Ozonation of synthetic versus natural textile tannins: recalcitrance and toxicity towards Phaeodactylum tricornutum

F. Germirli Babuna*, Z. Yilmaz*, O.S. Okay**, I. Arslan Alaton* and G. Iskender*

*Istanbul Technical University, Civil Engineering Faculty, Environmental Engineering Department, 34469, Maslak, Istanbul, Turkey (E-mail: fgbabuna@ins.itu.edu.tr; arslanid@itu.edu.tr; giskender@ins.itu.edu.tr)

**Istanbul Technical University, Faculty of Naval Architecture and Ocean Engineering, 34469, Maslak, Istanbul, Turkey (E-mail: okayoy@itu.edu.tr)

Abstract A sound in-plant pollution control strategy can only be defined by paying due attention to bio-recalcitrance and toxicity. In this context the levels of toxicity and inert COD introduced to textile dyebath discharges by two alternative auxiliary chemicals, namely natural tannin (NT) and synthetic tannin (ST), were investigated. The effect of 40 minutes ozonation at 1,000 mg h$^{-1}$ at pH 3.5 on the segregated effluent streams containing the above-mentioned tannin formulations was evaluated in terms of changes in toxicity and recalcitrance. The effect of ozonation on the COD distribution of raw and ozonated NT and ST samples according to their molecular weight cut-offs was also assessed. Both untreated tannin formulations exerted high acute toxicity towards marine microalgae Phaeodactylum tricornutum. Moderate decrease in the toxicity levels of both tannins was observed upon ozonation. The raw NT formulation with a COD content more than twice that of its alternative raw ST had an initially inert soluble COD content of only 25 mg/L, while the initially inert COD was 135 mg/L for ST. As the initially inert soluble COD content of NT was considerably lower, this textile auxiliary did not need chemical pretreatment to improve its biodegradability. On the other hand, the initially inert soluble COD content of ST was reduced by 70% by ozone pretreatment. In terms of residual COD contents achievable after passing through a biological treatment system, raw NT and pretreated ST formulations yielded 100 and 95 mg/L COD, respectively. The highest proportion of COD (46% for NT and 88% for ST) was found in the < 1 kDa range. The same fraction increased to 93% for NT after ozonation, while for ST no significant change was observed in the COD distribution of the molecular weight cut-offs after ozonation.

Keywords Activated sludge inhibition; acute toxicity; chemical treatment; dye carriers; segregated effluents; textile industry

Introduction Dyes and associated auxiliaries are continually being upgraded and replaced by more superior products. There is extreme pressure on dye manufacturers to develop novel dye-stuffs that can be successfully applied using less auxiliaries (salt, heavy metals, etc.) or shift to more ecological chemicals (“ecochemicals”) so as end environmental problems arising from textile dyeing and finishing activities. A single dyeing process stage (i.e. pad-batch dyeing) can use an appreciable number of dyestuffs and assisting chemicals from different chemical classes resulting in a very complicated and hence difficult to treat wastewater (O’Neill et al., 1999). Among the xenobiotic pollutants originating from the textile preparation, dyeing and finishing processes, textile tannins have attracted great interest due to their frequent use and extremely refractory nature. Synthetic tannins and tannic acids are high-molecular-weight oligomeric compounds with multiple structured, poliphenolic moities. They are frequently used during polyamide dyeing with acid, metal-complex or direct dyes at the after-rinsing stage to increase the fixation rates and wet fastness onto the dyed fabric (e.g. nylon). In spite of their rather refractory character,
tannins provide a more environmentally acceptable alternative to heavy metal treatment of polyamide fabrics for the same purpose (Burkinshaw and Bahojb-Allafan, 2003). On the other hand, according to the current understanding of industrial pollution control, in-plant control practices covering issues such as the substitution of auxiliary chemicals by more ecological ones and application of specific treatment schemes to segregate effluent streams for either effective removal and/or degradation of a target pollutant (Dogruel et al., 2003; Schoeberl et al., 2004) are gaining importance. To improve the toxicity and/or to reduce the recalcitrance of segregated tannin-containing industrial effluents, a treatment scheme composed of either ozone (Perkowski et al., 2003; Saroj et al., 2005) or advanced oxidation processes (AOPs) alone or a combination of these followed by biological treatment can be applied.

