Water Air Soil Pollut (2019) 230:167
https://doi.org/10.1007/s11270-019-4212-z

Toxicity Study of a Textile Effluent Treated with Electrohydraulic Discharge and Coagulant/Flocculants

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Received: 19 February 2019 / Accepted: 23 June 2019

Abstract Exposure to complex organic substances present in textile wastewater has been considered a threat to human health and aquatic organisms. Development of appropriate treatment mechanisms, as well as sensitive monitoring assays, is considered important in order to safeguard and protect the delicate natural equilibrium in the environment. In this study, combined coagulation/flocculation and electrohydraulic discharge (EHD) system were explored for treatment of textile wastewater. Pre- and post-treatment samples were used to evaluate process efficiencies. Process efficiencies were evaluated using physicochemical characteristics, and cytotoxicity and inflammatory activities induced in macrophage RAW264.7 cell line. The RAW264.7 cell line was evaluated as an alternative to animals and human blood culture models, whose routine applications are hindered by stern ethical requirements. The toxicity of effluent was evaluated using WST-1 assay. The inflammatory activities were evaluated in RAW264.7 cell culture supernatant using nitric oxide (NO) and interleukin 6 (IL-6) as biomarkers of inflammation. The levels of NO and IL-6 were determined using the Griess reaction assay and double-antibody sandwich enzyme-linked immunoassay (DAS ELISA), respectively. Overall, the results of this study show that combined approaches and not the single EHD system are sufficient for complete removal of chemical oxygen demand (COD) and total organic carbon (TOC), toxicity and inflammatory activities in textile wastewater. The study shows that induction of NO and IL-6 secretions in macrophage RAW264.7 cells is a very sensitive model system to monitor the efficiency of textile effluent treatment processes.

Keywords Coagulation/flocculation · Textile effluent · Toxicity · Inflammation · Electrohydraulic discharge · Nitric oxide · Interleukin 6

1 Introduction

The increase in industrial activities has been recognised as a fundamental ingredient of economic growth and improvement of standard of living. On the other hand, industrial expansion and textile activities in particular remain environmentally unfriendly. This is because textile industry uses a large amount of water and generates a large amount of wastewater. Textile wastewater is normally characterised by strong colour, chemical oxygen demand (COD), biochemical oxygen demand (BOD) and total organic carbon (TOC) (Chequer et al. 2013). Direct discharge of raw textile wastewater effluent into the environment has been widely acknowledged

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Published online: 08 July 2019

to cause water pollution with various adverse environmental effects on human and aquatic species (Lacasse and Baumann 2004; Savin and Butnaru 2008). To avoid toxic effects of effluent, various conventional wastewater treatment processes have been implemented (Oller et al. 2011; Maletz et al. 2013). The conversional methods include coagulation, flocculation, biological treatment, precipitation, adsorption and chlorination, among others (Kasprzyk-Hordern et al. 2009; Maletz et al. 2013). However, the efficiency of these methods is low in terms of decomposition of non-biodegradable dyes. To achieve complete removal of dye molecules, a combination of different conversional and advanced oxidation treatment technologies is recommended (Oller et al. 2011; Enjarlis 2013; Anvari et al. 2014). Advanced oxidation technologies involving electrohydraulic discharge (EHD) systems have been widely regarded as promising and effective treatment techniques particularly in the decomposition of persistent organic pollutants present in textile wastewater (Sharma et al. 2011; Oller et al. 2011; Olivier et al. 2013).

Discharge of inadequately treated textile effluent has been associated with many biological effects. For example textile dyes are known to cause toxicity, carcinogenicity, mutagenicity and allergic reactions (Puvaneswari et al. 2006; Malinauskiene et al. 2012). Allergic reactions are forms of chronic inflammatory reaction involving many inflammatory factors including cells such as basophils, eosinophils, T lymphocytes and macrophages (Kang and Biswas 2013). Inflammatory activities of textile effluent treated with EHD may be due to inflammatory pollutants such as dyes which are the main components of textile effluents (Nygaard et al. 2013; Leme et al. 2014). Another possible cause of toxicity and inflammatory effects in effluent treated by the EHD method is the presence of intermediate products such as reactive radicals. The intended effect of generated reactive radicals is to react with pollutants in water leading to the reduction of organic pollutants. However, excess reactive radicals such as the hydroxyl radical (•OH) form more stable radicals such as hydrogen peroxide (H_2O_2) which can be detected in environmental water (Li et al. 2012).

