Environ Monit Assess (2016) 188:471
DOI 10.1007/s10661-016-5441-x

Evaluation of cytotoxicity and inflammatory activity of wastewater collected from a textile factory before and after treatment by coagulation-flocculation methods

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Received: 24 March 2015 / Accepted: 21 June 2016

Abstract Effective treatment of textile effluent prior to discharge is necessary in order to avert the associated adverse health impacts on human and aquatic life. In the present investigation, coagulation/flocculation processes were evaluated for the effectiveness of the individual treatment. Effectiveness of the treatment was evaluated based on the physicochemical characteristics. The quality of the pre-treated and post-flocculation treated effluent was further evaluated by determination of cytotoxicity and inflammatory activity using RAW264.7 cell cultures. Cytotoxicity was determined using WST-1 assay. Nitric oxide (NO) and interleukin 6 (IL-6) were used as biomarkers of inflammation. NO was determined in cell culture supernatant using the Griess reaction assay. The IL-6 secretion was determined using double antibody sandwich enzyme linked immunoassay (DAS ELISA). Cytotoxicity results show that raw effluent reduced the cell viability significantly ($P < 0.001$) compared to the negative control. All effluent samples treated by coagulation/flocculation processes at 1 in 100 dilutions had no cytotoxic effects on RAW264.7 cells. The results on inflammatory activities show that the raw effluent and effluent treated with 1.6 g/L of Fe-Mn oxide induced significantly ($P < 0.001$) higher NO production than the negative control. The inflammatory results further show that the raw effluent induced significantly ($P < 0.001$) higher production of IL-6 than the negative control. Among the coagulants/flocculants evaluated $Al_2(SO_4)_3.14H_2O$ at a dosage of 1.6 g/L was the most effective to remove both toxic and inflammatory pollutants. In conclusion, the inflammatory responses in RAW264.7 cells can be used as sensitive biomarkers for monitoring the effectiveness of coagulation/flocculation processes used for textile effluent treatment.

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

Introduction

Environmental pollution due to textile industry activities has become a global concern that has attracted considerable attention among researchers. The textile industry is one of those industries that utilize high volumes of clean water and complex chemicals during processing operations (Oller et al. 2011). As a result, there is a huge amount of wastewater generated that is composed of different complex chemical substances. The chemicals in the wastewater are mostly high molecular weight, non-biodegradable and highly recalcitrant in the environment (Zapata et al. 2009a, b). Examples of common pollutants in textile wastewater include heavy metals, detergents, reactive dyes, dye fixing agents and hydrocarbon compounds used as softeners (Ghaly et al. 2014).

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Published online: 15 July 2016

In addition, textile effluents usually have extremely high pH values, strong colour, high chemical oxygen demand (COD), biochemical oxygen demand (BOD), turbidity, total organic carbon (TOC), suspended and dissolved solids and soluble organic compounds among others (Mazumber 2011; Martín et al. 2011 and Hussaini et al. 2013).

The potential adverse effects on humans and the entire ecosystem due to the direct disposal of textile effluent into the aquatic environment without proper treatment have been widely reported (Gatidou et al. 2007; El-Gohary and Tawfik 2009; Pothitou and Voutsa 2008). According to Martín et al. (2011), direct discharge of coloured wastewater can cause eutrophication and reduce the available dissolved oxygen level. Such adverse conditions make aquatic life difficult and tend to accelerate genotoxicity and microtoxicity in aquatic organisms (Foo and Hameed 2010; Verma et al. 2012). Similarly, continuous exposure to untreated coloured wastewater may lead to effects such as suppression of the immune system, the respiratory system, cause neurobehavioral disorders, leukaemia, hyperventilation, eye infections and lung edema among others (Verma et al. 2012). Furthermore, reactive dyes, which form the main component in textile effluents, can also cause harmful effects like acute toxicity (Klemola et al. 2007; Verma 2008; Verma et al. 2012), carcinogenic effects (Mathur et al. 2005) and contact allergies (Ryberg et al. 2006; Malinauskiene et al. 2011).