In this context this study is devoted to presenting the outcomes of the last part of a comprehensive research conducted on the treatability of two different commercial tannin formulations, namely natural tannin (NT) and synthetic tannin (ST) used in textile dyeing and finishing mills. The investigated tannin formulations are commonly applied in the polyamide (nylon) dyeing processes to improve the acid dye fixation rate. The previous findings showed that for both tannin formulations under investigation the optimum partial pretreatment outcomes in terms of COD, TOC removal efficiencies and BOD₅/COD ratios can be obtained by applying 40 minutes of ozone at a rate of 1,000 mg/h with a pH of 3.5 (Koyunluoglu et al., 2006). However, not considering any other quality parameter apart from the mentioned conventional collective ones can lead to immature and even misleading conclusions. In other words, evaluating the results of such partial ozone pretreatment must be performed by referring to the amount of toxicity introduced into the receiving water bodies and the level of biodegradability improvement. Therefore, this experimental study outlines the effect of pre-ozonation on inert COD fractions, acute toxicities (towards a marine microalgae) and COD values of different molecular weight cut-off fractions of segregated effluent streams containing two commercial tannin formulations widely applied in textile dyeing and finishing operations.

**Materials and methods**

**Samples used**

The two commercial textile tannin formulations, i.e. a natural tannin (NT) and a synthetic tannin (ST), investigated in this study were obtained from a local textile dyeing and finishing plant located near Istanbul and used as received. Table 1 tabulates some of the physicochemical and ecotoxicological properties of the selected dye tannin preparations.

The information given in Table 1 highlights that even though both of the tannin formulations have considerably low acute oral toxicities, ST especially exerts serious fish toxicity towards the selected test organism. When the fish toxicity data and ST and NT concentrations that are most often encountered in dyehouse discharge (i.e. the initially applied tannin concentration in the dyebath multiplied by a factor of 0.250 to estimate its actual concentration in the exhausted dyebath; and a factor of 0.375 for their ultimate concentration in the total dyehouse effluent) are comparatively evaluated, it can be concluded that both tannins divulge potential toxicity (the final concentration of NT in the total dyehouse effluent is 150 mg/L; the final concentration of ST in the total dyehouse effluent is 120 mg/L) to the total effluent.

All the experimental studies were performed on tannin samples prepared to simulate batch acid dyebath discharge of the actual polyamide dyeing process by adding appropriate amounts of each tannin formulation to deionised water. NT is a formulation containing tannic acid (gallotannin) and is generally used at a concentration of 1.56 g/L in acid dyebaths (i.e. 4% of the fabric weight is usually applied and 25% remains unfixed in the
spent acid dyebath). ST is commercially called “syntan” and used at a concentration of 1.25 g/L (i.e. 4% of fabric weight is used in the dyebath of which 25% contributes to the exhausted acid dyebath). Aqueous discharge of acid dyebath containing acid dyestuffs along with either ST or NT has a typical pH of around 3.5–4.0 and a temperature of approximately 80–85°C. In the presence of tannins acid dyestuffs fixate onto polyamide fabrics with high efficiencies. Therefore tannin formulations are the main components of exhausted acid dyebaths and constitute the major organic load in acid dyebath effluent. Thus, there is practically no difference between real acid (polyamide) dyebath effluent and the aqueous tannin solutions (i.e. simulated tannin effluent samples).

As indicated in the above discussion on toxicity and the literature information on the biorecalcitrance of tannins (Burkinshaw and Bahjob-Allafan, 2003), the segregated batch acid dyebath discharges containing acid dyestuffs along with either ST or NT has a typical pH of around 3.5–4.0 and a temperature of approximately 80–85°C. In the presence of tannins acid dyestuffs fixate onto polyamide fabrics with high efficiencies. Therefore tannin formulations are the main components of exhausted acid dyebaths and constitute the major organic load in acid dyebath effluent. Thus, there is practically no difference between real acid (polyamide) dyebath effluent and the aqueous tannin solutions (i.e. simulated tannin effluent samples).

As indicated in the above discussion on toxicity and the literature information on the biorecalcitrance of tannins (Burkinshaw and Bahjob-Allafan, 2003), the segregated batch acid dyebath discharges containing acid dyestuffs along with either ST or NT has a typical pH of around 3.5–4.0 and a temperature of approximately 80–85°C. In the presence of tannins acid dyestuffs fixate onto polyamide fabrics with high efficiencies. Therefore tannin formulations are the main components of exhausted acid dyebaths and constitute the major organic load in acid dyebath effluent. Thus, there is practically no difference between real acid (polyamide) dyebath effluent and the aqueous tannin solutions (i.e. simulated tannin effluent samples).