To ascertain efficiency removal of pollutants in wastewater, evaluation of quality of effluent is very important. In most cases, the quality of effluent has been evaluated based on physicochemical parameters such as pH, COD and TOC (Sonune et al. 2015; Osuolale and Okoh 2015). However, these physicochemical parameters lack information on the efficiency removal of biological effects (Sacan and Balcioglu 2006). Therefore, in order to assess the efficiency removal of pollutants with biological effects, appropriate bioassays must be used. Several studies have reported on evaluation of performance of wastewater treatment methods by bioassays using organisms like invertebrates and fish (Meriç et al. 2005; Soni et al. 2006; Rizzo 2011; Nagel-Hassemer et al. 2011). Other studies evaluated inflammatory effects of wastewater and environmental water using whole human blood culture (Pool and Magcwebeba 2009; Faul et al. 2014) or isolated peripheral human mononuclear cell culture (Wichmann et al. 2004; Adebayo et al. 2014). The induction of inflammatory reaction by oxidative stress is similar to stimulation with bacterial lipopolysaccharides (LPS) and dyes, which is via NF-kB leading to the synthesis of inflammatory modulators such as nitric oxide (NO) and interleukin 6 (IL-6) (Hoesel and Schmid 2013). NO and IL-6 are secreted by many cells including macrophages. These inflammatory mediators can be induced in animals and blood culture; however, their routine uses for assessment of effluent quality may be hindered by ethical requirements. In order to avoid stern ethical requirements, similar inflammatory responses can be induced in established cell lines. One of the cell lines commonly used in inflammatory response studies is a mouse macrophage RAW264.7 cell line. The cell line has been successfully used in many inflammatory studies as well as in anti-inflammatory studies (Kim et al. 2014; Xu et al. 2014). Nevertheless, so far there is no study reported on bioassay using RAW264.7 cells for evaluating the performance of the electrohydraulic discharge system combined with conversional methods for treatment of textile effluent. Therefore, the present study is aimed at evaluating the efficiency of the electrohydraulic discharge system combined with coagulant/flocculant treatment in terms of reduction of the COD, TOC, toxicity and inflammatory activities in textile effluent.

2 Material and Methods

2.1 Wastewater Sample Collection

The wastewater samples were collected from a nonwoven textile industry in Cape Town, South Africa, and stored in 200-L acid-washed plastic drums. The raw effluent was taken from the central storing tank that received effluent from all the processing units in the

factory. The effluent is completely dark in colour and highly odoriferous. Prior to the sampling, the drums were washed with diluted HNO_3 and millipore water and thereafter rinsed with the effluents. The samples were transported to the laboratory and refrigerated at 4 °C prior to laboratory investigations.

2.2 Coagulation and Flocculation Processes

The collected textile effluent was subjected to gravitational settling for 30 min due to the presence of a substantial quantity of suspended solids although the suspended particulate matter was poorly settled within 30 min. Thus, a coagulation-flocculation study was undertaken to remove the colour and other suspended particulate matters. The study was performed by varying the mass of either $Al_2(SO_4)_3$ or $Ca(OH)_2$ or Fe–Mn oxide from 0.2–1.0 g. Analytical-grade $Al_2(SO_4)_3{\cdot}14H_2O$ and $Ca(OH)_2$ were procured from Sigma-Aldrich and were used without any further treatment. Raw ferromanganese wad (Fe–Mn) oxide was obtained from Vaal Triangle, Gauteng, and was also used without further purification. Firstly, the specified dose of the coagulant/flocculant was added to a 0.5-L wastewater sample. Thorough mixing on magnetic stirrers was performed for 1 min at 100 rpm, and then, flocculation was carried out at a speed of 20 rpm for 20 min. Finally, the wastewater content in the beaker was allowed to settle for 40 min, and the supernatant was collected for analysis. The experiments were performed at room temperature (25 °C ± 2) and constant pH of 6.02. The experiment was repeated twice and the average values obtained from the duplicate of experiments were recorded.

2.3 Electrohydraulic Discharge Treatment

The raw textile effluent and the best treated sample with optimised dose of coagulant/flocculant were further subjected to EHD treatment. A power supply set at 25 V, delivering a current of 3 A and a power of 75 W, was directly connected in series to a transformer that steps the AC voltage up to a DC peak voltage of ~ 8 kV directly delivered into the EHD reactor. A 0.5-mm-diameter silver electrode directly connected to the high voltage (output of the transformer) was immersed in a 50 g/L of sodium chloride electrolyte placed in the inner tube of a single-cell reactor. This reactor tube containing the electrode was placed in a 1.8-L beaker filled with 1.5 L of raw effluent. An air pump with a high and low flow speed switch was connected to both air flow meter and the single-cell reactor tube for air (mostly oxygen) production. A flow rate of 3.0 L/min was used. The reactor consisted of an inner and outer tube. The diameter of the inner tube was approximately equal to 1 mm and that of the outer tube was 7 mm. The reactor was 23 cm long with an inlet and outlet for air circulation. The ground electrode was submerged into the raw effluent as the case may be in the beaker to complete the circuit and also grounded to the earth to avoid any electrocution. The EHD experiment was conducted for 60 min and sampling was done every 10 min (Table 1).