Thus, considering the negative impacts of direct discharge of untreated textile wastewater on humans and the ecosystem, textile effluents need to be treated before being discharged into the environment. The review of literature revealed that several physico-chemical and biological methods such as microfiltration, activated carbon adsorption, coagulation, flocculation, activated sludge treatment either in the form of a pre or post-treatment step have been exploited to treat these effluents. However, one of the major environmental concerns is the removal of colour, suspended solids and perhaps reduction of COD in the effluent before discharge into the environment (Zayas et al. 2007; Oller et al. 2011). The presence of these complex mixtures make the treatment quite extensive, time demanding and as such single step clean-up systems are normally not effective for complete colour or COD reduction, instead a combinatory approach is required (Oller et al. 2011).

The use of coagulation-flocculation is one of the most widely utilized and practised techniques in developed and developing countries due to its simplicity as well as effectiveness in the removal of colour, suspended matter and COD reduction (Ciabatti et al. 2010; Oller et al. 2011; Verma et al. 2012). Different coagulant-flocculant mixtures such as ferric salts, aluminium salts, synthetic organic polymers, polyelectrolytes and lime have been applied to treat conventional wastewater (Bolto and Gregory 2007; Verma et al. 2012). These coagulants/flocculants accelerate the rate of aggregation of colloidal particles by reducing the electrostatic surface charges between the coagulants/flocculants and particles in the acidic pH region (Sher et al. 2013). The overall efficiency of the process depends on factors such as coagulant-flocculant dosage and type, stirring speed, reaction time, effluent pH and others (Bolto and Gregory 2007; Verma et al. 2012).

In most cases, during treatment of wastewater from textile industries, great attention has been placed on colour removal. As a result, the assessment of effectiveness of the techniques employed is normally based on the physico-chemical properties of the treated effluent such as removal of colour, COD and TDS. For instance, the reductions of COD and TDS by coagulation and precipitation methods have been reported (Parmer et al. 2011; Sabur et al. 2012). Sabur et al. (2012) found that both processes were pH dependent and at pH 6, 90.17 and 74.09 % COD and TDS reduction were observed. Mukhlish et al. (2013) have applied the coagulation-flocculation process to treat textile effluent and achieved a 61.3 % COD reduction at the optimum dosage of 2.5 kg/m^3 of CaO and 2.0 kg/m^3 of $FeSO_4$, respectively. Very recently, Di Bella et al. (2014) applied coagulation-flocculation as a pre-treatment step for saline wastewater and found that 50 mg/L of aluminium sulphate was effective for the removal of 70–80 % TOC.

Although reactive dyes are dominant components in textile effluents and are well known to cause numerous adverse biological effects, assessment of such biological effects are relatively rare. Several studies have demonstrated that reactive dyes can cause allergic reactions (Ryberg et al. 2006; Malinauskiene et al. 2012; Nygaard et al. 2013). Allergic reaction is a chronic inflammatory response involving many immunological factors and cells like basophils, eosinophils, T cells and macrophages (Kang and Biswas, 2013). The role of macrophages in allergic reactions has been detailed in allergic asthma (Moreira and Hogaboam, 2011) and in atopic dermatitis (Kasraie and Werfel, 2013). In early inflammatory phase of allergic reaction, macrophages

play a key role in initiating inflammatory processes. After activation by foreign bodies such as an allergen, macrophages release many inflammatory factors like NO and pro-inflammatory cytokines such as IL-6 (Kasraie and Werfel 2013). The IL-6 is a pro-inflammatory cytokine involved in many reactions like acute phase responses and chronic inflammation processes (Mihara, et al. 2012; Barnes et al. 2011). Secretion of IL-6 is induced by inflammatory agents such as pollutants, pathogens or their product through activation of receptors. Expression and secretion of IL-6 in macrophages are regulated by activation of NF-kB (Scheller et al. 2011). NO is synthesized by enzymatic oxidation of L-arginine by nitric oxide synthase (NOS) (Koyabashi 2010). In macrophages, the main NOS is inducible NOS (iNOS); however, endothelial NOS (eNOS) isomer is also expressed (Connelly et al. 2003). The synthesized NO is very reactive with a short half-life (Koyabashi 2010). The excess of NO produced is readily converted to nitrite, which is used to estimate the NO produced.

