Combination technology of ceramic microfiltration and biosorbent for treatment and reuse of tannery effluent from different streams: response of defence system in *Euphorbia* sp.

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**Abstract**

**Background:** The present study was undertaken to evaluate the efficiency of combined technology involving ceramic microfiltration and biosorbent for the treatment of tannery effluent from different streams, viz. composite effluent, effluent from primary clarifier and secondary clarifier. The membranes were prepared from a cost-effective composition of alumina and clay.

**Results:** The effluents had high organic loading of 12,895, 3,890 and 410 mg/L, respectively, in terms of chemical oxygen demand (COD). Apart from these, the effluents consisted of toxic heavy metals, turbidity, biochemical oxygen demand (BOD), etc. It was observed that COD reduction was about 96.5% for effluent 1, 96.6% for effluent 2 and 96.9% for effluent 3. Considerable reduction in suspended solids, total nitrogen, and total organic carbon was obtained. Turbidity for all three types of effluent was below 1 NTU. The average flux value for effluents 1, 2 and 3 was about 13, 19 and 24 L/m²h (LMH), respectively. Response of the antioxidative defences of *Euphorbia hirta* was observed which resulted in considerable decrease in the activity of peroxidase, superoxide dismutase and catalase.

**Conclusions:** The treatment resulted in the reduction of toxicity thereby restoring normal activity when compared to control values. Changes in various biochemical parameters like protein, amino acid, carbohydrate, DNA, RNA and chlorophyll content were observed.

**Keywords:** Tannery effluent; Biosorbent; Ceramic membrane; Microfiltration; Reuse; *Euphorbia* sp

**Introduction**

The most polluting and oldest industry of the world is tanning industry. The demand for leather products is increasing with increasing population, thereby creating huge pollution load on the environment. The different processes associated with leather processing and finishing require huge amount of toxic chemicals, lime, chloride and the hides itself that contain huge amount of organic matter, protein and fats which contribute to the effluent when discharged into the environment (Altaf et al. 2008). Therefore, the most important challenge that scientists all over the world are facing is the treatment of tannery wastewater to make it reusable. Various methods have been explored for the treatment of tannery wastewater. The most conventional treatment option is biological treatment which has been successfully applied for many years (Vasudevan et al. 2012; Sivaprakasam et al. 2008). But due to the generation of large volume of sludge, this option has its own drawbacks. Apart from this, other processes like Fenton’s and electro-oxidation (Rameshraja and Suresh 2011), photolytic and electrolytic oxidation (Anglada et al. 2009), ozonation (Dogruel et al. 2004), coagulation (Espinoza-Quinones et al. 2009), UV/H₂O₂ (Rodrigues et al. 2008), etc. have been applied. Adsorbents prepared from natural materials have been used for the removal of chromium from tannery wastewater. The applicability of novel biosorbents had been studied for the removal of chromium from tannery wastewater (Chena et al. 2011; Bhattacharya et al. 2013).
Membrane-based treatment of wastewater is being explored recently due to its efficiency and less generation of sludge. Membrane bioreactors are being widely used for the treatment of tannery and other wastewater (Cassano et al. 2001; Stephenson et al. 2000; Suthanthararajan et al. 2004.) Scholz et al. (2005) has studied the use of a membrane bioreactor for the removal of organic pollutants and suspended solids from tannery effluent and followed it up with the use of reverse osmosis. Goltara et al. (2009) studied membrane sequencing batch reactor for the treatment of beam house effluent. Use of a hybrid membrane bioreactor consisting of electro-coagulation, biological treatment and microfiltration was investigated for the removal of colour and chemical oxygen demand (COD) from tannery wastewater (Keerthi et al. 2013).