2.4 Chemical Characterization

The raw and treated effluents were analysed for physicochemical characteristics such as the COD, TOC and pH value. All the physicochemical characteristics were measured in accordance with "Standard methods for treatment of water and

Table 1 Experimental protocol for wastewater treatment

Sample treatment	Treatment protocol
Raw effluent	Before treatment
Electrohydraulic discharge system alone	Treated with electrohydraulic discharge alone for 60 min
$Al_2(SO_4)_3{\cdot}14H_2O$ and electrohydraulic discharge system	1.6 g/L of $Al_2(SO_4)_3{\cdot}14H_2O$ and electrohydraulic discharge treatment for 60 min
$Al_2(SO_4)_3{\cdot}14H_2O$, $Ca(OH)_2$ and electrohydraulic discharge system	1.6 g/L of $Al_2(SO_4)_3{\cdot}14H_2O$, 1.6 g/L of $Ca(OH)_2$ and electrohydraulic discharge treatment for 60 min
Fe–MnO_2, $Al_2(SO_4)_3{\cdot}14H_2O$, $Ca(OH)_2$ and electrohydraulic discharge system	1.6 g/L of Fe–MnO_2, 1.6 g/L of $Al_2(SO_4)_3{\cdot}14H_2O$, 1.6 g/L of $Ca(OH)_2$ and electrohydraulic discharge treatment for 60 min

wastewater" (American Public Health Association 1999). Aliquot of each sample was sterile filtered using a 0.45-μM filter paper and stored at − 20 °C.

2.5 Cell Culture

Mouse macrophage RAW264.7 cell line from American Type Collection ATCC-TB-71 was maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated foetal bovine serum (FBS), 1% antibiotic/antimycotic (Sigma, Germany), 0.05% gentamycin (Sigma, Germany) and 1% Glutamax (Gibco, Life Technologies), in a humidified incubator at 37 °C and 5% CO_2. The cells were cultured at 5×10^5 cells/mL in 96-well plates till confluence. After confluence, medium was replaced with fresh culture medium containing the effluent samples at 1 in 100 dilutions in respective wells. Control treatments included normal medium for negative control and medium supplemented with 1 μg/mL lipopolysaccharides (LPS) from *Escherichia coli* 0111:B4 (Sigma, Germany) as positive control. The cell culture was incubated overnight at 37 °C and 5% CO_2. After the overnight incubation, culture supernatants were collected for NO and IL-6 assays. The cells were used for cell viability assays.

2.6 Cell Viability

Toxicity of effluent samples was evaluated by cell viability assays using the chromogenic-based water-soluble tetrazolium WST-1 assay. The WST-1 assay is based on the principle of breakdown of tetrazolium to water-soluble formazan dye by the action of dehydrogenase enzyme. The assay was done using WST-1 reagent (Roche, Germany). Briefly, after removal of culture medium, each cell culture well received 100 μl of medium supplemented with 10% WST-1 reagent. The absorbance was read immediately after addition of WST-1 medium, and a second reading was done after incubation for 30 min at 37 °C and 5% CO_2. The change in absorbance at 450 nm over 30 min was used as a measure of cell viability.

2.7 Nitric Oxide Assay

Nitric oxide (NO) production was determined in culture supernatants using the Griess reaction in 96-well plates (Nunc, Denmark). The supernatant was mixed with equal volume of Griess reagent made up of 1% sulphanilamide (Sigma, Germany), 0.01% naphthyl ethylenediamine dihydrochloride (Sigma, Germany) and 2.5% phosphoric acid. The colour developed after 15-min incubation was measured at 540 nm using a microplate reader (Thermo Electron). The concentration of NO was determined from a standard curve generated using 100–1.56 μM sodium nitrite (Sigma, Germany).

2.8 Interleukin 6 Assay

Interleukin 6 in cell culture supernatant was determined using a commercially available mouse ELISA kit (e-Bioscience, Germany). The assay system is a double-antibody sandwich enzyme-linked immunosorbent assay (DAS ELISA). The assays were done on NUNC Maxisorp 96-well plates (Nunc, Denmark). The assay kit's procedure was followed as provided in the kit. Briefly the protocol involved coating of a plate overnight at 4 °C with capture antibody (antimouse IL-6 diluted in coating buffer, PBS). The plate was washed five times in wash buffer consisting of PBS with 0.1% Tween. The plate was then blocked with assay diluent for 1 h at room temperature. After another five times of washing, IL-6 standard or cell culture supernatants were added to each well accordingly. The plate was then incubated for 2 h at room temperature. The plate was washed again five times, after which the detection antibody (biotinylated antimouse IL-6 in block diluent) was added to each well and incubated for 1 h at room temperature. After another five-time wash, avidin-horse radish peroxidase (HRP) conjugate was added and incubated for 30 min at room temperature. The plate was washed seven times; then, substrate TMB solution was added and incubated in the dark for 15 min at room temperature. The reaction was stopped with 0.5 M H_2SO_4 stop solution, and the absorbance read at 450 nm with a microplate reader (Thermo Electron).

