiation showed promising efficiency for detoxification of pollutants in wastewater. The microbial load was reduced to zero, and increases in root length (RL) and root count (RC) were 31.10% and 38.34%, respectively, at 5 kGy. The reduction in cytotoxicity was recorded to be 39.56%, 49.65%, and 79.63% in the case of human and sheep red blood cells (RBC) and brine shrimp, respectively. Before treatment, the samples were mildly mutagenic and after treatment mutagenicity was not detected. Results showed that gamma radiation has promising efficiency for the degradation of toxic agents present in effluents.

Keywords: absorbed dose, cytotoxicity, mutagenicity, wastewater, bioassays

Introduction

Advanced oxidation processes (AOP) are employed for wastewater treatment, especially for the degradation of organic compounds and mineralization. During AOP treat-

ment a strong oxidizing species (i.e., $\cdot\text{OH}$ radical) is produced *in situ*, which breaks down the complex organic molecule into harmless substances such as CO_2 , H_2O , and inorganic ions through chain reactions [1-5]. Although AOPs have been employed successfully for the degradation of organic molecules (i.e., dyes, pesticides, antibiotics and other recalcitrant compounds), toxicity is still a mystery

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and the development of an efficient, cost-effective, and green technique that can degrade and detoxify the toxic substances instead of transforming them into other phases is required [4, 6-10]. These processes are easy to handle, produce less residual effects as compared to classical treatment approaches, and are being employed for color removal, and mineralization of toxic chemicals and industrial wastewater [2, 11-13]. Currently, the trend in the development of innovative technologies is focused on those processes that eventually cause destruction of the organic contaminants rather than transferring them into another form. The application of high-energy radiation has been determined to be efficient in this regard [3, 14, 15]. The radiation-induced degradation of wastewater by gamma radiation, electron beam, and UV radiation is drawing more attention around the world. The treatment of wastewater effluents by radiation has advantages such as degrading non-biodegradable organic systems, not causing secondary pollution, and being cost-effective and eco-friendly [2, 16].

In view of partial oxidation of organic contaminants [17-20], the monitoring of treated effluents by AOPs is necessary and bioassays are routinely used to evaluate toxicity of treated effluent [21, 22]. Bioassays have been used to evaluate toxicity of raw and treated industrial effluents [2, 23, 24]. *Allium cepa* among higher plants is regarded as efficient for toxicity evaluation [24, 25], and today brine shrimp and heamolytic are also in use for cytotoxicity monitoring of raw and treated effluents [3]. The Ames test is a reference test in chemical mutagenicity testing and was extensively validated [26]. Previously, AOPs such as TiO₂/UV, O₃, electro-Fenton, wet-air oxidation, UV/electro-Fenton, photo-Fenton, sunlight irradiation, TiO₂-based photocatalysis, H₂O₂/UV, O₃/UV, and TiO₂/H₂O₂/UV [23] treatment efficiency have been evaluated using bioassays.

Therefore, it has been hypothesized that gamma radiation has the ability to detoxify textile effluents and that bioassays can be used to measure the extent of detoxification. The textile wastewater collected from different industries was treated by gamma radiation to absorbed doses of 5 kGy, 10 kGy, and 15 kGy, and the treatment effect was evaluated on the basis of microbial load, cytotoxicity, and mutagenicity reduction.

Material and Methods

Wastewater was collected from three textile industries from Faisalabad, Pakistan. The sampling was performed precisely using standard sampling methods [3]. Briefly, the plastic gallon jugs were pre-cleaned by soaking in nitric acid 1% (v/v) for 24 h and rinsed with distilled water. Samples in triplicate were collected from each industry. The collected samples were transported to the laboratory and kept at 4°C. After preliminary evaluation, samples were irradiated to gamma radiation absorbed doses of 5, 10, and 15 kGy using a Cesium-137 gamma radiation source at the Nuclear Institute for Agriculture and Biology (NIAB) in

Faisalabad, Pakistan. The dose rate at the time of sample irradiation was 1.25 kGy·h⁻¹ (March 2011). The gamma radiation source was calibrated with a Fricke dosimeter and the absorbed dose was calculated by relation shown in Eq. (1):

$$D = \left[\frac{N_A \cdot \Delta A \cdot 100}{\epsilon \cdot \rho \cdot G (Fe(III))} \right] \quad (1)$$