Ceramic membranes have been developed indigenously from a cost-effective composition of clay and alumina by the CSIR-Central Glass and Ceramic Research Institute. These membranes have been successfully used for the treatment of various types of wastewater (Bandyopadhyay et al. 2006; Bhattacharya et al. 2010, 2011). Therefore, in the present study, an attempt was undertaken to treat tannery effluent collected from different streams using the combined technology of biosorbent and ceramic microfiltration. To observe the reuse efficiency of the treated water, effect on various biochemical parameters and antioxidative enzymes was observed on the herb Euphorbia hirta L. The reason for choosing this plant was that this plant was widely grown in and around areas near the tannery industry. These herbs are widely grown and very common in tropical countries (Tabugo et al. 2013). It is widely distributed throughout India near roadsides (Prajapati et al. 2003). In this case, the herbs were found to grow along the roadside of the effluent collection plant. E. hirta is a weed that grows profusely in waste places. It is an invasive plant which spreads very quickly and is a species of much brightened environment that grows so well on dry grounds as well as in the wetter zones. The herb prefers sandy grounds or gravels and grows in sunny to lightly shaded, not too moist, grassy sites, between stones and waste areas (Holm et al. 1991). But interestingly, the plants grown adjacent to the tank from where composite wastewater was collected were highly affected in terms of leaf growth, appearances, browning of leaves, etc. This might have occurred due to wastewater that seeped through cracks on the tank wall. But the plants that grew near a secondary clarifier tank were quite healthy. This drew attention, and the authors observed the effect of treated and untreated wastewater on this plant. The plant belongs to the family Euphorbiaceae and is characterized by the presence of white milky latex. This plant and other species of this genus have been used to prepare traditional medicines as it has antibacterial, antifungal, antimalarial activity, etc. Effect on E. hirta L. exposed to coal smoke pollutants for a long time was studied. Reduced plant growth and variations in stem anatomy, as well as chlorophyll content, resulting from the pollutants were observed (Gupta 1987). In the current study, the effects on E. hirta with respect to oxidative stress enzymes like superoxide dismutase (SOD), catalase (CAT) and guaiacol peroxidase (POD) in response to treated and untreated tannery effluent from different streams were observed. Moreover, the effect on chlorophyll content, amino acid, carbohydrate, DNA and RNA content was also studied. These studies were undertaken to investigate the toxicity response of living plants towards treated effluent in comparison with untreated effluent to understand the environmental impact of the proposed treatment scheme.

**Methods**

**Collection of effluent**

The study was conducted on three different loadings of wastewater, viz. composite wastewater (effluent 1), effluent from primary clarifier (effluent 2) and secondary clarifier (effluent 3). The effluents were collected from a common effluent treatment plant of tannery wastewater located at Kolkata, India. A schematic representation of the common effluent treatment plant is depicted in Figure 1. Immediately after collection, the wastewater was subjected to various characterizations according to the standard methods for water and wastewater (Greenberg et al. 2005), as shown Table 1.

**Batch biosorption study**

Biosorption study was conducted at varying pH (1 to 9) and biosorbent dose (0.25 to 5 g/L) for three different effluents to optimize the process parameters. A novel biosorbent prepared from fruit peels of Trewia nudiflora was used. The biosorbent was prepared by subjecting the fruit peels to phosphoric acid treatment for surface activation (Bhattacharya et al. 2013).

**Crossflow microfiltration study**

Crossflow microfiltration study was conducted using an indigenously developed ceramic multichannel element with a diameter of 35 mm, length of 200 mm and channel diameter of 4 mm. Average pore size of the membrane was about 1 μm. The membrane was placed inside a stainless steel module horizontally aligned in the setup. The ceramic membrane was stable at a wide range of pH (2 to 12). The operating pressure limit of the current study was 0.4 to 2.2 kg/cm² although the membrane could withstand an external pressure up to 6 kg/cm². A centrifugal pump with 1 hp capacity was provided for recirculation of the feed water, and permeate samples were collected from the bottom port. The capacity of the feed tank was 10 L. A schematic representation of
the experimental process was shown in Figure 2. The study was conducted using three different types of feed, i.e. effluents 1, 2 and 3. Each study was conducted using three different feeds for each type of effluent. The effluent along with the optimized dose of biosorbent was taken to the feed tank and recirculated for about 30 min to ensure proper mixing. The pH of the effluent was adjusted to optimum value by adding dilute acid or alkali solution. The setup was allowed to run at 1 kg/cm² transmembrane pressure for about 180 min to observe the effect of time on permeate flux. Effect transmembrane pressure was observed by varying the pressure from 0.4 to 2.2 kg/cm². Permeate samples were collected at definite time intervals to measure COD (Spectralab, Mumbai, India), total kjeldahl nitrogen (TKN) (Pelican Instruments, Chennai, India), turbidity (Hach, Loveland, CO, USA), pH (Hach), conductivity (Hach), total dissolved solids (TDS) (Hach), total suspended solids (TSS) (Tarsons, Kolkata, India), biochemical oxygen demand (BOD) (Hach), etc. After each run, the experimental setup was cleaned with distilled water, and after completion of all the experiments, it was cleaned thoroughly with 0.1 (N) of nitric acid solution, then 0.1 (N) of sodium hydroxide solution followed by washing with deionized water. Washing was continued until clean