2.9 Statistical Analysis

The data are presented as mean ± standard deviation (SD) and were statistically analysed with one-way variance analysis (ANOVA) using SigmaStat (SigmaStat software, Inc., CA). The mean values of each treatment were compared with those of the control. The P value < 0.001 was considered statistically significant.

3 Results and Discussion

3.1 Physicochemical Characteristics of Effluent Samples

The results of the physicochemical characterisation of raw textile effluents supplied by the factory are shown in Table 2. The high value of electrical conductivity indicates that a significant amount of ionized species is present in the raw effluent, and thus, direct discharge might have an adverse impact upon aquatic and other species. The total suspended solids (TSS) and total dissolved solids (TDS) reveals suspended organic and inorganic matter in the raw effluent, and the individual values are well above the recommended limit. The COD value of the raw wastewater supplied is greater than 20,000 mg/L, and water with high value of COD is toxic to aquatic life and the entire ecosystem. It is evident from Table 2 that the wastewater does not meet the applicable 5000 mg/L COD minimum effluent discharge limit values as contained in City of Cape Town wastewater and industrial effluent bylaw schedule 2 page 10 (www.capetown.gov.za/en/Water/Documents/wwater_bylaw_eng.pdf). The high COD, TOC, BOD, TDS, TSS, pH and intense colour can be attributed to the use of complex organic substances that are highly non-biodegradable. It is imperative, based on the obtained values, that the effluents need to be treated prior to discharge into the environment as this will definitely have adverse effects on the environment, especially on the aquatic species.

All values are in milligrams per litre except for pH, electrical conductivity (mS/cm) and turbidity in Nephelometric Turbidity Units (NTU).

Table 2 Physicochemical characteristic of the raw wastewater

Parameters	Symbols	Value
pH		5.99 ± 0.03
Electrical conductivity		1238.6 ± 1.53
Alkalinity as carbonate	CO_3^{2-}	212.3 ± 12.5
Nitrate + nitrite	NO_3-	17.5 ± 0.70
Nitrite	NO_2-	11.7 ± 1.15
Ortho phosphate	PO_4^{3-}	203 ± 2.62
Sulphate	SO_4^{2-} dissolved	190.3 ± 0.61
Total phosphorus	P	199 ± 3.62
Turbidity		8145.3 ± 5.74
Dissolved organic carbon	DOC	2438.7 ± 1.52
Total organic carbon	TOC	5952 ± 2.64
Chemical oxygen demand	COD	20,457 ± 1.52
Biochemical oxygen demand	BOD	807 ± 1.00
Total suspended solids	TSS	2758.7 ± 8.08
Total dissolved solids	TDS	744 ± 6.55
Chlorides	Cl−	55.7 ± 0.09
Fluorides	F	16.3 ± 0.14
Colour	Dark	
Odour	Highly unpleasant (odiferous)	

3.2 Effects of Coagulation/Flocculation Processes and the EHD System on Physicochemical Properties

It had earlier been observed that application of individual coagulant/flocculants mentioned above was not effective to reduce the COD and TOC values to the limit set by City of Cape Town, South Africa (www.capetown.gov.za/en/Water/Documents/wwater_bylaw_eng.pdf). Thus, after pre-treatment with coagulation/flocculation processes, the optimal conditions were determined, and the treated effluents were further subjected to electrohydraulic discharge treatment. The results of combined treatment processes are presented in Table 3. Coagulation/flocculation process was applied as a form of pre-treatment to remove colour, suspended particulate matters and other interfering matrix and thereafter subjected to advanced oxidation process such as electrohydraulic discharge treatment. The results presented in Table 2 represent an optimum dosage of each coagulant and flocculant in combination with the electrohydraulic discharge system at a specific pH. With EHD alone, 11.7 and 16.7% COD and TOC removal was observed. The maximum COD and TOC removal for aluminium sulphate/EHD treatment was 77.8 and 83.4%, respectively. Combined treatment with 1.6 g/L of $Al_2(SO_4)_3{\cdot}14H_2O$, 1.6 g/L of $Ca(OH)_2$ and electrohydraulic discharge system successfully reduced the COD and TOC values by 95.3 and 96.23%, respectively. The combination of 1.6 g/L Fe–Mn oxide, 1.6 g/L $Al_2(SO_4)_3{\cdot}14H_2O$ and 1.6 g/L $Ca(OH)_2$, followed by electrohydraulic discharge system, further enhanced the COD and TOC reduction by 96.79 and 96.6%, respectively. Compared with the raw effluent, it can be seen that the most feasible and effective combined

Table 3 COD, TOC and pH values before and after flocculation/EHD treatment for 60 min