As indicated in the above discussion on toxicity and the literature information on the biorecalcitrance of tannins (Burkinshaw and Bahjob-Allafan, 2003), the segregated batch acid dyebath discharges containing acid dyestuffs along with either ST or NT has a typical pH of around 3.5–4.0 and a temperature of approximately 80–85°C. In the presence of tannins acid dyestuffs fixate onto polyamide fabrics with high efficiencies. Therefore tannin formulations are the main components of exhausted acid dyebaths and constitute the major organic load in acid dyebath effluent. Thus, there is practically no difference between real acid (polyamide) dyebath effluent and the aqueous tannin solutions (i.e. simulated tannin effluent samples).

As indicated in the above discussion on toxicity and the literature information on the biorecalcitrance of tannins (Burkinshaw and Bahjob-Allafan, 2003), the segregated batch acid dyebath discharges containing acid dyestuffs along with either ST or NT has a typical pH of around 3.5–4.0 and a temperature of approximately 80–85°C. In the presence of tannins acid dyestuffs fixate onto polyamide fabrics with high efficiencies. Therefore tannin formulations are the main components of exhausted acid dyebaths and constitute the major organic load in acid dyebath effluent. Thus, there is practically no difference between real acid (polyamide) dyebath effluent and the aqueous tannin solutions (i.e. simulated tannin effluent samples).

As indicated in the above discussion on toxicity and the literature information on the biorecalcitrance of tannins (Burkinshaw and Bahjob-Allafan, 2003), the segregated batch acid dyebath discharges containing acid dyestuffs along with either ST or NT has a typical pH of around 3.5–4.0 and a temperature of approximately 80–85°C. In the presence of tannins acid dyestuffs fixate onto polyamide fabrics with high efficiencies. Therefore tannin formulations are the main components of exhausted acid dyebaths and constitute the major organic load in acid dyebath effluent. Thus, there is practically no difference between real acid (polyamide) dyebath effluent and the aqueous tannin solutions (i.e. simulated tannin effluent samples).

As indicated in the above discussion on toxicity and the literature information on the biorecalcitrance of tannins (Burkinshaw and Bahjob-Allafan, 2003), the segregated batch acid dyebath discharges containing acid dyestuffs along with either ST or NT has a typical pH of around 3.5–4.0 and a temperature of approximately 80–85°C. In the presence of tannins acid dyestuffs fixate onto polyamide fabrics with high efficiencies. Therefore tannin formulations are the main components of exhausted acid dyebaths and constitute the major organic load in acid dyebath effluent. Thus, there is practically no difference between real acid (polyamide) dyebath effluent and the aqueous tannin solutions (i.e. simulated tannin effluent samples).

As indicated in the above discussion on toxicity and the literature information on the biorecalcitrance of tannins (Burkinshaw and Bahjob-Allafan, 2003), the segregated batch acid dyebath discharges containing acid dyestuffs along with either ST or NT has a typical pH of around 3.5–4.0 and a temperature of approximately 80–85°C. In the presence of tannins acid dyestuffs fixate onto polyamide fabrics with high efficiencies. Therefore tannin formulations are the main components of exhausted acid dyebaths and constitute the major organic load in acid dyebath effluent. Thus, there is practically no difference between real acid (polyamide) dyebath effluent and the aqueous tannin solutions (i.e. simulated tannin effluent samples).

As indicated in the above discussion on toxicity and the literature information on the biorecalcitrance of tannins (Burkinshaw and Bahjob-Allafan, 2003), the segregated batch acid dyebath discharges containing acid dyestuffs along with either ST or NT has a typical pH of around 3.5–4.0 and a temperature of approximately 80–85°C. In the presence of tannins acid dyestuffs fixate onto polyamide fabrics with high efficiencies. Therefore tannin formulations are the main components of exhausted acid dyebaths and constitute the major organic load in acid dyebath effluent. Thus, there is practically no difference between real acid (polyamide) dyebath effluent and the aqueous tannin solutions (i.e. simulated tannin effluent samples).