...where D is absorbed dose, N_A is Avogadro's number (6.02×10^{23}), ΔA represents absorbance difference of irradiated and un-irradiated samples, ρ is the density of dosimetric solution (1.024 g/cm^3 for $0.4 \text{ M H}_2\text{SO}_4$), ϵ is the molar extinction coefficient of ferric ion ($0.2205 \text{ M}^{-1}\text{cm}^{-1}$ at 304 and 25°C), and $G (Fe(III))$ is the number of Fe³⁺ ions produced/100ev of absorbed energy (15.6) for Fricke solution. The water quality parameters before irradiation were measured using standard methods. pH, dissolved oxygen (DO), biological oxygen demand (BOD), and chemical oxygen demand (COD) were measured using pre-calibrated pH, DO, BOD, and COD meters (Lovibond), whereas TDS and TSS were measured using the already reported method [27].

To evaluate the effect of gamma radiation on toxicity reduction, the toxicity of wastewater sample was measured before and after gamma radiation treatment. Bioassays such as *Allium cepa*, heamolytic, brine shrimp (cytotoxicity tests), and Ames tests (mutagenicity test) were performed [27]. The microbial load (total bacterial and total coliform) was also recorded before and after gamma radiation treatment. All samples were seeded in triplicate except *Allium cepa* test (five repetitions) and responses thus obtained were averaged and reported as mean±standard deviation. The *Allium cepa*, [24, 25], brine shrimp, and heamolytic tests are used for cytotoxicity evaluation [28], whereas Ames test is a standard test in chemical mutagenicity testing [26]. These tests were performed in the guideline of chemical testing using bioassays [29, 30].

Results and Discussion

The effect of gamma radiation treatment on microbial load, cytotoxicity and mutagenicity was investigated in the present study. The textile wastewater samples were irradiated to the gamma radiation absorbed doses of 5, 10, 15 kGy and subjected to toxicity analysis and results, and thus obtained were compared with control (un-treated samples). Before treatment, the measured values of microbial load, cytotoxicity, and genotoxicity are shown in Table 1. The total bacterial count (TBC) and total coliform (TC) populations were recorded as $>1 \times 10^6$ CUF and $>1 \times 10^5$ CUF, respectively.

Allium cepa, hemolytic, and brine shrimp tests showed that the textile wastewater was cytotoxic in nature. In comparison to positive control, 41.0%, 54.44%, 19.0%, 29.0%, and 32.0% higher cytotoxicity was recorded through RC, RL, human, sheep, and shrimp tests, respectively, which revealed that cytotoxic agents are commonly present in tex-

Table 1. Microbial load, cytotoxicity, and mutagenicity of textile wastewater before treatment.

Bioassays	Types	Units	Wastewater			Control	
			Industry I	Industry II	Industry III	Positive	Negative
Microbial load	TBC	CFU	$>1 \times 10^6$	$>1 \times 10^6$	$>1 \times 10^6$	–	–
	TC	CFU	$>1 \times 10^5$	$>1 \times 10^5$	$>1 \times 10^5$	0.000	0.000
Cytotoxicity	ACT						
	I) RC	counts	10±0.15	9±0.25	11±0.20	17±0.26	08±0.20
	II) RL	cm	4.1±0.06	3.9±0.04	3.8±0.05	9.0±0.15	2.5±0.04
	Heamolytic						
	I) human	%	81±1.6	78±1.8	72±1.9	100±0.0	0.000
	II) sheep	%	71±0.9	69±1.5	75±1.3	100±0.0	0.000
	Shrimp	-	68±0.80	64±0.85	54±0.81	100±0.0	0.000
Mutagenicity	Ames test						
	I) TA98	counts	62±2/96	59±2/96	60±1/96	19±2/96	0.000
	II) TA100	counts	68±2/96	63±2/96	61±3/96	21±2/96	0.000

TBC – total bacterial count, TC – total coliform, ACT – *Allium cepa* test, RC – root count, RL – root length, “–” = not measured, ND – not detected, PC – positive control, NC – negative control.

For heamolytic test, PC and NC were TritonX-100 (0.1%) and phosphate buffer saline, respectively.

For ACT, PC, and NC were distilled water and methyl methanesulfonate (MMS) (10 mg/L), respectively.

For shrimp test, PC and NC were cyclophosphamide (10 µg/mL) and sea water, respectively.

For Ames test, PC for TA98 and TA100 were $K_2Cr_2O_7$ (0.01 g/L) and NaN_3 (0.5 µg/100 µl), respectively, and background (without standard and tested compound) was used as NC.

tile effluents. The Ames test also indicated the presence of mutagenic agents in textile wastewater. However, the mutagenic level was mild, detected through TA98 and TA100 (Table 1). Previous studies also showed that the textile wastewater has the potential to cause toxicity, which is attributed to a different variety and nature of dyes and other compounds present in wastewater [31-34].