![Figure 1 Schematic representation of conventional treatment in common effluent treatment plant.](image)

Table 1 Characteristics of tannery wastewater

| Parameters          | Effluent 1 | Effluent 2 | Effluent 3 | Discharge norms<sup>a</sup> |
|---------------------|------------|------------|------------|-----------------------------|
|                     | Untreated  | Treated (AD + MF) | Untreated | Treated (AD + MF) | Untreated | Treated (AD + MF) | Untreated | Treated (AD + MF) | Unregulated |
| pH                  | 8.21       | 7.23       | 7.34       | 7.21                       | 6.29       | 6.24                       | 6.0 to 9.0 |
| COD (mg/L)          | 12,895     | 442        | 3,890      | 261                        | 410        | 35                        | -          |
| BOD (mg/L)          | 1,089      | 52         | 439        | 21.1                       | 47         | 6.2                       | 30         |
| TSS (mg/L)          | 1,088      | 2.4        | 282        | 1.11                       | 34         | 4.8                       | 100        |
| TDS (mg/L)          | 8,852      | 7,421      | 6,605      | 5,881                      | 3,895      | 2,899                      | -          |
| TKN (mg/L)          | 29.5       | 4.1        | 21.2       | 2.9                        | 8.6        | 0.5                       | -          |
| TOC (mg/L)          | 58.0       | 46.8       | 374        | 26.8                       | 10.8       | 0.75                       | -          |
| Conductivity (mS/cm)| 14.89      | 11.25      | 13.41      | 10.6                       | 7.87       | 2.99                       | -          |
| Turbidity (NTU)     | 1,193      | 0.664      | 256        | 0.625                      | 12.2       | 0.341                      | -          |
| Chromium (mg/L)     | 60         | 0.02       | 20         | ND                         | 0.3        | ND                         | 0.1        |
| Magnesium (mg/L)    | 920        | 27.26      | 730        | 15.44                       | 22.1       | 1.91                       | -          |
| Potassium (mg/L)    | 550        | 11.2       | 490        | 9.5                        | 61.4       | 0.71                       | -          |
| Phosphorus (mg/L)   | 710        | 20.6       | 580        | 17.4                       | 8.0        | 1.55                       | -          |
| Nickel (mg/L)       | 80         | 1.12       | 70         | 7.6                        | 0.1        | 0.04                       | -          |
| Lead (mg/L)         | 60         | 0.06       | 40         | 0.04                       | 0.4        | ND                         | -          |
| Sulphide (mg/L)     | 382        | 11.4       | 382        | 8.4                        | 70.1       | 1.3                       | 2.0        |

Data represent average of triplicates. ND, not detected. *The Environment (Protection) Rules (1986).
water permeability value could be restored almost completely which was about 45 to 60 min.

Collection of E. hirta L.
The plant was collected from an unaffected area near the lakeside around Kolkata. Affected plants were also collected from the adjacent area of the common effluent treatment plant to compare the results. The plants were allowed to grow in laboratory conditions in hydroponic solutions with proper illumination and temperature. After 7 days time of acclimatization, the plants were immersed in seven different solutions in three replicates. The solutions were effluent 1 and permeate, effluent 2 and permeate, effluent 3 and permeate, and fresh water (control).

Antioxidative stress enzymes and biochemical assays
Guaiacol peroxidase was measured by adding 990 μL of guaiacol solution (0.25% v/v in 10 mmol/L of phosphate buffer and 0.125% H₂O₂) to 10 μL of enzyme extract (extracted in phosphate buffer). The brown-coloured complex formed was measured spectrophotometrically at 470 nm and expressed as A470 per gram of fresh tissue weight per minute (Cipollani 1998). Superoxide dismutase was measured according to the method described by Giannopolitis and Ries using nitro blue tetrazolium (NBT) with slight modifications. After reaction, the sample was measured spectrophotometrically at 560 nm and expressed as unit per milligram of protein per minute (Giannopolitis and Stanley 1977). Catalase activity was measured by observing the disappearance of H₂O₂ spectrophotometrically at 240 nm. The reaction mixture consisted of phosphate buffer, H₂O₂ and enzyme extract. The activity was expressed as unit per milligram of protein per minute (Aebi 1983).