Inflammatory responses have been studied in vitro using established macrophage cell lines. One of the cell lines used widely in inflammatory studies is the mouse macrophage RAW264.7 cell line (Roponen et al. 2001; Jeon et al. 2014). When RAW264.7 cells are stimulated, they can secrete NO and IL-6 (Kim et al. 2014). Therefore, both NO and IL-6 levels in RAW264.7 cells can be useful biomarkers of inflammatory activities (Xu et al. 2014; He et al. 2014). Despite an extensive literature search, there are few or no studies that report on the evaluation of inflammatory activities of textile effluent and on efficient removal of the inflammatory pollutants. The current study focused on the evaluation of coagulant/flocculant treatment processes to effectively remove of toxic and inflammatory substances from textile wastewater. The study also investigated the effects of treatment procedures on pH, COD and TOC of the pre and post-treatment wastewater.

Material and methods

Wastewater samples

Wastewater samples that are odoriferous and dark in colour were supplied by a non-woven textile industry in Cape Town. This industry has different production lines where wastewaters are being generated and so the most concentrated effluents stored at the central compartment was collected and used for the treatment. The samples were stored in 200 L litre acid washed plastic drums. Prior to the sampling, the drums were washed with diluted HNO_3 and millipore water and thereafter rinsed with the effluents. For the laboratory investigations, the samples were stored in plastic vials at 4 °C.

Wastewater treatment

In order to identify the best suitable coagulant or flocculant for the treatment of effluent, the coagulant-flocculant dosages were optimized. The wastewater sample was subjected to gravitational settling for 30 min due to the presence of a substantial quantity of suspended solids. But, it was found that the suspended solids were poorly settled within 30 min. The following analytical grade chemicals $Al_2(SO_4)_3.14H_2O$, $Ca(OH)_2$, $FeCl_3$, $FeSO_4.7H_2O$ were procured from Sigma Aldrich and were used without to any further treatment. Raw ferromanganese wad (Fe-Mn) oxide was obtained from Vaal Triangle, about sixty kilometres South of Johannesburg and was also used without further purification. The study was performed by varying the mass of Fe-Mn oxide, $Al_2(SO_4)_3.14H_2O$, $FeSO_4.7H_2O$, $FeCl_3$, and $Ca(OH)_2$ from 0.2–1.0 g in a batch mode (Table 1).

Firstly, 500 ml each of wastewater sample was added to five beakers to which were added specified amounts

Table 1 Experimental protocol for wastewater treatment

Sample treatment	Treatment protocol
Raw effluent	Before treatment
Fe-Mn oxide	Addition of 0.2–1.0 g of Fe-Mn oxide to 0.5 L effluent, stirred rapidly for 120 s and then slowly for 60 min
$Al_2(SO_4)_3.14H_2O$	Addition of 0.2–1.0 g $Al_2(SO4)_3.14H_2O$ to 0.5 L effluent, stirred rapidly for 120 s and then slowly for 60 min
$FeSO_4.7H_2O$	Addition of 0.2–1.0 g $FeSO_4.7H_2O$ to 0.5 L effluent, stirred rapidly for 120 s and then slowly for 60 min
$FeCl_3$	Addition of 0.2–1.0 g $FeCl_3$ to 0.5 L effluent, stirred rapidly for 120 s and then slowly for 60 min
$Ca(OH)_2$	Addition of 0.2–1.0 g of $Ca(OH)_2$ to 0.5 L effluent, stirred rapidly for 120 s and then slowly for 60 min

of the coagulant and flocculants without adjusting the pH of the effluent with either acid or base. Thorough mixing was performed for 1 min at 200 rpm, and then the coagulation-flocculation was carried out at a speed of 20 rpm for 20 min. Finally, the wastewater mixture content in the beaker was allowed to settle for 60 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 parameters measured for the supernatant before and after treatment were COD, TOC and pH. The experiments were repeated three times and the average result was computed including the standard deviation of the mean. The best treated samples obtained at the optimal dosage were further evaluated for toxicity and inflammatory activities.

Chemical analysis

The collected sample was characterized for pH, electrical conductivity (EC), Total Suspended Solids (TSS), Total Dissolved Solids (TDS), Biochemical oxygen demand (BOD), total organic carbon (TOC), and chemical oxygen demand (COD) as shown in Table 2. All the parameters were measured in accordance with "Standard methods for treatment of water and wastewater" (American Public Health Association, 1999).