The results of microbial load, cytotoxicity, and mutagenicity reduction of gamma radiation-treated samples to the absorbed doses of 5 kGy, 10kGy and 15 kGy, evaluated through different bioassays are shown in Table 2. Microbial loads such as TBC and TC are reduced to zero, even at 5 kGy absorbed dose irradiation. The TBC and TC counts before treatment were $>1 \times 10^6$ and $>1 \times 10^5$ CUF, respectively, which indicates that the gamma radiation treatment hampered the microbes and after treatment even a single microbe was not detected. Regarding cytotoxicity reduction, evaluated through *Allium cepa*, heamolytic (human and sheep RBC), and shrimp tests, promising responses are seen. The increase in RC and RL for wastewater irradiated at the absorbed dose 5 kGy was 28.57% and 31.87%, respectively, and by increasing the absorbed dose to 10 kGy and 15kGy, the RC and RL improvement was insignificant. The heamolytic and brine shrimp tests also showed a similar trend regarding toxicity reduction, at high doses the effect was insignificant as compared to low doses. The reduction in RBC cell death was 49.38% (human) and 46.47% (sheep) for the absorbed dose of 10 kGy, whereas it was 50.61% and 52.11% for the absorbed dose of 15 kGy,

respectively, which indicates that by increasing the dose from 10 kGy to 15 kGy, the effect regarding toxicity reduction was non-linear. In the case of brine shrimp test, the reduction in shrimp *nauplii* death was observed up to 77.77%, 77.77%, and 88.88% for the absorbed doses of 5 kGy, 10 kGy, and 15 kGy, respectively. On average basis, 31.10%, 38.34%, 39.56%, 49.65%, and 79.63% toxicity reduction was recorded in the case of RC, RL, human, sheep, and shrimp tests, respectively, whereas 100% reduction in microbial load was achieved and cytotoxicity reduction was highly significant as compared to control for the absorbed 5 kGy, and at higher absorbed doses (10 kGy and 15 kGy) the effect was not significant as compared to 5 kGy. The preliminary test performed regarding water quality parameters also indicated that the water quality parameter values were beyond the permissible limits and might be responsible for toxicity of effluents because the nature of textile wastewater is complex due to presence of dyes, auxiliary compounds, and other salt additives.

The water assurance parameters (i.e., pH, COD, BOD, TDS, and TSS) were recorded up to 11.8, 2,100 mg/L, 800 mg/L, 1,590 mg/L, and 505 mg/L, respectively, and dissolve oxygen (DO) was also recorded to be low. The insignificant effect of gamma radiation treatment at higher absorbed doses might be due to the high pollutant concentration in textile effluents and, secondly, there may be some compounds unable to degrade even at high-absorbed doses and the degradable agent are destructed at low dose.

Table 2. Microbial load, cytotoxicity, and mutagenicity of textile wastewater after gamma radiation treatment to the absorbed doses of 5 kGy, 10 kGy, and 15 kGy.

Bioassays	Types	Units	Industry I			Industry II			Industry III			Average
			5 kGy	10 kGy	15 kGy	5 kGy	10 kGy	15 kGy	5 kGy	10 kGy	15 kGy	
Microbial load	TBC	CFU	ND	ND	–	ND	ND	–	ND	–	–	–
	TC	CFU	ND	ND	–	ND	ND	–	ND	ND	–	–
Cytotoxicity	ACT											
	I) RC	count	14±0.25	14±0.3	–	15±0.54	16±0.3	16±0.5	14±0.43	15±0.5	15±0.3	–
	I) RL	cm	6.2±0.15	6.0±0.2	6.4±0.2	5.8±0.30	6.8±0.5	–	6.6±0.60	7.0±0.5	6.9±0.6	–
	Heamolytic											
	I) human	%	–	41±1.0	40±1.3	53±2.10	45±1.1	44±1.3	51±1.99	50±2.8	44±1.5	–
	II) sheep	%	38±2.00	37±2.1	34±1.9	41±2.50	39±2.9	33±1.7	37±1.10	–	26±2.0	–
	Shrimp	-	4±1.00	4±1.00	2±1.00	5±1.00	4±2.10	–	6±1.00	4±2.0	2±1.0	–
Mutagenicity	Ames test											
	I) TA98	count	33±2.00	29±1.0	–	35±1.50	33±1.0	33±1.0	30±3.00	31±2.0	28±2.0	–
	II) TA100	count	41±1.00	38±1.0	36±1.0	39±2.00	–	34±2.0	35±1.50	33±1.0	29±1.0	–
Percentage toxicity reduction after treatment												
Microbial load	TBC	%	100	100	100	100	100	100	100	100	100	100
	TC	%	100	100	100	100	100	100	100	100	100	100
Cytotoxicity	ACT											
	I) RC	%	28.57	28.57	–	40	43.75	43.75	28.57	33.33	33.33	31.10
	I) RL	%	31.78	32.66	33.87	32.75	42.64	–	42.42	45.71	44.92	38.34
	Heamolytic											
	I) human	%	–	49.38	50.61	32.05	42.3	43.58	29.16	30.55	38.88	39.56
	II) sheep	%	46.47	46.47	52.11	40.57	43.47	52.17	50.6	–	65.33	49.65
	Shrimp	-	77.77	77.77	88.88	73.68	78.94	–	70	80	90	79.63
Mutagenicity	Ames test											
	I) TA98	%	46.77	53.22	–	40.67	44	44	50	48.33	53.33	47.54
	II) TA100	%	39.7	44.11	47	38	–	46	42.62	49.18	54.09	45.09