The protein content of plant tissue was measured according to Lowry et al. (1951) using folin reagent and bovine serum albumin (BSA) standard. The colour intensity was measured spectrophotometrically at 660 nm and expressed as milligram per gram of fresh weight. Total carbohydrate was measured by using anthrone with glucose as standard (Hedge and Hofreiter 1962). The samples were measured at 620 nm and expressed as milligram per gram of fresh weight. Amino acid content was measured by the formation of a red-coloured complex, i.e. formazone when reacted with ninhydrin in acidic medium and soluble in organic solvents like toluene (Bates et al. 1973). The samples were measured at 520 nm and expressed as micromole per gram of fresh weight. The chlorophyll content of plant leaves were measured according to APHA (Greenberg et al. 2005). Chlorophyll a and b were measured at 664 and 647 nm by extraction in ethanol. It was expressed as milligram per gram of fresh weight. For nucleic acid (DNA and RNA) determination, the plant tissues (0.25 g) were homogenized in 2 mL of cold methanol, and insoluble pellet was washed twice with same volume of methanol. The residue was extracted with 4 mL of cold perchloric acid (0.2 M) and then by 4 mL of absolute ethanol. Further, the residue was extracted with 5 mL of 2:1 ethanol/ether mixture at 50°C for 30 min. The residue formed was extracted with 5 mL of 5% perchloric acid at 70°C for 40 min. The supernatant (1 mL) was collected and 4 mL of diphenylamine reagent was added. It was kept in a water bath for 10 min and measured spectrophotometrically at 595 nm for DNA content. RNA content was measured by adding 1 mL of orcinol reagent to 1 mL of hydrolysate and measured at 660 nm (Cherry 1962). The chemicals used were purchased from Merck, GR, Bangalore, India.

Statistical analysis
Three replicates were performed for each treatment, and standard deviation (S.D.) was calculated and expressed in X ± S.D. using GraphPad InStat 3 software (GraphPad, San Diego, CA, USA) by one-way analysis of variance (ANOVA) in order to compare means of different treatments, taking p ≤ 0.05 and p ≤ 0.01 as levels of significance.
Results and discussion

Biosorptive treatment of tannery effluent

From batch equilibrium study conducted to optimize dose of biosorbent and pH of sample solution, it was observed that for effluent 1 the optimum dose was 3 g/L, for effluent 2 the dose was 2 g/L and for effluent 3 the dose was 0.5 g/L (Figure 3a, b, c). The association of biosorbent of toxic metals or organic matter present in effluent is generally electrostatic in nature. The interaction occurs between the available sites on biosorbent and metals or organic matter. At a given biosorbent dose, adsorption occurs until all the binding sites are exhausted and no more sites are available for sorption, i.e. the point of saturation is reached. Therefore, further increasing the dose does not increase the biosorption. Moreover, a higher amount of biosorbent results in blocking of binding sites from being available for uptake of organic matter or metal ions (Meena et al. 2004; Chhikara and Dhankhar 2008). Figure 3 indicated that the optimum pH for effluents 1 and 2 was 4, and for effluent 3, it was 5. With increasing the pH of any system, the number of negatively charged sites increases and the number of positively charged sites decreases. As explained by Neelavathi et al. (2004), negatively charged surface on the sorbent sites does not favour the adsorption of anions due to electrostatic repulsion. This explains the reduced biosorption at higher pH which might be due to the abundance of OH\(^{-}\) ions that caused decreased availability of adsorbable components for biosorption.

