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Reuse of textile effluent for dyeing using combined technology of ceramic microfiltration and surface treated sugarcane bagasse: toxicity evaluation using *Channa punctatus* as model

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ABSTRACT

An attempt was undertaken for treatment and reuse of highly concentrated textile dyebath effluent. Ceramic microfiltration membrane prepared from a cost effective composition of alumina and clay was used in combination with a biosorbent prepared from sugarcane bagasse. The combined process was highly effective for removal of chemical oxygen demand (COD), turbidity, colour and total suspended solids. For the concentrated dyebath effluent with initial COD value of 2220 mg/L, COD reduction was about 91%. Dye removal was about 99%, turbidity removal was > 99% and TSS reduction was > 90%. The treated samples were utilized in the dyeing process of cotton fabric under different conditions. The reusability study showed high potential with respect to water reclamation, as well as, reduction of the associated chemicals consumption. Dye uptake using membrane treated water was more compared to that of freshwater in case of light and medium shade dyeing. Toxicity effect of treated and untreated effluent on environment was observed on *Channa punctatus* (Bloch) in terms of various antioxidant enzymes, bioassay, comet assay, micronucleus, etc. It was observed that 48-h acute toxicity tests resulted in LC_{50} value of 43.5%. Untreated effluent at 25% dilution resulted in considerable increase in antioxidative enzymes with formation of comet cells and micronuclei.

Keywords: Reactive dye; Dyehouse effluent; Biosorbent; Ceramic membrane; Reuse; Toxicity effect; *Channa punctatus*

1. Introduction

Large amount of freshwater is consumed in textile industry for their dyeing and finishing processes. The effluent contains organic surfactants, alkalis, solvents, salts, as well as, dyes which are persistent in nature, non-biodegradable and toxic components which when discharged without treatment affects various segments of the environment [1]. The dyes and their metabolites have toxic, mutagenic and

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carcinogenic effect on aquatic life and human. Colouration of the effluent results from wastage and washing during dyeing, with the degree of colouration being dependent on the colours/shades of fabric and the type of dye used [2]. The existing treatment technologies include coagulation, membrane separation, electrochemical oxidation, ion exchange and adsorption. Surfactants and dyes with high molecular weight are successfully removed by coagulation/flocculation processes followed by sedimentation, flotation and filtration [3]. These processes involve use of different chemicals or are expensive and often not effective for removal of wide range of dyes, like reactive and other anionic dyes [4,5]. Adsorption processes are effectively used for reactive dye removal [6]. Activated carbon although widely used, is a costly option. Therefore, various researchers have utilized biosorbents prepared from waste materials, especially agricultural waste materials like sawdust, sugarcane, fruit peel, etc. [7]. Biosorbent prepared from fruit peel had been utilized for removal of Chromium from synthetic and tannery wastewater [8]. Various other combinations of physico-chemical processes like coagulation–flocculation by polyaluminium hydroxide (PAH) and bentonite, followed by adsorption on powdered activated carbon (PAC) techniques were used for treatment of reactive dye solutions [9].

Membrane-based processes have been used in combination with adsorption for treatment of textile wastewater [10]. The hollow fibre membrane microfiltration dead-end process combined with adsorption on PAC was used to separate organic dye from aqueous solutions [11]. Nanofiltration with polymeric membranes is increasingly used for dye removal from coloured effluent [12,13]. However, polymeric membranes are often affected in the harsh operating conditions [14]. The ceramic membranes exhibit high resistance to extremes of operating parameters, with respect to temperatures, chemical environment, operating pressure or backflushing conditions. Porous ceramic membranes have been developed by the Central Glass and Ceramic Research Institute (CSIR) in mono and multichannel configuration from a cost effective composition of α-alumina and clay and used for separation of iron and arsenic from contaminated ground water [15]. This membrane has been used in combination with various physico-chemical methods for treatment of sulphur black effluent from garment processing industry [16]. In the present study, performance of this membrane was studied using effluent collected from a hosiery dyehouse where 100% cotton knits wear are dyed in winch machine using various reactive dyes. A biosorbent developed from sugarcane bagasse was used in combination with microfiltration and the treated effluent was reused in dyeing of cotton fabric. Attempts have been undertaken by Nazan et al. to reuse treated textile effluent in bleaching of cotton fabric [17]. The authors of this work have tried to use membrane treated effluent for reuse in the overall dyeing process so that freshwater consumption may be minimized.

The discharge of industrial effluent having substantial amount of dyes and other chemicals impart toxicity in the aquatic environment. Hence there is a need for evaluation of toxicity of treated water produced to analyse the environmental impact it might have, if discharged apart from reusing it. Researchers have proposed that fish can serve as indicators for monitoring land based pollution as they are in constant contact with water and accumulate pollutants in their tissue [18]. Effect of non-lethal concentration of textile mill effluent was observed on Nile tilapia in terms of superoxide dismutase (SOD), catalase, lipid peroxidation of gills and liver [19]. Comparative toxicological study of treated and untreated textile wastewater was conducted on freshwater fish *Gambusia affinis* [20]. Effect of cadmium on catalase activity of brain, gill, kidney and liver tissues of catfish was observed resulting in non-significant increase up to 20 d and significant increase was noticed after 45 d of exposure in liver [21].

The main objective of the present study was to evaluate the efficiency of ceramic microfiltration process towards treatment of textile effluent in combination with a biosorbent prepared from waste biomass. Observing the feasibility of recycling the treated effluent in the fabric dyeing process was another important aspect of this study aiming to minimize the consumption of freshwater in textile industry. The present study had an environmental significance too. The combined process aimed at reducing the toxic and organic load of the industrial effluent so that upon discharge, the treated water would not cause any harm to the aquatic life. For this, evaluation of toxicity was conducted using *Channa punctatus* as a fish model. The reason for choosing *C. punctatus* as model was its easy availability and high response to toxicity. Moreover, caudal vein of this type of fish is easy to locate which helps in drawing blood for micronuclei and comet assay. This fish has high nutritive value, as well as, can act as bioindicator of streams, lakes, ponds etc. [22].

2. Experimental

2.1. Textile effluent

Raw textile effluent was collected from a reputed hosiery unit in Kolkata, India. The dye concentrations varied in the effluent depending on the different shades of fabric, viz. light, medium and dark. In the present study, composite effluent from a dark shade dyeing process was collected. The effluents were characterized in terms of dye concentration, turbidity, pH, total dissolved solids (TDS), conductivity, TSS, COD, etc. Turbidity, pH, conductivity and TDS of samples were measured using instrument of Hach, USA. TSS was measured by a membrane filter holder-47 mm, Tarsons make. COD of the samples was measured using COD Digestor, [Spectralab, India] using dichromate reflux method. Absorbance spectra of the samples were measured in a UV–vis. spectrophotometer by Varian, Australia. The dye concentration of effluent was estimated by measuring the optical density corresponding to a wavelength, λ_{max} of 503.9 nm. The methods followed for characterization of the wastewater samples were in accordance with the standard methods of APHA [23].

2.2. Biosorption study

2.2.1. Preparation of biosorbent

Biosorbent was prepared from sugarcane bagasse collected from local cane juice manufacturer. Bagasse was thoroughly washed, dried and soaked in 1(N) sodium hydroxide solution for 3–4 h for delignification. The biomass was washed with distilled water and treated with 30% phosphoric acid for 1 h. The material was washed successively with distilled water till the pH of the washed water became neutral. This was dried at 120 °C for 24 h and charred in a furnace at 500 °C for 30 min. The biosorbent thus prepared was powdered, sieved through 100 μm mesh and stored in airtight container for further use [24].

2.2.2. Characterization of biosorbent

The biosorbent was subjected to characterization in terms of surface area (Autosorb-1, Quantachrome Instruments, USA), moisture content, ash content and bulk density. Functional groups were analysed by fourier transform infrared (FTIR) spectrometry using KBr pellet method for solid samples. FTIR analysis was performed in diffuse reflectance mode in Nicholet 380 FTIR spectrophotometer. Surface morphology of the biosorbent was obtained by field emission scanning electron microscopy (FESEM) (Supra 35 VP, Zeiss, Germany). X-ray diffraction (XRD) of biosorbent was observed in Philips 1710 diffractometer using CuKα radiation ($\alpha = 1.541$ Å).

2.2.3. Batch scale biosorption study

Batch equilibrium studies were conducted taking 0.25 l of effluent in 500-ml Erlenmeyer flasks. Equilibrium studies were conducted to observe the effect of solution pH (1–9) and biosorbent dose (0.25–3.0 g/L) on dye removal. The experiments were carried out in a temperature controlled magnetic stirrer and temperature was maintained at 25 °C (±1 °C) which was the room temperature. Samples were collected at definite time intervals and characterized in terms of dye concentration. Optimum pH and biosorbent dose were utilized for conducting microfiltration study.

2.3. Microfiltration process

Microfiltration study was conducted using ceramic membranes with tubular configuration having 19 numbers of small channels (each 4 mm dia.) inside the tube. Length of the membrane was about 200 mm and outer diameter was 35 mm. Porosity of the ceramic material was about 36%. The membrane used for this study was indigenously developed by CSIR from a cost effective composition of α-alumina and clay. The ceramic membranes exhibited a unique feature of high chemical and mechanical resistances against harsh operating environment.

The current study was undertaken in a laboratory scale set-up where feed solution was taken in a rectangular covered feed tank made of stainless steel with eight litre capacity. The membrane casing was tubular (SS made) with 200 mm length and 60 mm outer diameter. This module was horizontally fitted in the set-up. Cross-flow mode of microfiltration was used where feed solution was passed through the membrane module tangentially at high flow rate. A port was at the bottom of membrane module to collect the permeate samples. Prior to cross-flow microfiltration (CMF), the effluent pH was adjusted to an optimum value and optimum dose of biosorbent was added to the effluent followed by recirculation time of about 30 min after which the filtration study was conducted. For each experiment, about 6 l of wastewater was taken. Microfiltration process is a low pressure process and operating pressure generally lies within 0.2–3.5 kg/cm^2. Depending on the capacity of liquid recirculation pump used in the present study, the transmembrane pressure for this process was varied

from 0.4–2.2 kg/cm^2 to observe the effect of operating pressure on the permeate flux. Thereafter, operating pressure was kept constant at 1.0 kg/cm^2 for about 3 h during which permeate samples were collected at specific time intervals. The system was cleaned successively after each run by passing dilute acid solution followed by dilute alkali solution and finally by deionized water at high velocity and low pressure. The ceramic membrane element was cleaned with dilute nitric acid solution followed by deionised water.