Sample	pH	COD (mg/L)	TOC (mg/L)
Before treatment	5.99 ± 0.03	20,457 ± 1.52	5952 ± 2.64
After treatment with electrohydraulic discharge system alone	5.83 ± 0.16	18,057 ± 2.36	4953 ± 5.46
After treatment with 1.6 g/L of $Al_2(SO_4)_3{\cdot}14H_2O$ + electrohydraulic discharge system	4.56 ± 0.24	4, 532 ± 0.43	986 ± 0.71
After treatment with 1.6 g/L of $Al_2(SO_4)_3{\cdot}14H_2O$ 1.6 g/L of $Ca(OH)_2$ + electrohydraulic discharge system	9.63 ± 1.23	958 ± 0.06	224 ± 0.05
After treatment with 1.6 g/L of Fe–Mn oxide, 1.6 g/L of $Al_2(SO_4)_3{\cdot}14H_2O$, 1.6 g/L of $Ca(OH)_2$ + electrohydraulic discharge system	8.51 ± 0.09	656 ± 0.89	200 ± 1.23

treatment is 1.6 g/L of $Al_2(SO_4)_3{\cdot}14H_2O$, 1.6 g/L of $Ca(OH)_2$ and electrohydraulic discharge system as well as 1.6 g/L of Fe–Mn, 1.6 g/L of $Al_2(SO_4)_3{\cdot}14H_2O$, 1.6 g/L of $Ca(OH)_2$ and electrohydraulic discharge system with significant reduction in COD and TOC values.

The raw textile effluent was treated using the EHD system; the EHD produces reactive radicals such as O_3, OH, H_2O_2 and UV, which degrade organic compounds. From Table 2, there was only a small reduction of about 10% in the COD, TOC, and pH values of the raw and treated effluent by the EHD even after 60 min. This might be due to the competition between the radical scavengers such as nitrates, sulphates, carbonates and chlorides and the organic pollutants present in the effluents for free reactive species produced by the EHD system. Depending on the solution pH, the scavenging effect lowers and suppresses the oxidation potential of the free reactive species and that possibly affects the process efficiency leading to lower COD and TOC values (Bacardit et al. 2007; Oller et al. 2011). Equally, the formation of a series of intermediate products and the possible occurrence of secondary reactions among the by-products might also be responsible for lower degradation efficiency by the EHD (Kiwi et al. 2000). According to Enjarlis (2013), incomplete degradation of organic pollutants gives rise to formation of different intermediate products that can affect the overall process efficiency. It is also noteworthy based on the results presented in Table 2 that the raw effluent contained an appreciable amount of carbonate, nitrate, sulphate, phosphates and chlorides. These major species control the solution pH and quench the free reactive species. Wu et al. (2008) had earlier reported that the quenching effect of hydroxyl radical by carbonates appreciably contributed to impeding the COD and TOC reduction levels. Treatment of the raw effluent with EHD is potentially less effective due to the presence of radical scavengers (Kiwi et al. 2000). Furthermore, interesting behaviour was noticed with the treatment of the raw effluent by combination of aluminium sulphate/EHD. The combined approach lowers the COD and TOC values to 4532 and 986 mg/L with a removal rate of 77.8 and 83.4%, respectively. This observed trend indicates that a significant portion of organic pollutants and interfering species have been removed from the raw effluent as sludge by the aluminium sulphate whereas a portion transformed into intermediate compounds due to the influence of the free radicals produced by the EHD. Thus, both the aluminium sulphate and EHD played a crucial role in lowering the COD and TOC values. Not only that, it has been previously demonstrated that continuous exposure of the treated effluent to ozone, UV, OH and H_2O_2 at a minimum dose is effective for complete degradation of organic pollutants in wastewater (Zayas et al. 2007). Furthermore, after 60 min of treatment with combination of 1.6 g/L of $Al_2(SO_4)_3{\cdot}14H_2O$ and 1.6 g/L of $Ca(OH)_2$ followed by electrohydraulic discharge system, the COD and TOC values were further decreased to 95.3 and 96.23%, respectively. This was due to the destabilization of organic flocs which settled very fast and resulting in almost stable COD values. Correspondingly, with the addition of 1.6 g/L $Ca(OH)_2$, the pH value increased to 9.36, which is slightly higher than the favourable pH range (6–7). At high pH value, decomposition of ozone takes place prior to eventual reaction with pollutants in the presence of hydroxyl ions (Muruganandham et al. 2014). As the solution pH increased, the concentration of hydroxyl radical increased and later combined to produce hydrogen

peroxide. Thus, the combined effect of hydrogen peroxide/ozone at high pH value eventually increased the decomposition of organic compound bonding framework. This view has been widely expressed in a number of studies (Jung et al. 2012). The obtained COD and TOC values are lower than the City of Cape Town permissible limit. At this level, treated wastewater is safe to be discharged into the City of Cape Town wastewater system. Table 3 shows that with the incorporation 1.6 g/L of Fe–Mn oxide and 1.6 g/L of aluminium sulphate/calcium hydroxide/EHD system, the COD and TOC values further reduced by 96.79 and 96.6%, respectively. This is due to the improved generation of free reactive species caused by absence of interfering anions. Integration of coagulation-flocculation and electrohydraulic discharge system is considered to be advantageous for lowering the COD and TOC levels in the effluent. On the other hand, COD and TOC values cannot be completely removed from raw effluent. The residual COD and TOC in the treated effluent may plausibly be due to the formation of intermediate macromolecules or secondary reactions. Overall, there was a substantial decrease in the investigated parameters of the treated effluent compared with the raw effluent with the exception of EHD itself alone and 1.6 g/L of $Al_2(SO4)_3$/EHD treatment. The decrease was due to the synergistic effect of the individual coagulant/flocculant agents as well as the EHD system. The treated effluents were further subjected to toxicity and inflammatory test.