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
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
Flourides	F	16.3 ± 0.14
Colour	Dark	
Odour	Highly unpleasant (odiferous)	

All values are in mg/L except for pH, electrical conductivity (mS/cm), turbidity (NTU)

Cell culture

Mouse macrophage RAW264.7 cells, American Type Culture Collection (ATCC-TB-71) were cultured 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 Technology). The cells were cultured in 96-well plates at density of 5×10^5 cells/ml in a humidified incubator at 37 °C and 5 % CO_2 until confluence. Then, the medium was replaced with fresh culture medium containing sterile filtered textile effluent water samples treated with adsorption and coagulation techniques. To the cell cultures, samples were added as follows: normal medium for negative control, medium supplemented with 1 μg/ml lipopolysaccharides (LPS) from *Escherichia coli* 0111:B4 (Sigma, Germany) as positive control, medium containing effluent samples at 1 in 100 dilutions in respective wells. The effluent samples included raw effluent, effluent treated with 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 $FeSO_4.7H_2O$, 1.6 g/L of $FeCl_3$, and 1.6 g/L of $Ca(OH)_2$. Effluent samples were sterilized using 0.45 uM filters and applied to culture medium at 1 in 100 dilutions. After overnight incubation at 37 °C and 5 % CO_2, culture supernatants were collected for NO and IL-6 assays. The cells were used for cell viability assays.

Cell viability

Cell viability was determined using chromogenic-based water-soluble tetrazolium salts (WST-1). The assay is based on the principle of the breakdown of tetrazolium to water soluble formazan dye by the action of dehydrogenase enzyme. Briefly, the assay procedure was as follows; after removal of cell supernatant from culture, each well received 100 μl of medium supplemented with 10 % WST-1 reagent (Roche, Germany). 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.

Nitric oxide determination

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). NO value of each water sample was subtracted from respective treatment as reading of background blank.

Interleukin-6 determination

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 (anti-mouse 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 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 anti-mouse IL-6 in block diluent) was added to each well and incubated for 1 h at room temperature. After another five times wash, Avidin-horse radish peroxidise (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).

Statistical analysis

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

Results

Effluent sample chemical characteristics

The results of chemical oxygen demand (COD), total organic content (TOC) and pH values of effluent before treatment and after treatment with 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 $FeSO_4.7H_2O$, 1.6 g/L of $FeCl_3$, and 1.6 g/L of $Ca(OH)_2$ are shown in Table 3. Treatment with 1.6 g/L of Fe-Mn oxide produced no appreciable reduction in COD and TOC value compared to the raw effluent, although a significant reduction in odour was noticed. With the addition of 1.6 g/L of $Al_2(SO_4)_3.14H_2O$ to the raw effluent, 63.13 and 75.5 % reduction in COD and TOC value respectively was observed. Similarly, addition of 1.6 g/L of $FeSO_4.7H_2O$ to the raw effluent decreased the COD and TOC value by 45.6 and 61.1 %, respectively. The maximum COD and TOC removal at optimal dosage of 1.6 g/L of $FeCl_3$ was 59.65 and 72.26 %, respectively. The optimal concentration of $Ca(OH)_2$ for treating effluent was 1.6 g/L with 38.1 and 56.8 % reduction in COD and TOC, respectively.

Effects of effluent samples on cell viability

The effects of effluent samples on RAW264.7 cell viability are shown in Fig. 1. Raw effluent reduced cell viability significantly ($P < 0.001$) as compared to negative control. None of the effluent samples effluent treated with 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 $FeSO_4.7H_2O$, 1.6 g/L of $FeCl_3$, and 1.6 g/L of $Ca(OH)_2$ induced toxicity in RAW264.7 cell culture.

Table 3 Chemical oxygen demand (COD), total organic content (TOC) and pH values of effluent before and after treatment at optimal conditions

Sample treatment	pH	COD (mg/L)	TOC (mg/L)
Before treatment (raw effluent)	5.99 ± 0.03	20,457 ± 1.52	5952 ± 2.64
After treatment with 1.6 g/L of Fe-Mn oxide	5.74 ± 0.06	19,987 ± 3.42	5893 ± 2.12
After treatment with 1.6 g/L of $Al_2(SO_4)_3.14H_2O$	2.44 ± 0.15	7543 ± 0.45	1456 ± 0.31
After treatment with 1.6 g/L of $FeSO_4.7H_2O$	5.03 ± 0.26	11,134 ± 16.57	2310 ± 17.81
After treatment with 1.6 g/L of $FeCl_3$	1.88 ± 0.04	8369 ± 11.45	1650 ± 12.53
After treatment with 1.6 g/L of $Ca(OH)_2$	11.32 ± 1.00	12,653 ± 4.18	2568 ± 5.34

Effects of effluent samples on nitric oxide production

The effects of effluent samples on secretion of NO by RAW264.7 cells are shown in Fig. 2. Raw effluent and effluent treated with Fe-Mn oxide induced significantly ($P < 0.001$) higher NO production than the negative control. The effluent samples treated with 1.6 g/L of $Al_2(SO4)_3$, 1.6 g/L of $FeSO_4$, 1.6 g/L of $FeCl_3$ and 1.6 g/L of $Ca(OH)_2$ did not induce significant amounts of NO.