2.4. Reuse study

Reuse study was conducted at batch scale using cotton fabric samples provided by reputed cotton mill according to the procedure shown in Fig. 1. Dyes were provided by Clariant Chemicals India Ltd. Three types of dyes were used, i.e Drimarene yellow CL-3G (C.I. reactive yellow 84) for light shade, Drimarene red CL-B (C.I. reactive red 198) for medium shade and Drimarene Navy HF-B (C.I. reactive blue 171) for dark shade. Experiments were conducted using treated effluent as well as with freshwater for comparison. Since the treated effluent contained reasonable amount of salt (about1.5 g/l) effect of varying salt concentration and soda ash concentration on dyeing was observed. Colour intensity (k/s value) of dyed cotton fabric was measured in spectrophotometer (Premiere Scan 5100A, India).

2.5. Effect of treated and untreated effluent on fish

The effect of untreated and combined treated effluent on aquatic life was studied to observe whether the water was suitable for discharge. The study was conducted on *C. punctatus* as a model and effect on various oxidative stress enzymes viz. POD (guaiacol peroxidase), SOD, CAT (catalase), GR (glutathione reductase), GST (glutathione –S-Transferase), GSH-GPx (glutathione peroxidase), ALP (alkaline phosphatase), ACP (acid phosphatase), protein, total carbohydrate, etc. of fish liver, gill, kidney and fins were observed. Also, 96 h bioassay was conducted. To observe changes in DNA micronuclei and comet assay of fish blood was conducted.

2.5.1. Experimental fish

Healthy fish specimens of *C. punctatus* of length 15 ± 1.5 cm and weight 35 + 4.8 g was collected from fish farm and acclimatized to the laboratory condition for 2 weeks in large tank (80 L capacity) in dechlorinated tap water (temperature: 25 ± 0.5 ℃, pH: 7.5, DO: 6–7 mg/L). The water was aerated properly and replaced every 48 h to remove fish faecal matter and unconsumed food particles. During acclimatization, fish were fed commercial fish food. Prior to experiment, feeding of fish was stopped and photoperiod was maintained with 12 h light and dark, respectively. The

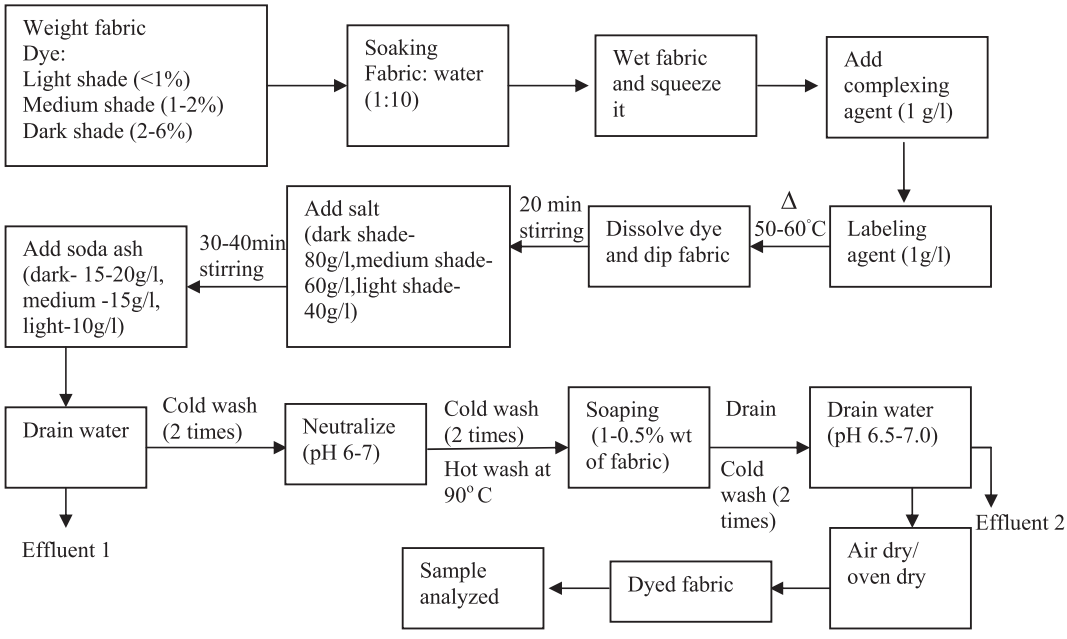

Fig. 1. Flow chart explaining the dyeing process.

experimental solutions were replaced every 24 h with same concentration and water.

2.5.2. Toxicity bioassay study

Acute toxicity tests are useful in defining adverse effects of toxicants occurring in an organism within a short period of time. Chemical analysis of any industrial effluent is not sufficient to predict the toxicity of the effluent. Moreover, different chemicals might combine together to cause more severe effect that might not be detected by chemical analysis. Whole effluent toxicity (WET) test are required in order to measure and control toxicity and is measured as part of discharge limit. In this study, toxicity bioassay test was conducted as per standard method described by United States Environmental Protection Agency (USEPA: EPA-821-R-02-012). Although these toxicity evaluation methods are time consuming but for better understanding of toxicity effect on living organisms these tests are being conducted. These methods are relatively cost effective and safe methods of toxicity evaluation [25]. In the present work, bioassay study was conducted to determine the median lethal concentration after 48 h (LC_{50}) for textile effluent. The fish were exposed to different dilutions (%) of raw textile effluent i.e. 25, 50, 75 and 100%, membrane treated permeate (100%) and freshwater served as control. It was observed that fish exposed to 50, 75 and 100% effluent reacted severely and maximum suffered death within 48 h of exposure and therefore 48 h-LC_{50} was calculated. This might be due to high alkalinity and toxic compounds. The LC_{50} was calculated using the probit analysis. Further study was conducted with 25% diluted effluent.

2.5.3. Enzymes assay

For enzyme assays three fish from each group i.e Group I (25% effluent), Group II (permeate) and Group III (control) were sacrificed and dissected carefully to remove liver, kidney, gills and fins. The tissues were cleaned with ice cold PBS (phosphate buffered saline) to remove any blood traces. The tissues were stored at −80 ℃ for further analysis. Tissues were homogenized with 50 mM cold phosphate buffer (pH 7.0) containing 0.5 mM EDTA and centrifuged (Kubota, 6,500, Japan) at 14,000 rpm for 15 min at 4 ℃. The supernatant collected was subjected to enzyme assay. For analysis of each enzyme, standard protocol described by various scientists were followed. POD was analysed as per methods described by Cipollani [26], SOD as per Giannopolitis and Ries [27,28], CAT as per Aebi (1983) [29], GR was measured as per method described by David and Richard [30], GST was measured as per Habig et al. [31] and GSH-GPx was determined as per Paglia and Valentine, 1967 [32]. Alkaline phosphatase and acid phosphatase were measured as per method described by Sadasivam and Manikam [33].

2.5.3.1. Guaiacol peroxidase assay (POD). The reaction mixture of 1 ml was prepared containing 10 µL of enzyme extract and 990 µL guaiacol solution. The guaiacol solution contained 0.25% guaiacol (v/v) in 10 mmol/L sodium phosphate buffer pH 6.0 and 0.125% H_20_2 (v/v). The change in the absorbance was recorded at 470 nm. Guaiacol was oxidized by hydrogen from H_2O_2 forming a brown coloured complex which was measured spectrophotometrically [26]. Peroxidase activity was expressed as A470 per gm fresh tissue weight per minute.

2.5.3.2. SOD assay. The reaction mixture for SOD analysis consisted of 1.3 µM riboflavin, 13 mM methionine, 63 µM NBT and 0.05 M sodium carbonate (pH 10.2). Final volume was adjusted to 3 ml using distilled water. The samples were kept in proper illumination and same set was prepared without illumination which served as blank. The samples were measured spectrophotometrically at 560 nm. SOD was calculated by change in absorbance with time [27]. According to Beauchamp and Fridovich, 1 unit of SOD is defined as the amount that inhibits the NBT photoreduction by 50% [28]. SOD activity was expressed as U/mg protein/min.

2.5.3.3. Catalase assay. The enzyme activity was determined by observing the disappearance of H_2O_2 spectrophotometrically at 240 nm. The reaction mixture consisted of phosphate buffer and H_2O_2 to the enzyme extract. One unit of enzyme decomposes one micromole of H_2O_2 per minute [29]. Catalase activity was expressed as U/mg protein/min.

2.5.3.4. GR, GST and GSH-GPx assay. The reaction mixture for analysis of Glutathione reductase consisted of 1 ml 0.1 M, pH 7.2 phosphate buffer, 0.1 ml 15 mM EDTA, 0.1 ml 10 mM sodium azide, 0.1 ml 6.3 mM oxidized glutathione and 0.1 ml enzyme. The volume was made up to 2 ml with distilled water and incubated for 3 min. At 340 nm, 0.1 ml 9.6 mM NADPH was added and analysed spectrophotometrically. The enzyme activity was expressed as µ moles of NADPH oxidised/min/g tissue [30].

The reaction mixture for Glutathione-S-Transferase (GST) consisted of 0.1 ml of 1 mM glutathione. About 0.1 ml of 1 mM CDNB and 2.7 ml of 0.1 M (pH 6.5)

phosphate buffer, 0.1 ml enzyme extract was added and measured at 340 nm. The activity was expressed as nmoles of CDNB conjugated/minute [31].

The reaction mixture for determination of Glutathione peroxidase (GSH-GPx) consisted of 100 µl of NADPH, 10 µl of glutathione reductase, 10 µl of sodium azide, 2.5 ml of phosphate buffer (pH 7.0), 100 µl H_2O_2 and 100 µl of enzyme extract. The enzyme activity was measured by decrease in absorbance at 340 nm and expressed as U/mg protein [32].

2.5.3.5. Alkaline and acid phosphatase assay. The reaction mixture for alkaline phosphatase (ALP) analysis consisted of 0.1 ml enzyme and 1 ml of paranitrophenyl phosphate (PNPP) substrate in 0.1 M glycine -NaOH buffer (pH 9.4). Sample was incubated at 37˚C for 30 min. The reaction mixture for acid phosphatase (ACP) consisted of 0.1 ml enzyme extract and 1 ml of PNPP substrate in citrate buffer (pH 4.5) and incubated at 37˚C for 30 min. Both the reactions were stopped with 1.5 ml of 01. N sodium hydroxide and measured at 405 nm against reagent blank. Enzyme activity was expressed as µmol of PNP/min/mg of enzyme [33].