3.3 Effects of Coagulation and Flocculation Processes/EHD System on Cell Viability

The toxicity of raw effluent and effluent treated with electrohydraulic discharge alone or combined coagulation/flocculation processes and electrohydraulic discharge system were tested for their effects on RAW264.7 cell viability. Raw effluent and effluent treated with electrohydraulic discharge alone reduced cell viability significantly ($P < 0.001$) compared with negative control (Fig. 1). Samples treated with 1.6 g/L of $Al_2(SO_4)_3 \cdot 14H_2O$ combined with electrohydraulic discharge system, 1.6 g/L of $Al_2(SO_4)_3 \cdot 14H_2O$, 1.6 g/L of $Ca(OH)_2$ and electrohydraulic discharge system or 1.6 g/L of Fe–Mn oxide, 1.6 g/L of $Al_2(SO_4)_3 \cdot 14H_2O$, 1.6 g/L of $Ca(OH)_2$ and electrohydraulic discharge system had no significant effect on the viability of RAW264.7 cells.

The decrease of cell viability induced by raw effluent and effluent treated by EHD alone may be due to toxicity effects of pollutants in the samples. The raw sample was characterised by high values of COD and TOC, which may be due to high content of organic pollutants. Toxicity of textile effluent has been previously reported using in vivo bioassays (Soni et al. 2006; Verma 2008). Toxicity due to specific reactive dyes in raw effluent has been reported by several studies (Klemola et al. 2007; Verma 2008). Similarly, toxicity of effluent treated with EHD alone could be partly attributed to inadequate removal of toxic pollutants by the EHD system. Inefficient degradation using EHD could be due to the

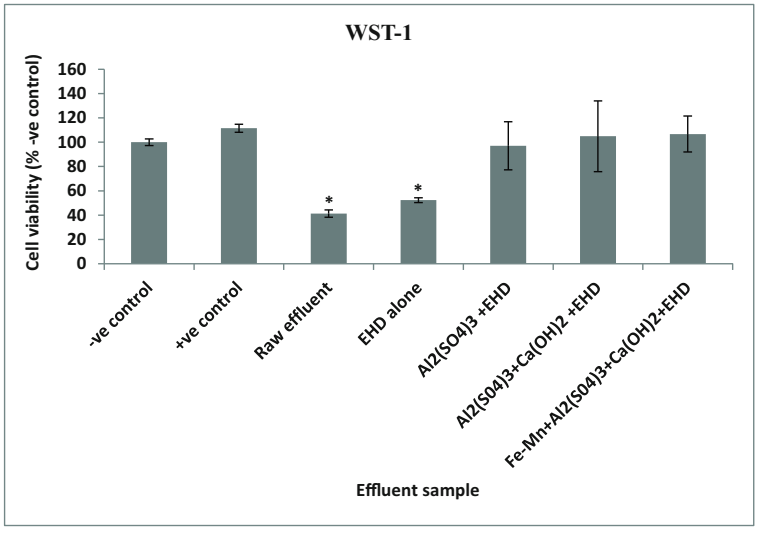

Fig. 1 Effects of effluent samples treated with different methods on RAW264.7 cell viability as determined by WST-1 assay. Negative (−ve) control was treated with normal medium; positive (+ve) control was treated with medium containing LPS (1 μg/ml). Wastewater samples were diluted to 1 in 100 in medium as described in Section 2. The results are presented as mean ± SD and * indicates that cell viability is significantly ($P < 0.001$) lower than negative control

presence of scavenging molecules in the effluent sample that react with produced intermediate by-products (Kiwi et al. 2000). This is because the performance of the EHD system depends on generation of free reactive radical species such as hydroxyl radical (•OH), perhydroxyl radical (•OOH), superoxide anion ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), ultraviolet light and shock waves (Bruggeman and Locke 2013). The free radicals are very reactive and non-selective, especially hydroxyl radicals, which cause degradation of complex organic substances (Malik 2010; Jiang et al. 2014). The degradation of dyes is achieved by cleavage of the double bond of dye molecules (Jiang et al. 2014). However, it has been observed that most of the free radicals have a very short life, hence may disappear before decolouration is complete (Tahara and Okubo 2014). Furthermore, the hydroxyl radical (•OH) is quickly converted to H_2O and H_2O_2, which is more stable and can be detected in water (Li et al. 2012). Therefore, accumulation of H_2O_2 can also be a source of toxicity in effluent treated with EHD alone.