Effects of effluent samples on IL-6 secretion

The effects of effluent samples on IL 6 secretion by RAW264.7 cells culture are shown in Fig. 3. Raw effluent induced significantly ($P < 0.001$) higher IL-6 secretion than the negative control. Effluent treated with 1.6 g/L of $FeSO_4$ induced production of IL-6, with non-significant different from the control. Effluent samples treated with 1.6 g/L of Fe-Mn oxide, 1.6 g/L of $Al_2(SO4)_3$, 1.6 g/L of $FeCl_3$, and 1.6 g/L of $Ca(OH)_2$ had no significant effects on production of IL-6.

Discussion

Different physicochemical methods have been developed for treatment of wastewater. In the present study, the raw textile effluents produced by the factory had an average COD and TOC value of 20,457 ± 1.52 and 5952 ± 2.64 mg/L, respectively. The COD value of the raw wastewater supplied was greater than 20,000 mg/L.

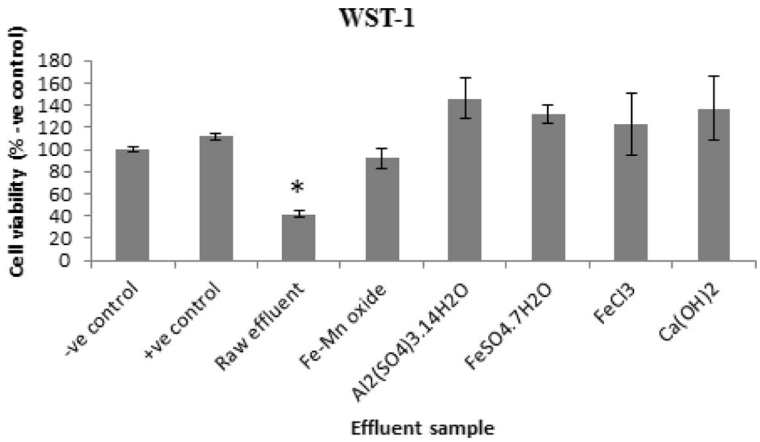

Fig. 1 Effects of effluent samples treated with coagulation/flocculation techniques on RAW264.7 cells viability as determined by WST-1 assay. Negative (−ve) control was treated with normal medium, positive (+ve) control was treated with LPS (1 μg/ml), and effluent samples 1 in 100 dilutions as follows: raw effluent; effluent treated with 1.6 g/L of Fe-Mn oxide, 1.6 g/L of Al2(SO4)3.14H2O, 1.6 g/L of FeSO4.7H2O, 1.6 g/L of FeCl3, and 1.6 g/L of Ca(OH)2. *Indicates that cell viability is significantly ($P < 0.001$) lower than the negative control

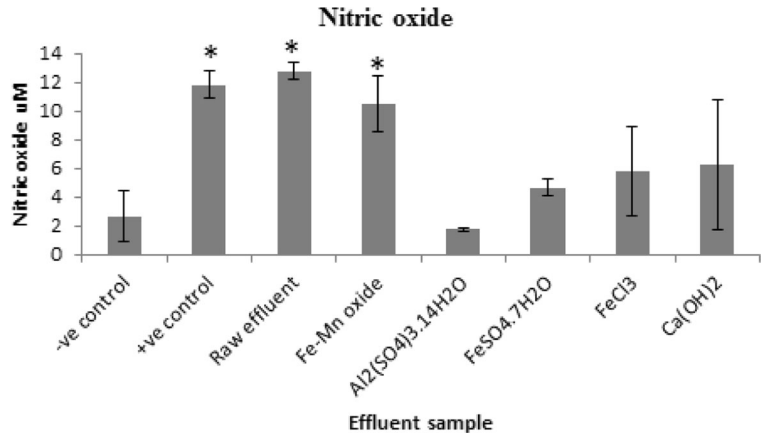

Fig. 2 Effects of effluent samples treated with adsorption and coagulation methods on induction of NO production in RAW264.7 cells culture. Negative (−ve) control was treated with normal medium, positive (+ve) control was treated with LPS (1 μg/ml), and effluent samples 1 in 100 dilutions as follows: raw effluent; effluent treated with 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 $FeSO_4.7H_2O$, 1.6 g/L of $FeCl_3$, and 1.6 g/L of $Ca(OH)_2$. *Indicates that NO level is significantly ($P < 0.001$) higher than the negative control