2.5.4. Protein and carbohydrate assay

Homogenized tissues were subjected to protein and carbohydrate analysis. Protein content was measured according to Lowry et al. [34]. The reaction mixture consisted of 1 ml protein reagent (2% sodium carbonate, 0.5% copper sulphate and 0.5% sodium tartarate), 1 ml sample solution (1: 2.3) and 100 µl folin reagent. The sample was incubated for 30 min and measured at 660 nm using BSA (bovine serum albumin) as standard. The protein content was expressed as mg/g fresh tissue weight. Total carbohydrate content was measured by anthrone method using glucose as standard [35]. The reaction mixture consisted of sample, distilled water and anthrone. The samples were incubated in water bath for 10 min and measured at 620 nm against reagent blank.

2.5.5. Micronuclei and comet assay

To observe any genotoxicity caused due to textile effluent, presence of micronuclei and comet assay of whole blood was conducted. For micronuclei tests, blood of fish exposed to three different treatments were drawn after 24, 48, 72 and 96 h. Peripheral blood samples were drawn from caudal vein of fish with the help of heparinized syringes. Three sets of slides were prepared for each sample. Immediately after collection, blood samples were smeared on clean dust free slides and air dried for overnight. Slides were fixed in methanol for 10 min followed staining with 5% giemsa for 10 min. Slides were washed with distilled water and air dried. Two thousand binucleated cells were observed per treatment at 100× magnification and results were expressed as mean value (±SD) of micronuclei per 1,000 binucleated cells [36]. To conduct comet assay of fish blood, blood drawn with syringe coated with anticoagulant (acid-citrate-dextrose) and stored in PBS solution. Half frosted slides were covered with sandwich gel of 1% and 0.6% normal melting point (NMP) agarose. About 10 µl (i.e. cells count ~ 2×10^4) of blood sample was mixed with 0.5% low melting point agarose (LMP) and spread on coated slides and then covered by single layer 0.5% LMP agarose. Slides were kept overnight immersed in lysing solution (2.5 M NaCl, 100 mM Na_2EDTA, 10 mM tris-HCl, 1% Na sarcosinate, at pH 10). Freshly prepared 1% triton X-100 and 10% dimethyl sulfoxide were added for DNA unfolding. Electrophoresis buffer was prepared freshly containing 300 mM NaOH, 1 mM Na_2EDTA at pH 13.0. Slides were placed carefully side by side in gel-electrophoresis tank facing anode and electrophoresis buffer was poured to cover the slides and kept for 20 min for DNA unwinding at 4 ˚C. Electrophoresis was carried out for another 20 min at 25 V. Slides were then washed three times for 5 min each with neutralization buffer containing 0.4 M tris-HCl, pH 7.5. Slides were then stained with 20 µg/L ethidium bromide solution and allowed to dry. About 100 cells were scored per slide and observed for presence of comet tail to evaluate DNA damage [37]. Images were captured in Olympus microscope (1X70) using Camedia software (Chicago, MT, USA) (E-20 P, 5.0 megapixel).

3. Results and discussions

3.1. Characterization of biosorbent

The surface area of the biosorbent was measured as 705 m^2/g, bulk density as 0.274 g/m^3, moisture content of 9.24% and ash content of 4.24%.

The biosorbent was characterized in terms of FE-SEM, EDAX and XRD.

XRD analysis (Fig. 2(a)) revealed the amorphous nature of silica with 2θ peaks between 18˚ and 30˚ [38]. After biosorption with textile effluent formation of new peaks were resulted corresponding to NaCl with 2θ peak at 45.6˚ present in huge amount in effluent. Apart from this, other peaks were present which might be due to other heavy metals like lead and zinc present in the effluent. For better understanding of

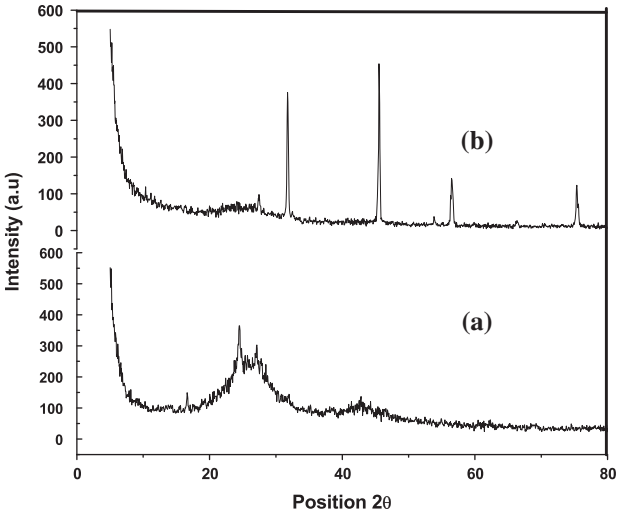

Fig. 2. XRD of biosorbent: (a) before treatment (b) after treatment.

this fact, EDAX was performed. From Fig. 2(b), it was observed that peaks of sodium, potassium, magnesium and sulphur were present in the biosorbent after adsorption which was not present in the biosorbent before adsorption (Fig. 2(a)). Moreover, peaks for calcium and chloride were increased in treated biosorbent compared to unused biosorbent. Interestingly, silica content reduced in biosorbent after treatment than that of used biosorbent suggesting biosorption occurred by replacing silica. A small peak of chromium was present in biosorbent after treatment of effluent. The data from EDAX suggested possible adsorption by the biosorbent. FESEM analysis (Fig. 3(a)) showed uniformly distributed fibrous structure of unused sugarcane bagasse. Holes in the fibres revealed that there was considerable removal of hemicelluloses and lignin. Fig. 3(b) showed deposition of certain salt like substances which might be present due to the biosorption of effluent.

FTIR analysis of the biosorbent (Fig. 4) revealed the groups involved in biosorption phenomenon before and after sorption. The peaks at 3,698 cm^{-1} and 3,702 cm^{-1} before and after biosorption might be due to O–H linking of polysaccharides. It was observed that there was no shift at peak 2,923 cm^{-1} which might be present due to C–H group. Peaks at 1826 cm^{-1} and 1816 cm^{-1} before and after biosorption could be attributed due to presence of C = O stretching [39,40]. Peak shift was observed at 1,478 cm^{-1} before biosorption, whereas after biosorption the peak was observed at 1,421 cm^{-1}. The peaks correspond to C–O stretching of lignin. This band might be involved in biosorption phenomenon. Peak shift was also observed at 1,611 cm^{-1} and 1,575 cm^{-1} before and after sorption. Peak at 1,575 cm^{-1} corresponds to C = O band present in quinone structure, whereas peak at 1,611 cm^{-1} might be due to C–O stretching band of lignin. Peaks at 1,176 cm^{-1} and 1,181 cm^{-1} might be attributed C–O–bands associated to phenolic structure. Peaks at 551 cm^{-1} and 667 cm^{-1} represented out of plane bending of C–OH present in cellulose [41–43].

3.2. Treatment of textile effluent

Batch equilibrium study was conducted using textile effluent for optimization of solution pH and dose of the biosorbent. It was observed that colour removal (%) decreased with increasing pH. Maximum biosorption occurred at pH 2 (about 97.4%), which decreased to about 21.3% at pH 9 (Fig. 5). pH of particular solution not only affects the surface charge of adsorbents but degree of ionization of pollutants are also being affected. Variation of pH affects the adsorptive process by dissociating the functional groups present in the adsorbent [44]. In this case, it was observed that biosorption of dyes was more in acidic pH. Reactive dyes are usually anionic in nature. Hence, at pH 2 there exists a strong electrostatic force of attraction between the positively charged biosorbent surface and the anionic groups, viz. the sulphonic groups of dye molecules. It might also be suggested that maximum removal occurred due to availability of more active sites on bagasse surface. Similar results were also obtained by Said et al. [45].

From Fig. 5 it might also be observed that maximum colour removal (about 97%) was obtained with 1 g/L of biosorbent dose. Initially, colour removal (%) increased with increasing dose. However, above 1 g/L dose, the removal efficiency with increasing dose reached equilibrium. Increasing biosorption with increase in dose could be explained due to greater availability of biosorbent sites and surface area. But after a certain dose, all the available sites might get exhausted and there could be chances of overlapping of available sites. Therefore, further increasing the dose did not increase the removal efficiency [46,47].

Based on the above results, the membrane study was conducted using pH 2 and biosorbent dose of 1 g/L.

3.2.1. Ceramic microfiltration study

The biosorbent induced textile effluent was further treated by microfiltration and the colour and COD removal were observed at definite time intervals. One of the prime objectives of this study was to reuse the

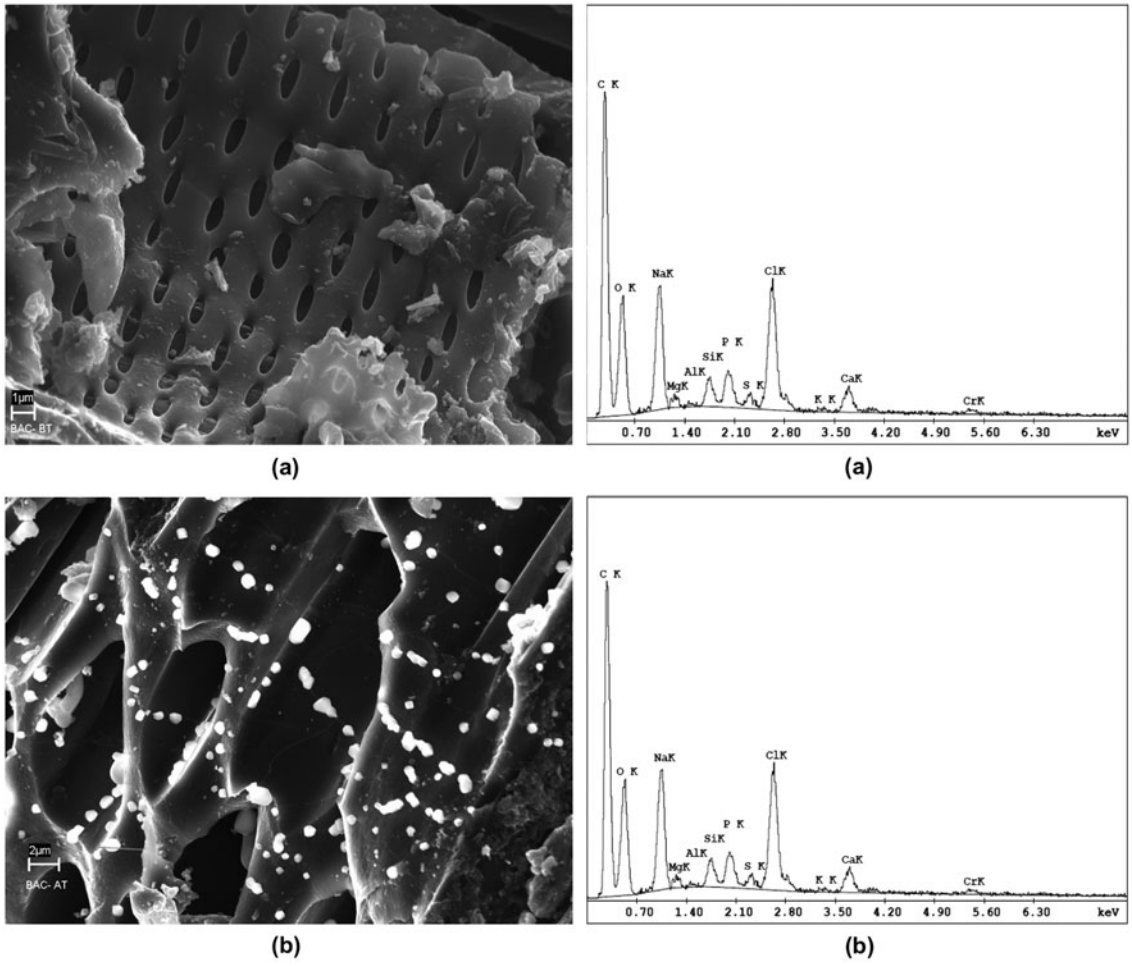

Fig. 3. FESEM and EDAX of biosorbent: (a) before treatment (b) after treatment.