3.4 Effects of Coagulation and Flocculation Processes/EHD System on Inflammatory Activities

Inflammatory effects of effluent samples were determined in RAW264.7 cell supernatant by NO and IL-6 as biomarkers of inflammation. Samples from raw effluent, effluent treated with electrohydraulic discharge alone and combined $Al_2(SO4)_3$/EHD induced significantly ($P < 0.001$) higher NO production in RAW264.7 cell culture than the negative control (Fig. 2). Effluent samples treated with $Al_2(SO_4)_3 \cdot 14H_2O/Ca(OH)_2$/EHD system as well as Fe–Mn oxide/$Al_2(SO_4)_3 \cdot 14H_2O/Ca(OH)_2$/EHD system did not induce significant different amounts of NO compared with negative control. Similar trend of inflammatory activities of effluent samples was also observed in the induction of IL-6 secretion as shown in Fig. 3. Raw effluent, effluent treated with electrohydraulic discharge alone and combined $Al_2(SO4)_3$/EHD induced significantly ($P < 0.001$) higher IL-6 secretion in RAW264.7 cell culture than negative control. Effluent samples treated with $Al_2(SO_4)_3 \cdot 14H_2O/Ca(OH)_2$/EHD system as well as Fe–Mn oxide/$Al_2(SO_4)_3 \cdot 14H_2O/Ca(OH)_2$/EHD system had no significant effects on production of IL-6 in RAW264.7 cell culture compared with negative control.

Both NO and IL-6 results showed a similar trend of inflammatory activities. A significant increase in NO and IL-6 induced by raw effluent may be associated with high content of inflammatory pollutants in the sample. Presence of dyes may be the main cause of inflammatory activities in raw effluent samples. Several studies have also associated textile dyes with allergic reactions (Malinauskiene et al. 2012; Nygaard et al. 2013). Allergic reactions are due to chronic inflammation, in which macrophages secrete inflammatory mediators. Treatment of raw effluent with EHD alone significantly ($P < 0.001$) reduced levels of NO and IL-6 as compared with raw effluent. However, the levels of both NO and IL-6 were still significantly ($P < 0.001$) higher than the negative control. In addition to presence of inflammatory pollutant as result of incomplete removal, inflammatory activities in effluent treated with EHD

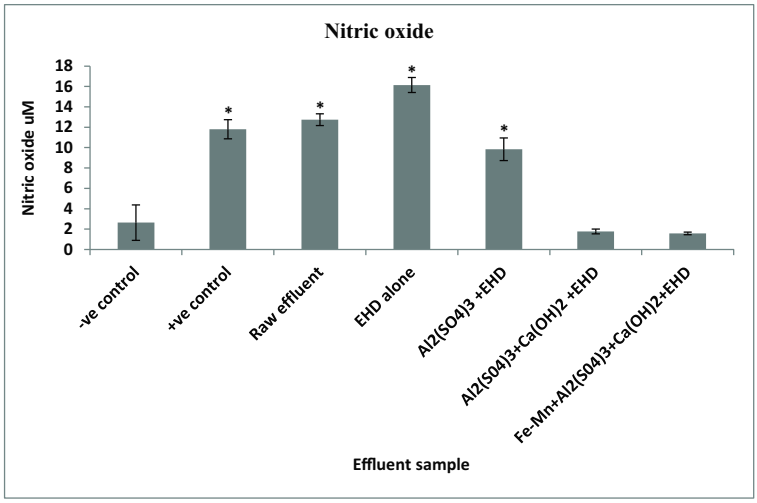

Fig. 2 Effects of raw and effluent samples treated with different methods on induction of NO production in RAW264.7 cell culture. Negative (−ve) control was treated with normal medium, positive (+ve) control was treated with medium containing LPS (1 μg/ml), and raw and treated effluent samples were diluted at 1 in 100 dilutions in medium. The results are presented as mean ± SD and * indicates that NO production is significantly ($P < 0.001$) higher than negative control