Ceramic microfiltration assisted by biosorption process

Three different types of effluents were treated using the combined process. In Figure 4, the COD and TKN removal were observed with time. Tannery effluent contains huge amounts of organic matter which are measured in terms of COD and TKN. It is necessary to consider the ratio of COD to TKN because chemical oxygen demand in wastewater results in the prevalence of anaerobic conditions in the biological treatment process. The presence of high TKN is due to the use of animal’s hides, skins which contribute nitrogen in organic and inorganic forms. High COD and TKN contribute to high nutrients in wastewater which must be removed or reduced before discharge or else it might result in eutrophication (Oke et al. 2006). From Figure 4a it might be observed that COD reduction for effluents 1, 2 and 3 for different days were about 96% to 97%, 92% to 94% and 90% to 93%, respectively. It might be observed that COD reduction for effluent 1 was slightly more than that for effluents 2 and 3. This might be due to the cake layer formation on the surface of the membrane resulting from the higher solute loading of effluent 1 which provided more resistance towards permeate flow, as well as partial adsorption of some of the components resulting in higher organic matter removal. TKN reduction for effluents 1, 2 and 3 was about 86%, 86.3% and 94.2%, respectively (Figure 4b). Table 1 showed detailed characteristics of the effluents after treatment. It might be observed that apart from the substantive reduction of organic loading, as well as suspended and turbid components, the biosorption-induced method could effectively reduce the heavy metal contents, viz. chromium, lead, nickel and other inorganic constituents, viz. potassium, phosphorus, sulphide, etc. Being biologically treated, the secondary clarifier effluent (effluent 3) had a lower concentration of TKN compared to effluents 1 and 2, and the combination method of biosorption with ceramic microfiltration could adequately reduce the TKN.

Figure 5a represented permeate flux at a constant pressure of 1 kg/cm\(^2\) for 180 min. Since COD loading of effluent 1 was higher compared to effluents 2 and 3, a flux value of about 9 to 15 L/m\(^2\)/h (LMH) was obtained. For effluent 2, about 17.9 to 21 LMH of flux was obtained and for effluent 3 it was about 23.5 to 27.5 LMH. A steady state flux was obtained for all three types of effluent. Microfiltration study was also conducted at varying pressure (Figure 5b) to observe the effect of pressure on steady state flux. Pressure was varied from 0.4 to 2.2 kg/cm\(^2\). Flux rate increased with increasing pressure.

Effect on antioxidative stress enzymes

From Table 2 it might be observed that the maximum enzyme activity (POD, SOD and CAT) was exhibited by effluent 1 followed by effluents 2 and 3. The plant when subjected to the treated effluent (approximately 1.72-fold) was much higher (p ≤ 0.01) than that of the control. Similarly, it might be observed that the plant grown in effluent 2 (approximately 1.32-fold) had a much higher (p ≤ 0.01) enzyme activity which was reduced (approximately 1.14-fold) for that in the treated effluent. Interestingly, since effluent 3 had a much lower loading, the enzyme activity (approximately 1.2-fold) was not much higher (p ≤ 0.01) and the treated effluent resulted in a much lower enzyme activity comparable to that of the control values. For SOD activity, it was observed that effluent 1 exhibited maximum activity (approximately four-fold) which was reduced (approximately 1.75-fold) for that in the treated water. Enzyme activity of the plant (approximately 3.7-fold) in effluent 2 was reduced after being subjected to the treated effluent (approximately 1.72-fold). Enzyme activity for effluent 3 (approximately 3.15-fold) was higher (p ≤ 0.01), but for the plant in the treated effluent, it was not significantly higher (p ≤ 0.01) than that of the control. Similarly, it might be observed that the
Figure 3 COD removal in batch biosorption study. Using varying pH at constant biosorbent dose and at constant pH with varying biosorbent dose: (a) effluent 1, (b) effluent 2 and (c) effluent 3.
Catalase activity of the plant in effluent 1 (approximately 3.23-fold) decreased when grown in the treated effluent (approximately 1.6-fold), and for effluent 2 (approximately 2.76-fold), it decreased to approximately 1.5-fold ($p \leq 0.01$). Enzyme activity of the plant in effluent 3 (approximately 2.06-fold) reduced to approximately 1.3-fold, and data was comparable to that of the control. Peroxidases are widely distributed in plant cells especially in plasma membrane. These enzymes show an increased activity when exposed to various stress-causing factors. These enzymes are located in the cytosol, cell wall, vacuole, etc. Studies have shown that an increase in POD activity was observed in mulberry leaves due to fluoride toxicity (Anil et al. 2009). Superoxide dismutase is generally associated with the first-line defence of organisms against reactive oxygen species. These enzymes catalyze the dismutation of superoxide radicals to oxygen and hydrogen peroxide and are widely distributed in different subcellular compartments of plant cells. The enzymes are generated to protect the plant cells from environmental stress (Bowler et al. 1994). Catalase enzyme activity changes with metabolic activity and environmental factors. Quantification of the enzyme activity determines the effect of stress or chemicals, etc. on plant metabolism. Catalase activity increased in soybean due to the application of zinc and salinity stress (Weisany et al. 2012).