treated effluent in the dyeing process. In order to generate a clear effluent free from dyes, suspended particles and lesser COD load, however rich in salt content, a combination of ceramic microfiltration process was used in combination with the biosorption. The ceramic membrane was chosen due to their excellent chemical stability. Characterization of the untreated and treated effluent was shown in Table 1. The data represented average values of five sets of experiments conducted. It may be noted that all the parameters of treated water were below the discharge norms [48].

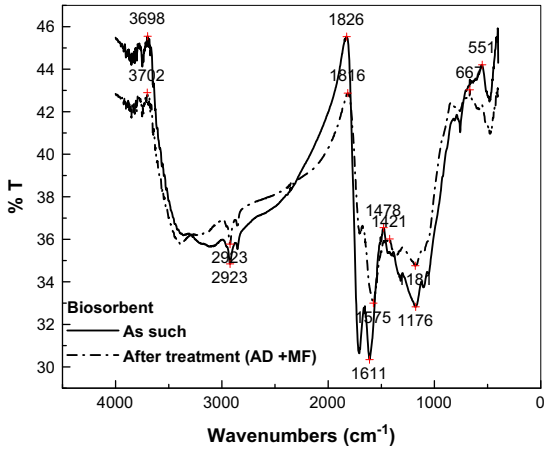

Fig. 4. FTIR study of biosorbent before and after treatment.

In Fig. 6 filtration performances with respect to colour and COD removal had been depicted. It might be observed that COD reduction increased with increasing time. At the onset of experiment removal was about 88% due to a combined effect of biosorption and membrane filtration. With time, gradual deposition of suspended matters including the biosorbent particles formed a dynamic layer on the membrane surface which restricted the permeation of finer organic matters present in the effluent resulting into further COD removal. After 90 min of operation COD

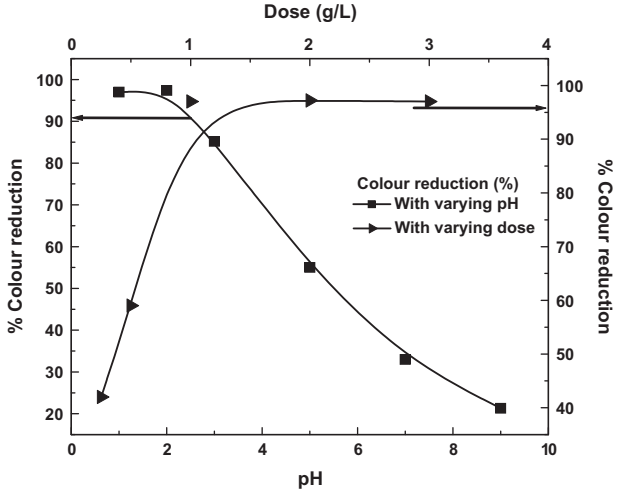

Fig. 5. Colour removal (%) in batch equilibrium study at varying pH and dose.

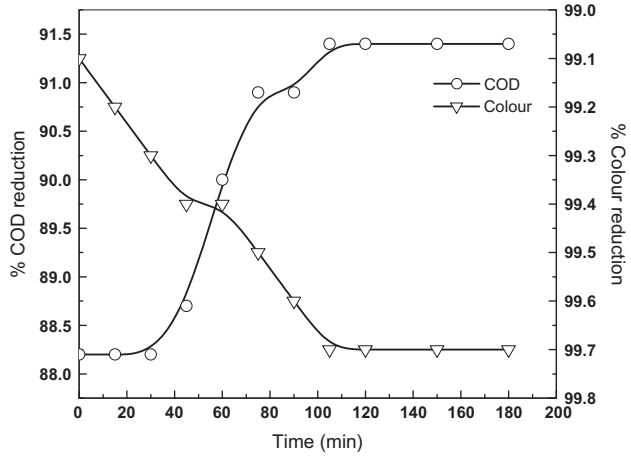

Fig. 6. Per cent reduction of COD and colour with time at constant transmembrane pressure (1 kg/cm^2).

removal was about 91%. Thereafter, COD removal did not increase significantly, after 180 min 91.4% removal was obtained. The incorporation of surface activated biosorbent in the microfiltration process resulted in adequate removal of colour from the effluent. In Fig. 6, initially, colour removal was about 99.1% which became about 99.7% after 180 min which could be attributed due to surface adsorption on the adhering layer of the membrane.

Fig. 7 represented effect of time on permeate flux at a constant transmembrane pressure of 1.0 kg/cm^2. It might be observed that at constant operating pressure, within one hour of filtration flux decline was about 5% which attained a steady state thereafter. The initial decline in flux possibly resulted from the partial blocking of membrane pores together with the accumulation of a polarized layer on membrane surface offering extra resistance to permeation. The same figure showed the effect of varying transmembrane pressure (0.4–2.2 kg/cm^2) on permeate flux. The

Table 1
Characterization of the untreated and treated textile wastewater

Parameters	Untreated effluent[b]	Permeate[b]	Discharge norms[a]
Turbidity (NTU)	26.8	0.275	–
COD (mg/l)	2,220	190	250
BOD (mg/l)	240	14.4	30
pH	8.61	2.88	5.5–9.0
Conductivity (mS/cm)	4.765	3.032	–
TDS (g/l)	2.33	1.55	–
Dye concentration (%)	127.33	0.4	–
TSS (mg/l)	124	12	100
TKN (mg/l)	14.01	0.25	–
TOC (mg/l)	170	11.4	–
Cr (mg/l)	20	0.12	0.1
Pb (mg/l)	50	0.09	0.1
S (mg/l)	2,898	2.4	2
Mg (mg/l)	393	13.02	–
K (mg/l)	146	27.54	–
P (mg/l)	22	0.43	–

[a]The Environment (Protection) Rules, 1986 and 2010.
[b]Average data.

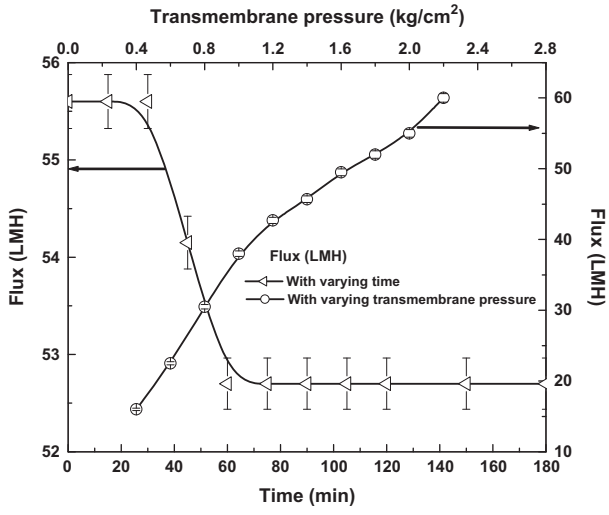

Fig. 7. Permeate flux profile at constant (1 kg/cm^2) and varying transmembrane pressure.

figure indicated that the effect of operating pressure on permeate flux was almost linear due to an increase in the driving force. At an enhanced operating pressure of 2.2 kg/cm^2, flux value was about 60 LMH.

The laboratory scale study indicated that about 46% recovery of water was obtained after 3 h which could be increased further by increasing the surface area of the ceramic membrane module. Unlike the concentrate stream produced in the conventional membrane separation processes, the retentate produced in this study contained trace amounts of colour, organic matter, as well as, other heavy metals due to the incorporation of the biosorbent in the effluent and had higher concentration of dissolved salt. Thus, the biosorptive effect had facilitated to generate a less toxic reject stream which was relatively easy and safe to handle. This reject volume could be further compacted by evaporation. The solid sludge consisted of adsorbed biomass which could be used as land fill or fuel since it was basically a carbonaceous agricultural waste biomass.