“–” – not measured, ND – not detected, ACT – *Allium cepa* test, RC – root count, RL – root length

The representative textile wastewater samples were also tested for mutagenicity before and after gamma radiation treatment and results (Table 2; Ames test). The untreated wastewater sample showed mild mutagenicity and, after gamma radiation treatment, the mutagenicity was reduced significantly and up to 46.77% and 39.7% reduction was observed in the case of TA98 and TA100, respectively, for the gamma radiation absorbed dose of 5 kGy. A reduction in mutagenicity was 53.22% in the case of TA98 for the absorbed dose of 10 kGy, whereas in the case of TA100 it was 44.11% and 47.00% for the absorbed doses of 10 kGy and 15 kGy, respectively, for effluents from industry I, and a similar trend was observed for industry II and III. Overall, reduction in mutagenicity was recorded to be 47.54% and

45.09%, respectively, for TA98 and TA100. From the results of the Ames test, it can be concluded that gamma radiation treatment has the ability to degrade a mutagenic agent present in industrial wastewater.

In recent years, different AOPs have been investigated for the removal of emerging contaminants from wastewater and drinking water, etc., and it also has been observed that the AOP are able to detoxify the toxic agent [2, 27]. The detoxification behavior might be due the degradation of toxic compounds into harmless by-product as a result of the action of the hydroxyl radical [3-5]. Few studies have reported the toxicity of treated samples by AOP; however, the studies regarding gamma radiation effect on toxicity are rare. Therefore, textile wastewater was subject to gamma

radiation treatment to the absorbed doses of 5 kGy, 10 kGy, and 15 kGy, and treatment effect was evaluated on the basis of cytotoxicity, mutagenicity, and microbial load reduction. It was found that the microbial population, cytotoxicity, and mutagenicity were reduced significantly after gamma radiation treatment, and that a low dose of gamma radiation is more effective as compared to higher doses, and this treatment could possibly be used for toxicity reduction by optimizing the gamma radiation dose. Some authors have studied the toxicity of treated wastewater by AOP other than gamma radiation treatment and positive results have been reported [3, 23]. Toxicity of four types of industrial wastewater, treated by Fenton's reagent, was analyzed using bioluminescent bacteria *Vibrio fischeri* NRRL B-11177 [35], *Daphnia magna* toxicity tests [36] and toxicity of soap and detergent wastewater by *Allium cepa*, hemolytic, brine shrimp, and Ames test is also reported [3]. The present investigation and previous studies have pointed out that the AOPs could be used for the detoxification of industrial effluents and gamma radiation treatment found to be very efficient for cytotoxicity, mutagenicity, and microbial load reduction.

Conclusion

The textile effluents were subjected to gamma radiation treatment to the absorbed doses of 5, 10, and 15 kGy, and treatment efficiency was judged on the basis of microbial load, cytotoxicity, and mutagenicity reduction. Gamma radiation treatment revealed promising efficiency for the detoxification of toxic agents present in effluents. The higher absorbed doses did not show a significant effect as compared to low-absorbed doses, and to enhance treatment efficiency there is a need to optimize the gamma radiation dose. On the basis of results of cytotoxicity and mutagenicity, it is concluded that this treatment can be used for the detoxification of textile effluents.

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