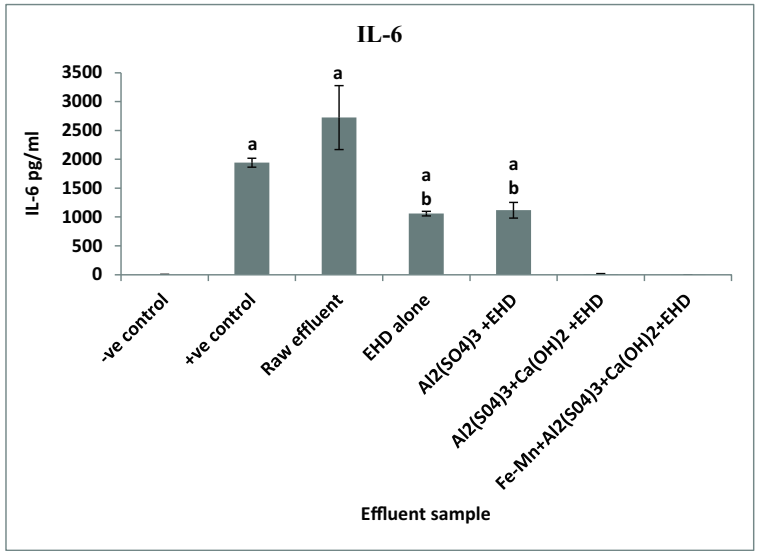

Fig. 3 Effects of raw and effluent samples treated with different methods on induction of IL-6 secretion in RAW264.7 cells culture. Negative (−ve) control was treated with normal medium, positive (+ve) control was treated with LPS (1 μg/ml), wastewater sample 1 in 100 dilutions in medium. The results are presented as mean ± SD and (a) indicate that IL-6 secretion is significantly ($P < 0.001$) higher than the negative control. (b) indicate that IL-6 secretion is significantly ($P < 0.001$) lower than raw effluent

alone may also be due to the presence of excessive intermediate free radicals. Excessive free radicals may lead to oxidative stress, which induces inflammatory mediators and pro-inflammatory cytokine through NF-kB pathways (Hoesel and Schmid 2013). Similar effects of oxidative stress have been studied in macrophages of the respiratory system (Hnizdo and Vallyathan 2003; Kirkham 2007).

Addition of 1.6 g/L $Al_2(SO_4)_3{\cdot}14H_2O$ alone followed by EHD system did not reduce inflammatory pollutants. As a result, effluent samples induced secretion of inflammatory cytokine IL-6 and NO production in RAW264.7 cells. The inflammatory activities could be due to two possible sources. One is incomplete removal of inflammatory pollutants, which correlates well with high values of physicochemical properties of the sample. The sample is characterised by high value of COD. High COD is an indication of inefficiency of $Al_2(SO_4)_3{\cdot}14H_2O$ to remove organic pollutants. At low pH (4.56), aluminium precipitates rapidly. Due to the rapid precipitation of aluminium, it led to short contact time that could be responsible for the incomplete removal of pollutants that are responsible for inflammatory activities.

Samples treated with EHD after addition of 1.6 g/L of $Al_2(SO_4)_3{\cdot}14H_2O$ and 1.6 g/L of $Ca(OH)_2$ or 1.6 g/L of Fe–Mn oxide, 1.6 g/L of $Al_2(SO_4)_3{\cdot}14H_2O$ and 1.6 g/L of $Ca(OH)_2$ did not induce significant amounts of NO and IL-6. This is an indication of efficient removal of inflammatory pollutants which correlates well with low values of COD and TOC. The presence of $Ca(OH)_2$ in the pre-treatment process might have improved faster settling of pollutants hence increasing efficiency removal of COD (Yang et al. 2012). Similar observations have been reported on efficiency of $Ca(OH)_2$ to decolourise wastewater containing dyes (Al-Hemiri et al. 2007). Furthermore, calcium hydroxide at a pH value of 9.63 dissociated in water and released highly reactive free radicals (hydroxyl ions) which are very reactive and very effective in removal of dyes, bacteria and other organic pollutants. Therefore, based on the results of this study, the best combined techniques are 1.6 g/L of $Al_2(SO_4)_3{\cdot}14H_2O$ and 1.6 g/L of $Ca(OH)_2$ or 1.6 g/L of Fe–Mn oxide, 1.6 g/L of $Al_2(SO_4)_3{\cdot}14H_2O$ and 1.6 g/L of $Ca(OH)_2$ followed by electrohydraulic discharge system. Wastewater samples treated with these combined techniques achieved above 95% effective for COD and TOC with neither toxicity nor inflammatory activities.

4 Conclusions

In this study, the physicochemical characteristics of the textile wastewater samples revealed high value of parameters such as COD, BOD, TOC, TSS and TDS among others. In addition to high values of physicochemical properties, the effluent also was characterised by high toxicity and inflammatory activities. Treatment of the raw effluent by combination of coagulation/flocculation process with electrohydraulic discharge system produced satisfactory results. The maximum

and adequate COD and TOC removal of 96.79 and 96.6%, respectively, was realised with a combination of 1.6 g/L of Fe–Mn oxide, 1.6 g/L of $Al_2(SO_4)_3.14H_2O$, 1.6 g/L of $Ca(OH)_2$ and EHD system. Similarly, the combined system removed toxic and inflammatory pollutants based on the results of NO and IL-6 in RAW264.7 cell cultures. This study demonstrated that a single treatment approach is not effective in removing all pollutants. However, a combined treatment approach effectively removed complex organic pollutants. The study has also shown that performance of the combined methods can be evaluated using biomarkers of inflammation such as NO and IL-6 in macrophage RAW264.7 cells as a model system.

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