3.3. Toxicity response and enzyme activity with respect to different tissues of fish

Acute toxicity tests (LC_{50}) for 48 h were conducted on *Channa* sp. in three aqueous systems resulted in LC_{50} value of 43.5%. Mortality rate in control and membrane-treated permeate was zero. It was observed

Table 2
Oxidative stress biomarkers in liver of *C. punctatus* exposed to textile effluent

		Liver exposure time			
Oxidative stress biomarkers	Experiment groups	24 h	48 h	72 h	96 h
POD (ΔA_{470} g fresh weight/min)	Control	17 ± 0.2	12 ± 0.11	10 ± 0.4	7 ± 0.24
	25%	28 ± 0.18	25 ± 0.12	21.7 ± 0.2	19 ± 0.28
	Permeate	17.9 ± 0.17	13 ± 0.14	11.1 ± 0.17	7.6 ± 0.21
SOD (U/mg protein)	Control	5.9 ± 0.41	5.7 ± 0.35	5.1 ± 0.27	5.5 ± 0.22
	25%	8.2 ± 0.77	7.5 ± 0.55	6.8 ± 0.38	6.2 ± 0.07
	Permeate	6.1 ± 0.72	6.1 ± 0.44	5.9 ± 0.45	5.9 ± 0.88
CAT (U/mg protein/min)	Control	8.3 ± 0.12	7.9 ± 0.12	7.8 ± 0.09	7.7 ± 0.11
	25%	17.7 ± 0.42	15.8 ± 0.32	13.7 ± 0.52	13.1 ± 0.12
	Permeate	10.5 ± 0.02	10.3 ± 0.36	10.0 ± 0.12	9.7 ± 0.62
Glutathione S- Transferase (nmoles of CDNB conjugated/minute/mg protein)	Control	2.8 ± 0.04	2.82 ± 0.04	2.81 ± 0.02	2.8 ± 0.03
	25%	8.5 ± 0.22	8.2 ± 0.21	7.7 ± 0.14	7.2 ± 0.07
	Permeate	3.4 ± 0.22	3.4 ± 0.07	3.35 ± 0.04	3.35 ± 0.13
GR (μmoles of NADPH oxidized/minute/mg protein)	Control	0.94 ± 0.08	0.94 ± 0.07	0.93 ± 0.02	0.92 ± 0.02
	25%	3.78 ± 0.24	3.5 ± 0.18	3.2 ± 0.14	2.7 ± 0.21
	Permeate	1.47 ± 0.11	1.44 ± 0.07	1.40 ± 0.17	1.37 ± 0.08
GP (U/mg protein)	Control	2.14 ± 0.04	2.08 ± 0.08	1.98 ± 0.02	1.88 ± 0.04
	25%	4.66 ± 0.11	4.58 ± 0.10	4.42 ± 0.04	4.27 ± 0.14
	Permeate	2.37 ± 0.09	2.24 ± 0.05	2.12 ± 0.15	1.97 ± 0.09
ALP (μmol/min/mg of enzyme)	Control	0.436 ± 0.07	0.433 ± 0.04	0.438 ± 0.01	0.438 ± 0.09
	25%	0.272 ± 0.01	0.278 ± 0.09	0.288 ± 0.07	0.290 ± 0.01
	Permeate	0.414 ± 0.04	0.416 ± 0.07	0.42 ± 0.09	0.424 ± 0.03
ACP (μmol/min/mg of enzyme)	Control	0.321 ± 0.01	0.320 ± 0.04	0.327 ± 0.04	0.327 ± 0.03
	25%	0.427 ± 0.04	0.420 ± 0.07	0.310 ± 0.04	0.231 ± 0.04
	Permeate	0.291 ± 0.07	0.297 ± 0.04	0.299 ± 0.05	0.308 ± 0.06
Biochemical parameters					
Protein (mg/g)	Control	422 ± 21	420 ± 12	418 ± 18	392 ± 21
	25%	290 ± 18	285 ± 15	278 ± 22	266 ± 19
	Permeate	395 ± 12	372 ± 18	371 ± 17	368 ± 15
Carbohydrate (mg/g)	Control	548 ± 10	542 ± 8	540 ± 11	537 ± 8
	25%	398 ± 11	392 ± 9	390 ± 7	387 ± 12
	Permeate	527 ± 8	522 ± 12	510 ± 15	507 ± 14

Note: Values are mean ± s.d; $p < 0.05$.

that textile effluent was highly toxic to fish due to the presence of higher concentration of toxic dyes and associated chemicals, as well as, various heavy metals. Behavioural response was observed for every 1–8 h and 12 h. Control group behaved normally but those subjected to higher dilutions of effluent (50, 75 and 100%) behaved severely. Erratic swimming, staying motionless at the bottom of aquarium, frequent gulping for air, etc. were some of the behaviour observed [49,50].

Various natural compounds that actively participate in antioxidative defence mechanism of fish are peroxidase, catalase, SOD, glutathione, etc. [51]. The effects of treated and untreated textile effluent on four tissues (liver, kidney, gill and fins) were given in Tables (2)–(5). Maximum enzyme activity was observed in liver with respect to peroxidase, catalase and SOD, i.e. about 2.7 folds, 1.7 folds and 1.13 folds, respectively, for 25% effluent. Enzyme activities were severely affected for fish exposed to untreated effluent even after 96 h of exposure.

Peroxidases [52] are those enzymes that catalyses dehydrogenation of large number of organic compounds like phenols, amines, etc. These enzymes are involved in several physiological and biochemical process including cell growth and development. The activity of these enzymes is suggested to be an indicator of pollution level and the tolerance level of organisms exposed to the pollution. In aquatic system increased level of the activity of these enzymes is used as biochemical indicator of effluent toxicity [53]. Peroxidase activity was more in 25%

Table 3
Oxidative stress biomarkers in kidney of *C. punctatus* exposed to textile effluent

		Kidney exposure time			
Oxidative stress biomarkers	Experiment groups	24 h	48 h	72 h	96 h
POD (ΔA_{470} g fresh weight/min)	Control	15 ± 0.18	13.5 ± 0.10	10.4 ± 0.22	6.4 ± 0.14
	25%	26 ± 0.10	23.7 ± 0.11	20 ± 0.21	17.5 ± 0.22
	Permeate	15.4 ± 0.12	13.8 ± 0.12	10.9 ± 0.10	7.0 ± 0.11
SOD (U/mg protein)	Control	5.8 ± 0.05	5.8 ± 0.04	5.4 ± 0.09	5.4 ± 0.07
	25%	10.7 ± 0.41	9.2 ± 0.33	8.7 ± 0.42	7.9 ± 0.12
	Permeate	7.0 ± 0.02	6.7 ± 0.07	6.7 ± 0.12	6.5 ± 0.13
CAT (U/mg protein/min)	Control	7.8 ± 0.27	7.7 ± 0.02	7.7 ± 0.28	7.6 ± 0.11
	25%	16.2 ± 0.22	14.8 ± 0.12	12.7 ± 0.72	10.1 ± 0.42
	Permeate	8.4 ± 0.22	8.3 ± 0.39	8.0 ± 0.19	7.9 ± 0.12
Glutathione S- Transferase (nmoles of CDNB conjugated/minute/mg protein)	Control	2.22 ± 0.01	2.2 ± 0.01	2.2 ± 0.09	2.17 ± 0.04
	25%	7.8 ± 0.04	7.7 ± 0.14	6.9 ± 0.47	6.2 ± 0.23
	Permeate	3.6 ± 0.17	3.59 ± 0.36	3.58 ± 0.19	3.56 ± 0.07
GR (μmoles of NADPH oxidized/minute/mg protein)	Control	0.98 ± 0.13	0.96 ± 0.10	0.96 ± 0.02	0.95 ± 0.04
	25%	3.9 ± 0.08	3.7 ± 0.25	3.51 ± 0.40	2.39 ± 0.09
	Permeate	1.7 ± 0.21	1.69 ± 0.14	1.67 ± 0.11	1.66 ± 0.12
GP (U/mg protein)	Control	1.99 ± 0.04	1.84 ± 0.05	1.80 ± 0.07	1.72 ± 0.08
	25%	3.92 ± 0.14	3.82 ± 0.12	3.77 ± 0.10	3.57 ± 0.11
	Permeate	2.07 ± 0.09	1.95 ± 0.05	1.88 ± 0.12	1.76 ± 0.04
ALP (μmol/min/mg of enzyme)	Control	0.386 ± 0.04	0.39 ± 0.14	0.392 ± 0.01	0.395 ± 0.07
	25%	0.263 ± 0.01	0.265 ± 0.04	0.266 ± 0.06	0.269 ± 0.01
	Permeate	0.369 ± 0.05	0.371 ± 0.08	0.373 ± 0.07	0.374 ± 0.08
ACP (μmol/min/mg of enzyme)	Control	0.287 ± 0.02	0.289 ± 0.04	0.291 ± 0.05	0.293 ± 0.05
	25%	0.311 ± 0.02	0.301 ± 0.06	0.118 ± 0.08	0.121 ± 0.05
	Permeate	0.221 ± 0.04	0.228 ± 0.05	0.231 ± 0.03	0.234 ± 0.02
Biochemical parameters					
Protein (mg/g)	Control	299 ± 18	296 ± 17	292 ± 22	287 ± 17
	25%	156 ± 12	155 ± 11	148 ± 21	146 ± 13
	Permeate	292 ± 14	288 ± 16	286 ± 22	285 ± 19
Carbohydrate (mg/g)	Control	388 ± 12	382 ± 10	380 ± 7	379 ± 11
	25%	208 ± 15	190 ± 5	187 ± 12	178 ± 18
	Permeate	381 ± 7	378 ± 9	372 ± 12	368 ± 14

Note: Values are mean ± s.d; $p < 0.05$.

effluent (~2.7 folds in liver, ~2.7 folds in kidney, ~1.95 folds in gills and ~2.2 folds in fin tissue) than that of control and membrane treated permeates indicating high toxicity and chemical polluting load of the effluent.

Catalase enzymes functions in catalysing decomposition of hydrogen peroxide to water and oxygen. It uses hydrogen peroxide as substrate as well as hydrogen acceptor [54]. Catalase activity in 25% effluent was more (~1.7 folds in liver, ~1.32 folds in kidney, ~2.38 folds in gills and ~1.9 folds in fin tissue) than that of control, while it reduced when exposed to treated effluent (permeate). Increased activity was due to effective antioxidant defence system that was acting against oxidative stress of the environment (textile effluent). The enzyme activity was more at 24 h which reduced after 96 h of exposure. For example, in liver tissue, CAT activity reduced to about 32% for 25% effluent (Table 2).

SOD belongs to metalloproteins that catalyses conversion of superoxide radicals thereby acting as primary preventive inhibitor. These enzymes are present in cytosol and mitochondria of mammalian cells and provide first line defence against free radical damage [55]. Toxic stress alters activity of SOD in important tissues of fish. Exposure of fish to toxic effluent (textile) resulted in initial elevation of SOD activity for 24 h which reduced significantly ($p < 0.05$) towards end of 96 h (~1.13 folds in liver, ~1.46 folds in kidney, ~1.39 folds in gills and ~1.7 folds in fin tissue).