It is evident that the wastewater does not meet the applicable 5000 mg/L COD maximum effluent discharge limits for discharge into water sources as per 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, pH and intense colour can be attributed to the use of complex organic substances that are highly non-biodegradable dyes (Anouzla et al. 2009). Apart from dyes, the industry utilizes different chemicals including organic polymers, surfactants, chelating agents and emulsifying oils. Considering the complexity of the chemical compounds used, it is difficult to attribute the high values of indicator parameters and toxicity level of the raw effluent or the treated effluent to the dye alone. Lack of determination of specific pollutants used in the industry that could be a source of high parameters is the main limitation of the present study. Given a complex mixture of dyes and polymers with a high TOC level of above 5000 mg/L in samples, it was somehow difficult to determine the

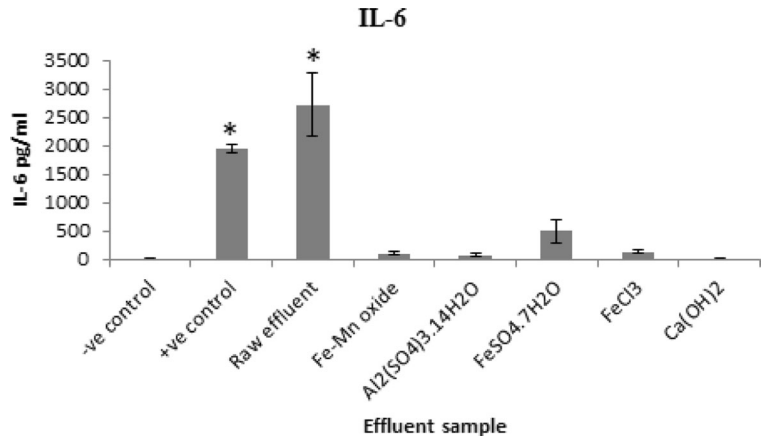

Fig. 3 Effects of effluent samples treated with adsorption and coagulation methods on IL-6 secretion in RAW264.7 cells culture. Negative (−ve) control was treated with normal medium, positive (+ve) control was treated with LPS (1 μg/ml), and effluent samples 1 in 100 dilutions as follows: raw effluent; effluent treated with 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 $FeSO_4.7H_2O$, 1.6 g/L of $FeCl_3$, and 1.6 g/L of $Ca(OH)_2$. *Indicates that IL-6 level is significantly ($P < 0.001$) higher than the negative control

concentration of specific pollutant both in the treated and untreated water samples. Nevertheless, high values of the indicator parameters of the samples revealed the pollution status of the effluent and thus direct discharge into the environment may cause adverse environmental and health effects, especially on aquatic species. In order to improve the effluent characteristics and comply with South African National Water Act waste discharge standards (DWA 2010 guidelines and City of Cape Town wastewater and industrial effluent bylaws), different dosages of coagulant/flocculants were added to the effluents and after the treatment the COD and TOC were evaluated.

In the present study, textile effluent samples were treated separately with specified amount of coagulant/flocculant. Thereafter, the optimal dose for each coagulant/flocculant was determined. Based on the effective reduction of the chemical oxygen demand (COD) and total organic carbon (TOC) value, $Al_2(SO_4)_3.14H_2O$ at a dose of 1.6 g/L seemed to be the most effective treatment option. The optimum dose of 1.6 g/L for $Al_2(SO_4)_3.14H_2O$ reduced the COD and TOC value by 63.13 and 75.5 %, respectively. The treatment with this coagulant/flocculant removed the complex organic substance. Similar observations have been reported by others (Kumar et al. 2008; Dulov et al. 2011). In order to further evaluate the efficiency of coagulant/flocculants to remove organic substances, treated samples were assessed for induction of toxicity and inflammatory activities in macrophage RAW264.7 cells.