Figure 4 COD vs time (a) and TKN vs time (b) for tannery effluent on different days.

Figure 5 Flux vs time (a) and variation of flux (b) with transmembrane pressure for tannery effluent on different days.
Effect on biochemical parameters

From Figure 6 it might be observed that there was considerable reduction in amino acid content of the plant exposed to untreated effluent, i.e. approximately 43% for effluent 1, 11% for effluent 2 and 7% for effluent 3. Interestingly, amino acid content for the plant exposed to treated effluent 3 was more than that of the control which might be due to the presence of macro- and micro-nutrients. Apart from that, treatment of both effluents 1 and 2 resulted in increased amino acid content compared to untreated effluent after 72 h of exposure. A similar trend was observed for carbohydrate content where there was also about 39%, 6% and 5.3% reduction of carbohydrate content observed in the plants exposed to effluents 1, 2 and 3. Carbohydrate content also increased compared to that of the control for plants grown in treated effluent 3. For both chlorophyll a and b, the value was comparable to that of the control for plants exposed to treated effluent 3. Scientists have observed that heavy metal toxicity can hamper the chlorophyll a/b ratio. Reduction in chlorophyll a compared to b is due to its faster hydrolysis. Changes in the concentration of chlorophyll a and b determine the effect of environmental stress on plants. Chlorophyll content decreases under stress due to inhibition of various enzymes like δ-aminolevulinic acid dehydratase and protochlorophyllide, subsequently affecting the photosynthetic machinery (Ahmad et al. 2007; Manios et al. 2003; Palma et al. 2002; Singh and Singh 2006; Sangeetha et al. 2012). DNA content for plants exposed to effluents 1, 2 and 3 was reduced to about 77%, 60.6% and 33%, which increased for plants grown in treated effluent. DNA content of plants decreased due to more mobilization of protein under stress condition. In the case of chlorophyll content, about 62%, 43% and 34% for chlorophyll a and 48%, 23% and 9%, respectively, for chlorophyll b were observed for the plants growing in effluents 1, 2 and 3. For both chlorophyll a and b, the value was comparable to that of the control for plants exposed to treated effluent 3.

Table 2 Oxidative stress enzymes of Euphorbia sp. plant exposed to various types of tannery effluent

| Oxidative stress enzymes | Experiment groups | Plant exposure time |
|--------------------------|-------------------|---------------------|
|                          | 24 h              | 48 h                | 72 h                |
| POD (ΔA470 g fresh weight/min) | Control        | 15.4 ± 0.2 | 14.8 ± 0.2 | 14.2 ± 0.1 |
|                          | Effluent 1 untreated | 23.8 ± 0.2 | 23.5 ± 0.1 | 22.9 ± 0.2 |
|                          | Effluent 1 treated    | 17.7 ± 0.1 | 17.2 ± 0.1 | 16.8 ± 0.1 |
|                          | Effluent 2 untreated | 19.5 ± 0.2 | 19.2 ± 0.2 | 18.8 ± 0.2 |
|                          | Effluent 2 treated    | 17.1 ± 0.1 | 16.8 ± 0.1 | 16.2 ± 0.2 |
|                          | Effluent 3 untreated | 18 ± 0.2    | 17.8 ± 0.1 | 17.2 ± 0.1 |
|                          | Effluent 3 treated    | 15.6 ± 0.2 | 15.0 ± 0.2 | 14.3 ± 0.1 |
| SOD (U/mg protein)       | Control        | 2.9 ± 0.1 | 2.6 ± 0.1 | 2.0 ± 0.1 |
|                          | Effluent 1 untreated | 9.1 ± 0.1 | 8.9 ± 0.1 | 8.6 ± 0.1 |
|                          | Effluent 1 treated    | 4.1 ± 0.1 | 3.8 ± 0.1 | 3.5 ± 0.1 |
|                          | Effluent 2 untreated | 7.9 ± 0.1 | 7.6 ± 0.1 | 7.4 ± 0.1 |
|                          | Effluent 2 treated    | 3.9 ± 0.1 | 3.6 ± 0.1 | 3.44 ± 0.1 |
|                          | Effluent 3 untreated | 6.8 ± 0.1 | 6.6 ± 0.1 | 6.3 ± 0.1 |
|                          | Effluent 3 treated    | 3.3 ± 0.1 | 3.1 ± 0.1 | 2.7 ± 0.1 |
| CAT (U/mg protein/min)   | Control        | 4.4 ± 0.2 | 3.2 ± 0.1 | 3.0 ± 0.2 |
|                          | Effluent 1 untreated | 11.3 ± 0.1 | 10.8 ± 0.1 | 9.7 ± 0.2 |
|                          | Effluent 1 treated    | 5.8 ± 0.2 | 5.1 ± 0.2 | 4.8 ± 0.2 |
|                          | Effluent 2 untreated | 9.6 ± 0.1 | 8.8 ± 0.2 | 8.3 ± 0.2 |
|                          | Effluent 2 treated    | 4.9 ± 0.1 | 4.7 ± 0.2 | 4.52 ± 0.1 |
|                          | Effluent 3 untreated | 7.7 ± 0.2 | 7.7 ± 0.2 | 6.2 ± 0.2 |
|                          | Effluent 3 treated    | 4.6 ± 0.1 | 4.1 ± 0.2 | 3.8 ± 0.1 |