Glutathione peroxidase is located in mitochondria and cytosol of skeletal muscle cells of fish. It is a sele-

Table 4
Oxidative stress biomarkers in gill of *C. punctatus* exposed to textile effluent

		Gill exposure time			
Oxidative stress biomarkers	Experiment groups	24 h	48 h	72 h	96 h
POD (ΔA_{470} g fresh weight/min)	Control	12.2 ± 0.09	10.2 ± 0.10	8.1 ± 0.11	6.2 ± 0.14
	25%	18 ± 0.10	16 ± 0.12	14.8 ± 0.11	12.1 ± 0.10
	Permeate	12.5 ± 0.07	10.8 ± 0.10	8.7 ± 0.11	6.9 ± 0.11
SOD (U/mg protein)	Control	4.7 ± 0.41	4.6 ± 0.37	4.6 ± 0.14	4.59 ± 0.28
	25%	7.9 ± 0.55	7.2 ± 0.09	7.0 ± 0.44	6.4 ± 0.42
	Permeate	5.2 ± 0.99	5.4 ± 0.24	4.1 ± 0.22	3.8 ± 0.72
CAT (U/mg protein/min)	Control	11.3 ± 0.52	10.1 ± 0.72	9.4 ± 0.28	8.7 ± 0.51
	25%	23.4 ± 0.08	22.8 ± 0.66	22.0 ± 0.22	20.7 ± 0.47
	Permeate	13.4 ± 0.32	13.2 ± 0.39	13.1 ± 0.22	12.9 ± 0.88
Glutathione S- Transferase (nmoles of CDNB conjugated/minute/mg protein)	Control	0.56 ± 0.04	0.55 ± 0.08	0.55 ± 0.02	0.54 ± 0.02
	25%	4.5 ± 0.22	4.2 ± 0.21	3.7 ± 0.14	3.2 ± 0.07
	Permeate	1.1 ± 0.04	1.02 ± 0.24	1.01 ± 0.11	1.0 ± 0.23
GR (μmoles of NADPH oxidized/minute/mg protein)	Control	0.33 ± 0.04	0.31 ± 0.08	0.31 ± 0.24	0.30 ± 0.18
	25%	1.26 ± 0.22	1.08 ± 0.08	0.98 ± 0.07	0.92 ± 0.22
	Permeate	0.67 ± 0.08	0.65 ± 0.05	0.65 ± 0.09	0.63 ± 0.29
GP (U/mg protein)	Control	1.88 ± 0.09	1.74 ± 0.06	1.63 ± 0.07	1.62 ± 0.07
	25%	3.29 ± 0.14	3.15 ± 0.17	3.02 ± 0.12	2.87 ± 0.11
	Permeate	1.97 ± 0.10	1.95 ± 0.08	1.84 ± 0.12	1.77 ± 0.11
ALP (μmol/min/mg of enzyme)	Control	0.42 ± 0.04	0.40 ± 0.04	0.44 ± 0.08	0.45 ± 0.01
	25%	0.203 ± 0.09	0.211 ± 0.09	0.217 ± 0.07	0.218 ± 0.07
	Permeate	0.38 ± 0.04	0.384 ± 0.07	0.391 ± 0.04	0.387 ± 0.02
ACP (μmol/min/mg of enzyme)	Control	0.238 ± 0.04	0.239 ± 0.01	0.241 ± 0.07	0.242 ± 0.02
	25%	0.409 ± 0.05	0.410 ± 0.05	0.117 ± 0.01	0.118 ± 0.03
	Permeate	0.209 ± 0.04	0.211 ± 0.05	0.212 ± 0.05	0.213 ± 0.07
Biochemical parameters					
Protein(mg/g)	Control	259 ± 11	252 ± 21	250 ± 22	242 ± 14
	25%	89 ± 9	85 ± 8	80 ± 6	76 ± 9
	Permeate	248 ± 14	247 ± 18	244 ± 15	238 ± 12
Carbohydrate(mg/g)	Control	224 ± 6	218 ± 7	206 ± 10	200 ± 8
	25%	140 ± 6	134 ± 5	130 ± 7	125 ± 3
	Permeate	218 ± 8	214 ± 9	201 ± 6	194 ± 5

Note: Values are mean ± s.d; $p < 0.05$.

nium containing enzyme that reacts both with lipid and hydrogen peroxide. It protects organisms from oxidative damage and helps in detoxification of hydrogen peroxide generated by SOD [56]. This enzyme activity was found to decrease with increase in time of exposure to textile effluent. Enzyme activity of control as well as treated effluent (permeate) was almost constant with time. Decreased levels of enzyme activity in fish exposed to untreated effluent suggested weakened defence system which might prove fatal in long run. Increased level of enzyme activity was observed in textile effluent (~2.3 folds in liver, ~2.07 folds in kidney, ~1.8 folds in gills and ~1.42 folds in fin tissue).

Glutathione reductase protects cellular membranes from peroxides by maintaining cellular level of GSH. It is a NADPH flavoprotein that reduces GSSG to GSH by oxidizing NADPH. NADPH in tissues is provided by pentose phosphate pathway. Considerable increase in this enzyme activity was observed in untreated effluent (~2.9 folds in liver, ~2.5 folds in kidney, ~3.1 folds in gills and ~3.5 folds in fin tissue). Heavy metals and organic matter present in effluent interfered in mitochondrial activity and triggered generation of ROS (reactive oxygen species). This caused increased enzyme activity of GR. Increase in GR activity resulted in accumulation of GSH thereby increasing stress tolerance of organisms [57].

Glutathione-S-Transferase metabolizes wide variety of electrophilic compounds. These enzymes plays active role in detoxification of carcinogenic compounds which would otherwise cause damage to DNA [58]. Significant ($p < 0.05$) duration dependant decrease in enzyme activity was noted in tissues of fish. The reduction in enzyme activity with more exposure time indicated that the detoxification mechanism of fish was impaired. Similar findings were observed by Sreejai and Jaya in fishes exposed to hydrogen sulphide [59]. Fish tissue exposed to untreated effluent showed increased enzyme (~2.6 folds in liver, ~2.8 folds in kidney, ~5.9 folds in gills and ~6.3 folds in fin tissue).

Alkaline phosphatase is a polyfunctional enzyme present highly concentrated in plasma membrane and

Table 5
Oxidative stress biomarkers in fin tissue of *C. punctatus* exposed to textile effluent

		Fin tissue exposure time			
Oxidative stress biomarkers	Experiment groups	24 h	48 h	72 h	96 h
POD (ΔA_{470} g fresh weight/min)	Control	10 ± 0.11	8.2 ± 0.08	6.4 ± 0.12	5.0 ± 0.09
SOD (U/mg protein)	Control	3.4 ± 0.04	3.0 ± 0.06	2.9 ± 0.01	2.9 ± 0.07
	25%	6.7 ± 0.24	6.1 ± 0.22	5.6 ± 0.47	5.0 ± 0.33
	Permeate	4.2 ± 0.05	4.1 ± 0.07	4.0 ± 0.06	4.0 ± 0.04
CAT (U/mg protein/min)	Control	5.1 ± 0.02	5.0 ± 0.07	4.8 ± 0.04	4.7 ± 0.01
	25%	12.1 ± 0.72	11.7 ± 0.24	9.7 ± 0.55	9.0 ± 0.09
Glutathione S- Transferase (nmoles of CDNB conjugated/minute/mg protein)	Control	0.36 ± 0.03	0.36 ± 0.02	0.35 ± 0.02	0.35 ± 0.07
GR (μmoles of NADPH oxidized/minute/mg protein) & GP (U/mg protein)	25%	3.8 ± 0.20	3.0 ± 0.01	2.4 ± 0.04	2.2 ± 0.72
	Permeate	1.2 ± 0.01	1.19 ± 0.10	0.98 ± 0.01	0.89 ± 0.04
ALP (μmol/min/mg of enzyme)	Control	0.289 ± 0.04	0.29 ± 0.07	0.32 ± 0.04	0.33 ± 0.01
	25%	0.063 ± 0.02	0.065 ± 0.04	0.067 ± 0.06	0.069 ± 0.01
	Permeate	0.272 ± 0.04	0.277 ± 0.01	0.278 ± 0.04	0.28 ± 0.02
ACP (μmol/min/mg of enzyme)	Control	0.219 ± 0.04	0.221 ± 0.01	0.224 ± 0.04	0.226 ± 0.01
	25%	0.098 ± 0.02	0.101 ± 0.04	0.109 ± 0.03	0.111 ± 0.07
	Permeate	0.211 ± 0.07	0.211 ± 0.01	0.215 ± 0.07	0.216 ± 0.07
Biochemical parameters					
Protein(mg/g)	Control	207 ± 17	202 ± 12	190 ± 14	182 ± 21
	25%	102 ± 12	95 ± 8	92+7	86 ± 5
	Permeate	198 ± 14	192 ± 20	187 ± 13	178 ± 10
Carbohydrate(mg/g)	Control	192 ± 8	188 ± 7	186 ± 8	185 ± 9
	25%	140 ± 6	134 ± 5	130 ± 7	125 ± 3
	Permeate	188 ± 7	184 ± 8	180 ± 6	177 ± 8

Note: Values are mean ± s.d; $p < 0.05$.

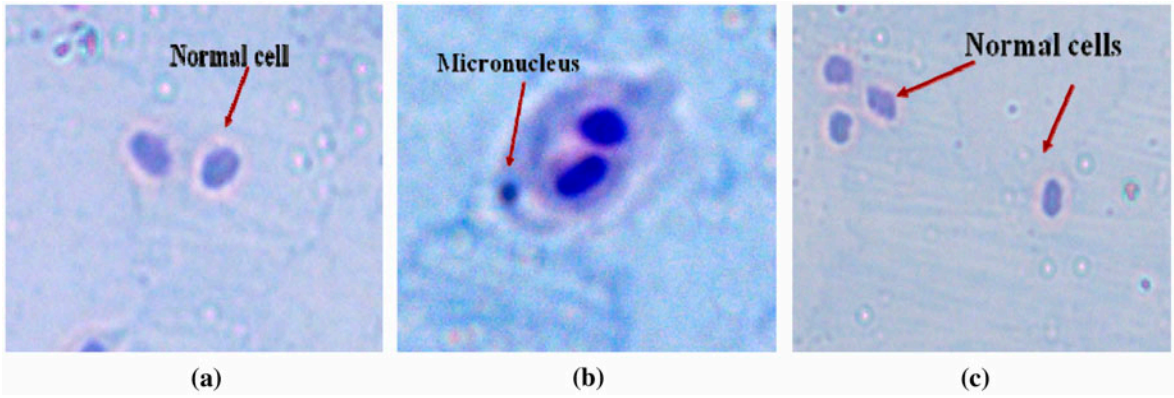

Fig. 8. Normal cells and micronucleus formation in *Channa* sp. blood exposed to different treatments: (a) control; (b) untreated effluent; (c) biosorbent + membrane treated effluent.