The cell viability results show that raw effluent induced toxicity to RAW264.7 cells after 24 h exposure. The results imply that raw effluent contains toxic pollutants, which can induce cytotoxicity effects to RAW264.7 cells. On the other hand, all effluents treated with optimized coagulation/flocculation processes did not induce toxic effects. Lack of toxicity partly indicates the efficiency of the treatment techniques. The lack of toxicity also could partly be due to low concentration of effluent samples used at 1 in 100 dilutions. Similar observations are commonly reported in previous toxicity studies (Pool et al. 2000; Hendricks and Pool 2012). The observation has been associated with high concentrations of pollutants required to induce toxicity as compared to that required for induction of other response like immunotoxicity (Heymery et al. 2014).

The results of inflammatory response in RAW264.7 cells after 24 h incubation with effluent samples show that raw effluent and effluent treated with 1.6 g/L of Fe-Mn oxide induced inflammatory response by inducing significantly ($P < 0.001$) higher NO production than the negative control. Wastewater sample treated with 1.6 g/L of $Al_2(SO_4)_3.14H_2O$ induced the lowest level of NO. Wastewater samples treated with 1.6 g/L of $FeSO_4.7H_2O$, 1.6 g/L of $FeCl_3$, and 1.6 g/L of $Ca(OH)_2$ induced NO production with no statistically difference to the negative control. Although the levels of NO induced by 1.6 g/L of $FeSO_4.7H_2O$, 1.6 g/L of $FeCl_3$, and 1.6 g/L of $Ca(OH)_2$ are not significant, the use of these coagulant/flocculants or the residual toxins in the water might have effects on NO production. The effect of ferric iron (Fe^{3+}) has been reported to regulate NO through transcription of iNOS (Weiss et al. 1994). Thus, the presence of iron ions can induce NO production through increased expression of iNOS (Galleano et al. 2004). Similarly, wastewater treated with 1.6 g/L of $Ca(OH)_2$ also induced a non-significant NO production. The production of NO in macrophage is normally induced by inflammatory agents via an iNOS pathway, which is a calcium independent pathway (Mattila and Thomas 2014). However, macrophages such as RAW2646.7 cells can also express eNOS, which is a calcium dependant isomer (Schmidt et al. 1992; Connelly et al. 2003). Therefore, the presence of calcium (Ca^{2+}) in treated sample from the coagulant/flocculants can influence NO production. In the same vein, the COD was still high after treatment, so the effect could be due to the residual toxins in the treated water.

The results of inflammatory responses further show that raw effluent induced significantly ($P < 0.001$) higher level of IL-6 secretion than the negative control. Effluent treated with 1.6 g/L of FeSO4.7H2O increased IL-6 secretion with no statistically different from the control. Treatment of effluents using 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 $FeCl_3$, and 1.6 g/L of $Ca(OH)_2$ did not induce IL-6 secretion. The increase of IL-6 secretion is an indication of the inflammatory response. Increased IL-6 secretion has been used as a sensitive biomarker for monitoring inflammatory activities in water (Pool et al. 2000; Abedayo et al. 2014). Therefore, the high level of IL-6 induced by raw effluent is an indication of the presence of inflammatory pollutants

in textile effluent. The increased IL-6 secretion in the effluent sample treated with 1.6 g/L of $FeSO_4.7H_2O$ can indicate inadequate removal of pollutants as effluent is characterized by high COD value or is due to effects of Fe^{2+} on the function of macrophages (Ward et al. 2011).

Conclusion

The results of this study show that raw effluent from a textile industry can induce cytotoxicity in RAW264.7 cells and inflammatory activities by increasing both NO production and IL-6 secretion. Treatment of textile industrial effluent using 1.6 g/L of $Al_2(SO4)_3.14H_2O$ is the most effective coagulation/flocculation processes for removing both toxic and inflammatory pollutants. The induction of IL-6 secretion in RAW264.7 cells is a more sensitive biomarker than NO production for evaluation of efficiency of coagulation/flocculation treatment of textile effluent. The induction of the inflammatory response in macrophage RAW264.7 cells can be used as a model bioassay system for monitoring the effectiveness of coagulation/flocculation processes in the treatment of textile industry wastewater.

Compliance with ethical standards

Ethics statement The project was funded by funds from the NRF of South Africa (Grant number 74016).

Conflict of interest The authors declare that they have no conflict of interest.

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