Values are mean ± S.D.; *p < 0.01.*
control for plants grown in treated effluent 3. Decreased content of DNA and RNA is associated with increased activity of DNase and RNAse, respectively, in response to stress. Certain heavy metals like Cu, Cd, Pb, Hg, Cr, etc. resulted in decreased DNA and RNA content (Prasad and Strzalka 2002).

**Conclusions**

The present study was conducted to evaluate the efficiency of ceramic membranes in combination with a biosorbent prepared from waste biomass. Three different loadings of wastewater were used in the experiment to observe the versatility of the combined process. The treated
effluents showed considerable reduction of organic load- 
ing, suspended solids, total organic carbon and nitrogen 
content. About 99% reduction of heavy metals like Cr, Ni 
and Pb was achieved in the combined process. Maximum 
removal was obtained for the secondary clarifier effluent 
where most of the parameters were far below the dis-
charge limit. At an operating pressure of 1 kg/cm², the 
flux value of effluent 1 was about 9 to 15 LMH, whereas 
for effluent 2 it was about 17.9 to 21 LMH and for effluent 
3 it was about 23.5 to 27.5 LMH.

The main aim of the study was to treat wastewater to an 
extent so that it could be utilized for irrigation and agri-
cultural purpose. For this, the authors have selected 
_E. hirta_ as a model plant being found growing abundantly 
in the complex of CETP in diseased condition. The target 
was to propose a single-step treatment technology for 
onsite application to reduce the level of pollutants from 
the wastewater so that less harm is imparted to the en-
vironment. The application of treated water resulted in 
considerable reduction in the activity of various stress 
enzymes like POD, SOD and CAT compared to that of 
treated wastewater. Considerable reduction in protein 
content (approximately 42%), carbohydrate content (ap-
proximately 39%), chlorophyll a and b content (ap-
proximately 62% and 48%), DNA (approximately 77%) 
and RNA (~44%) was observed for plants grown in effluent 1. 
A similar trend was also observed for effluents 2 and 3. 
Interestingly, amino acid, protein and carbohydrate con-
tent increased to about approximately onefold in plants 
exposed to treated effluent 3. Moreover, chlorophyll con-
tent of the plant was comparable to that grown in the 
control. Therefore, it might be concluded that the combined 
technology may be used for the treatment of highly toxic 
and pollutive tannery effluent and reused for various pur-
poses including agriculture, irrigation, etc.

Competing interests

The authors declare that they have no competing interests.

Authors’ contributions

SG carried out the design of the experiments, supervised the overall 
engineering aspects of the study, took care of the microfiltration study. 
PBI prepared the biosorbent, collected effluent samples, conducted the 
treatment study. AM looked into the environmental aspects of the study wrt 
to plant enzyme activity. All the authors read and approved the final 
manuscript.

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