Table 6
Frequency of micronuclei and nuclear abnormalities and cells with comet class of whole blood of fish

Experiment groups	Time of exposure (h)	MNs	NAs	Number of cells with comet class
Control	24	0.0 ± 0.0	1.4 ± 0.2	0.0 ± 0.0
	48	0.0 ± 0.0	1.6 ± 0.2	0.0 ± 0.0
	72	0.0 ± 0.0	1.7 ± 0.2	0.0 ± 0.0
	96	0.0 ± 0.0	1.9 ± 0.2	0.0 ± 0.0
25%	24	2.4 ± 0.2	4.4 ± 0.28	11.4 ± 0.21
	48	2.7 ± 0.34	4.7 ± 0.33	12.7 ± 0.34
	72	2.9 ± 0.32	4.9 ± 0.34	13.0 ± 0.24
	96	3.0 ± 0.33	5.3 ± 0.27	14.8 ± 0.29
Permeate	24	0.0 ± 0.0	1.4 ± 0.22	0.0 ± 0.0
	48	0.0 ± 0.0	1.7 ± 0.18	0.0 ± 0.0
	72	0.0 ± 0.0	1.8 ± 0.19	0.0 ± 0.0
	96	0.1 ± 0.1	1.8 ± 0.20	0.4 ± 0.1

Note: NB: for MN and NA 2000 cells were scored; for comet assay 1,000 cells were scored data are represented by mean ± s.d. ($p < 0.001$).

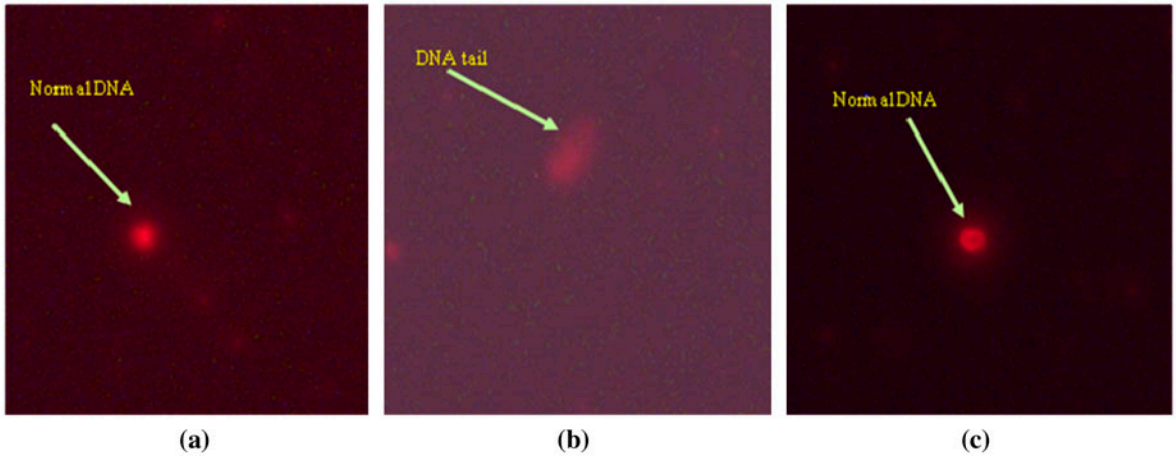

Fig. 9. Comet assay of *Channa* sp. blood exposed to different treatments: (a) control; (b) untreated effluent; (c) biosorbent + membrane treated effluent.

is involved in various metabolic activities like cell growth and division. These enzymes are very sensitive to heavy metal pollutants [60].

Acid phosphatases are present in lysosomes and involve in immune defence of organisms. These enzymes hydrolyse the phosphomonoesters produced by hydrolysis of other phosphates of cell [61,62]. It was observed that ALP in fish tissues exposed to untreated effluent was less than that of control or treated effluent i.e about 34% less in liver, 32% in kidney, 51% in gills and 79% in fin tissue. According to Sunmono et al. [63] decrease in ALP might be due to leakage of enzyme from cytosol into blood circulation or might be due to organ dysfunction. Interestingly, acid phosphatase showed increase in activity in untreated effluent compared to control followed by sharp decline after 48 h of exposure in liver, gills and kidney. Since the enzyme is related to lyososomal activity, the increase might be due to damage in tissues. For ACP, it was about 28% less in liver, 59% in kidney, 51% in gills and 50.8% in fin tissue. Similar findings were observed by Bakde and Poddar who observed that in liver there was an initial rise of 56.7 % in ACP activity followed by 32% decline for 30% effluent. They observed that within 48 h the acid phosphatase activity increased than that of normal and then gradually reduced [64].

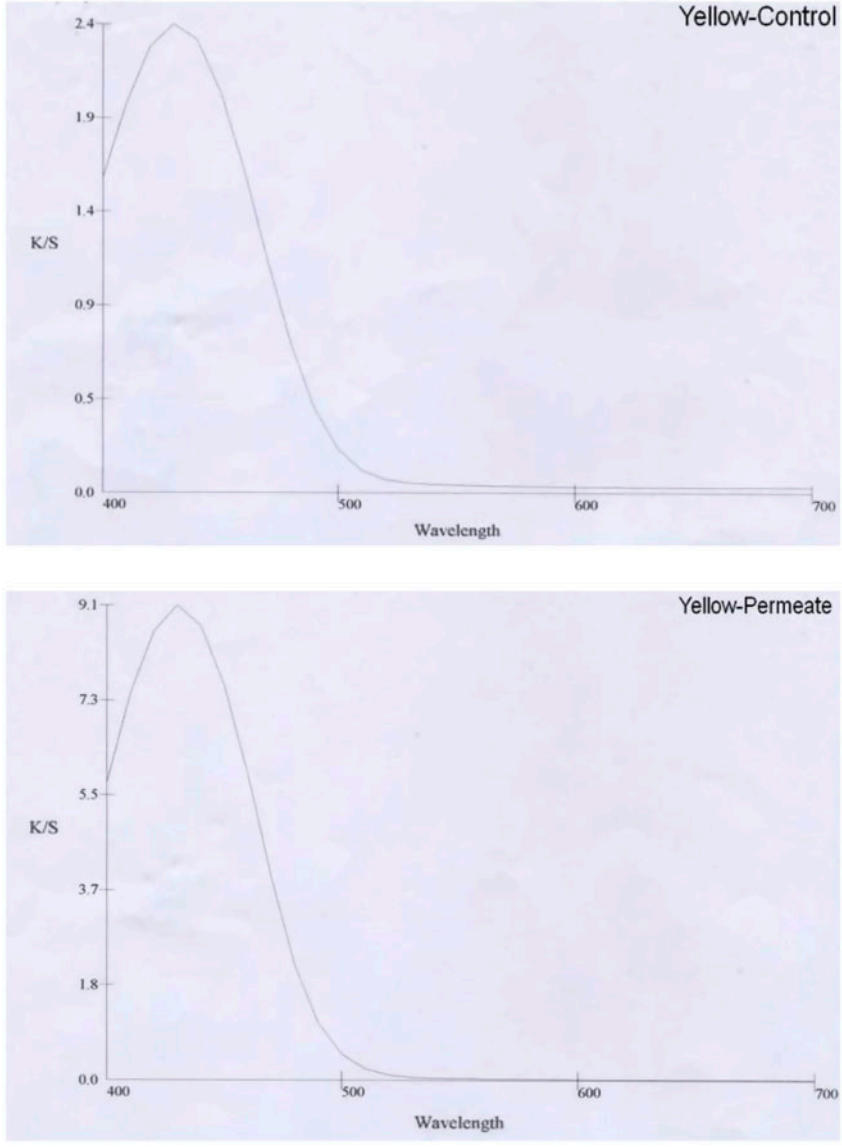

Fig. 10a. k/s values of cotton fabric after dyeing with yellow dye using fresh water (control) and permeate water produced by combined process.

3.4. Biochemical changes in different fish tissues

Protein and carbohydrate content of different fish tissue exposed to untreated effluent decreased compared to that of control and treated effluent (Tables (2)–(5)). Since liver is the most vital organ in carbohydrate metabolism, its content was highest compared to other tissues. Decrease in protein and carbohydrate content might be due to its utilization to maintain osmo- and ionic- regulation, or to counteract stress caused by toxicants present in effluent [65]. Treated effluent however showed results comparable to that of control. It was observed that reduction in protein content was about 32% in liver, 49% in kidney, 68% in gills and 51% in fin tissues compared to control after 96 h of exposure. Similarly, reduction in carbohydrate content was about 28% in liver, 53% in kidney, 38% in gills and 32% in fin tissue.

3.5. Comet assay and micronucleus assay

The results (Fig. 8(a)–(c) and Table 6) showed induction of micronuclei in fish erythrocytes exposed to 25% textile effluent at different time of exposure. Micronuclei induction was observed in peripheral

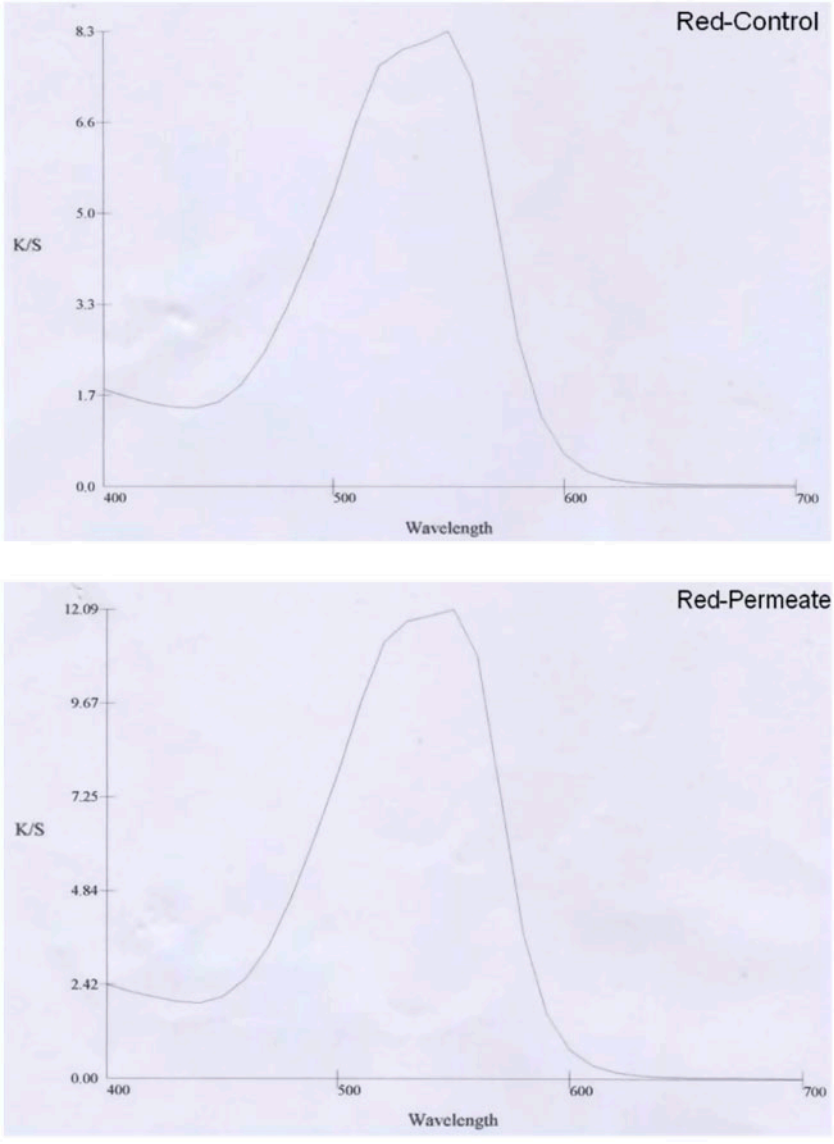

Fig. 10b. k/s values of cotton fabric after dyeing with red dye using fresh water (control) and permeate water produced by combined process.

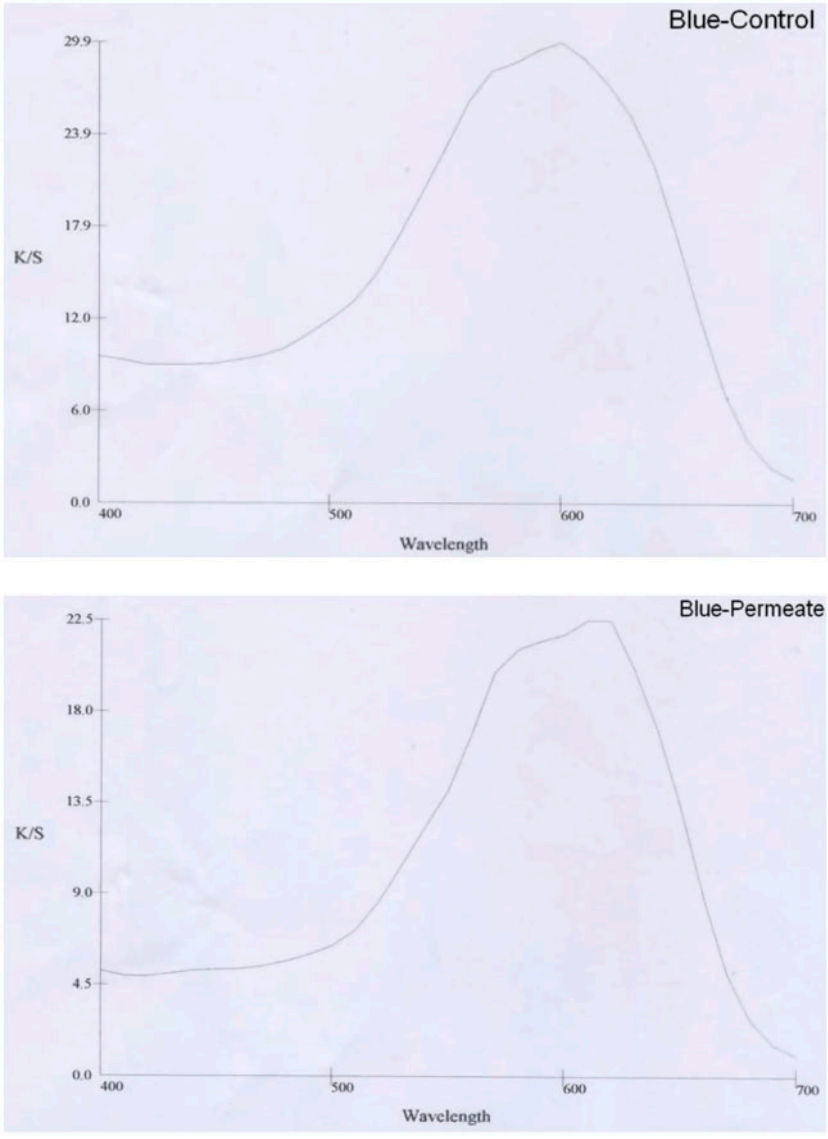

Fig. 10c. k/s values of cotton fabric after dyeing with blue dye using freshwater (control) and permeate water produced by combined process.

blood erythrocytes, gill epithelial cells and liver cells of various fishes exposed to cadmium [66]. Micronuclei formation and nuclear abnormalities in fish exposed to untreated effluent were much higher (i.e. 14.8 ± 0.29 comet cells, 5.3 ± 0.27 nuclear abnormalities and 3.0 ± 0.33 micronuclei) than that of control after 96 h of exposure. Results obtained in fish exposed to treated effluent were comparable with control values with negligible numbers of comet cells and micronuclei (i.e. 0.4 ± 0.1 comet cells, 1.8 ± 0.20 nuclear abnormalities and 0.1 ± 0.1 micronuclei) after 96 h of exposure. Exposure of cells to genotoxic chemicals results in formation of reactive oxygen species which interacts with DNA causing disruptive changes [67]. Heavy metals and other toxic chemicals present in textile effluent have resulted in nuclear abnormalities and micronucleus induction in blood cells. The frequencies of MN and NA increased with increase in time of exposure. Comet assay (Fig. 9(a)–(c)) of fish blood on the other hand provided an in depth knowledge of DNA damage. The distributions of damage in fish blood cells exposed to textile effluent were different from that of control. This assay can be used as an effective genotoxic biomarker to monitor effect of pollutants on aquatic life.

Table 7
Characteristic of effluent one and two produced after batch scale dyeing in the laboratory

	Samples	TOC (mg/l)	TKN (mg/l)	Dye concentration (mg/l)
Effluent one	Control (Red dye)	3,453	0.91	115
	Permeate water	3,500	1.1	109.2
	Control (Yellow)	2025	0.91	102
	Permeate water	2069	0.77	90
	Control (Blue)	3,845	0.91	88
	Permeate water	4,125	1.8	209
Effluent two	Control (Red dye)	5,518	0.91	18.5
	Permeate water	5,030	1.4	13.2
	Control (Yellow)	4,832	0.91	48
	Permeate water	4,955	0.82	42
	Control (Blue)	4,502	0.91	34
	Permeate water	5,464	2.2	43

3.6. Reuse of treated effluent

The permeate samples were used to test the reusability of treated effluent in the dyebath formulation for dyeing of wet fabric. It was observed that the effluent pH after treatment process slightly increased to about 2.8–3.0. The reason for this increase in pH might be attributed to the presence of alkali (−OH) group on biosorbent surface observed from the FTIR spectra. The availability of –OH groups possibly be due to the initial treatment of the biomass with sodium hydroxide for delignification. Similar results were obtained by Kan et al. where the pH of solution increased after contact with tourmaline adsorbent [68]. In general, the water used for dyeing operations requires a neutral pH (7–7.5) with no colour and odour, as well as, lower TDS value (65–150 mg/l). Hence for conduction of reuse study, pH of the treated effluent produced was adjusted to neutral values by addition of dilute sodium hydroxide solution. A parallel test using freshwater was also undertaken for comparative evaluation. Freshwater was replaced by treated effluent for conducting the entire dyeing process. Batch scale dyeing was performed on three different shades of colour i.e. light (yellow), medium (red) and dark (navy blue). Water produced during dyeing process was collected from the dye bath and marked as effluent one while the water collected after completion of total dyeing process was marked as effluent two (composite effluent). The effluents were characterized and compared with that generated from use of freshwater. From visual observation it was found that dyeing using treated effluent for dark shade was not satisfactory. On the other hand, light and medium shade showed better results compared to fresh water. The dyed cotton fabrics were analysed in spectrophotometer and from k/s values it was observed that dye uptake for light and medium shade was more compared to that of control. Interestingly, the treated effluent could be successfully reused for light and medium shade with less consumption of salt. It was observed that for yellow dye using standard dose of salt and soda ash, the k/s values (Fig. 10(a)) were more than control. Similar results were obtained for medium shade also (Fig. 10(b)). Values near to control were achieved at a lower salt dose (50 g/l). Dyeing conducted with salt dose of below 50 g/l did not provide satisfactory results. The treated effluent was not suitable for dark shade dyeing due to a high TDS content of the medium which caused poor dye fixation resulting into uneven dyeing. Here, the k/s values were much below the control values and the values were comparable only at higher salt dose (Fig. 10(c)). Hence the reuse limits of the treated water in the proposed treatment process were confined within light and medium shade dyeing. For dark shade dyeing further treatment with reverse osmosis might be incorporated. From TKN, TOC and dye concentration values (Table 7) of effluent one and effluent two produced during the batch dyeing of reuse study, it was observed that the values were in compatible with that of fresh water. Hence, the combination process resulted in less consumption of fresh water, as well as, reduction in the usage of salt. Further studies need to be conducted for optimization of process parameters for large scale reuse study.

4. Conclusion

The study showed the potential of ceramic microfiltration membrane along with biosorbent prepared from sugarcane bagasse for treatment of textile effluent. The combined process resulted in 99.2% reduction of colour and 91.4% reduction of COD. In addition,

there was about 93% reduction in total organic carbon and substantial reduction of heavy metals like chromium and lead. The treated effluent was reused for dyeing of cotton fabric for light, medium and dark shade. k/s values of control for light shade was 2.34, whereas for treated water it was 9.14. Similarly for medium shade, control k/s values were 7.97 and for treated water it was 11.79. The results obtained for treated water with lower salt dose were nearer to control. For medium shade dyeing with 50 g/l of salt dose, k/s value obtained was 7.98. Therefore, the water produced from combined process could be successfully reused without any tertiary treatment.

Study on evaluation of toxicity of permeate water on aquatic life was conducted. Various dilutions of textile effluent viz. 25, 50, 75 and 100% were used for the study of oxidative stress enzymes and toxicity tests like LC_{50}, comet assay and micronuclei on *C. punctatus* as model. The 48-h acute toxicity tests resulted in LC_{50} value of 43.5%. There was formation of comet cells (14.8 ± 0.29) and micronuclei (3.0 ± 0.33) in fish blood after 96 h of exposure in 25% diluted effluent. Considerable increase in stress enzymes like POD, CAT, SOD, GR, GPx, GST, etc. were observed in different tissues of fish like liver, kidney, gills and fins. Therefore, it might be concluded that the combined technology proved to be environmentally friendly one, where wastewater from one of the most polluting industry i.e. textile industry could be treated and the treated water could be successfully reused for dyeing of cotton fabric. The produced water did not have significant adverse impact on aquatic life.

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