As indicated in the above discussion on toxicity and the literature information on the biorecalcitrance of tannins (Burkinshaw and Bahjob-Allafan, 2003), the segregated batch acid dyebath discharges containing acid dyestuffs along with either ST or NT has a typical pH of around 3.5–4.0 and a temperature of approximately 80–85°C. In the presence of tannins acid dyestuffs fixate onto polyamide fabrics with high efficiencies. Therefore tannin formulations are the main components of exhausted acid dyebaths and constitute the major organic load in acid dyebath effluent. Thus, there is practically no difference between real acid (polyamide) dyebath effluent and the aqueous tannin solutions (i.e. simulated tannin effluent samples).

As indicated in the above discussion on toxicity and the literature information on the biorecalcitrance of tannins (Burkinshaw and Bahjob-Allafan, 2003), the segregated batch acid dyebath discharges containing acid dyestuffs along with either ST or NT has a typical pH of around 3.5–4.0 and a temperature of approximately 80–85°C. In the presence of tannins acid dyestuffs fixate onto polyamide fabrics with high efficiencies. Therefore tannin formulations are the main components of exhausted acid dyebaths and constitute the major organic load in acid dyebath effluent. Thus, there is practically no difference between real acid (polyamide) dyebath effluent and the aqueous tannin solutions (i.e. simulated tannin effluent samples).

As indicated in the above discussion on toxicity and the literature information on the biorecalcitrance of tannins (Burkinshaw and Bahjob-Allafan, 2003), the segregated batch acid dyebath discharges containing acid dyestuffs along with either ST or NT has a typical pH of around 3.5–4.0 and a temperature of approximately 80–85°C. In the presence of tannins acid dyestuffs fixate onto polyamide fabrics with high efficiencies. Therefore tannin formulations are the main components of exhausted acid dyebaths and constitute the major organic load in acid dyebath effluent. Thus, there is practically no difference between real acid (polyamide) dyebath effluent and the aqueous tannin solutions (i.e. simulated tannin effluent samples).

Analytical procedures
Apart from COD, all analyses were performed as defined in Standard Methods (APHA, AWWWA and WPCF, 1989). COD measurements were accomplished by ISO 6060 method (ISO, 1986). Sample filtrates previously subjected to vacuum filtration by means of 0.45 μm cut-off Millipore membrane filters were defined as soluble COD fractions. All experiments and analyses were conducted at room temperature and in duplicate.
Algal bioassays

The principle of the adopted batch algal bioassays is based on the US EPA bottle test (Miller et al., 1978). The toxicity tests performed on raw and ozonated NT and ST samples were performed in accordance with the method described by Okay et al. (2002), using the marine microalgae Phaeodactylum tricornutum. Apart from being the primary food source for many aquatic organisms and its common use in toxicity testing (Clarkson et al., 1999; Pavlic et al., 2005), this species was especially selected as the industrial wastewaters are mainly discharged into a marine environment in Istanbul.

The toxicity test was conducted at a constant temperature of 20°C and light irradiation at 3,500–4,000 Lux. Diluted solutions of raw (in filtered sea water) and ozonated, natural and synthetic tannin solutions were incubated together with algal species. Thirteen g/L NaCl was added to the ozonated tannin samples in order to supply the desired salinity concentration for the survival of the marine algae. Cultures were maintained in standard algal growth media of f/2 (Guillard, 1972). A starting concentration of 10,000 cells/mL was added and the production rate was monitored by counting the cells with Coulter Counter (Beckman Z2) for a period of 96 hours. The results were evaluated by comparing the control cultures of corresponding series for raw and ozonated samples. The flasks were repositioned daily within the experimental space to minimise possible spatial differences in illumination and temperature on growth.

Inert COD experiments

The inert COD content of both raw and ozonated NT and ST formulations was assessed according to an experimental procedure given in the literature (Germirli et al., 1993). The method involves running two aerated cylindrical batch reactors, one fed with the sample itself, and the other with glucose solution having the same dilution, of 3 L volumetric capacity. The feed was obtained from a laboratory-scale fill and draw aerobic reactor operated under steady state with a food to microorganism ratio of 0.7 mg COD (mg VSS. day)^{-1} for about 40 days. A mixture of 50% v/v glucose and 50% v/v raw (or ozonated) sample was used as the feed for this fill-and-draw aerobic reactor. An initial biomass concentration of 50 mg/L VSS was sustained in both of the batch reactors (run with sample and with glucose) where inert COD tests were performed. Aliquots removed periodically from the mixed liquor of each reactor were analysed for soluble COD. To avoid erroneous results, a strict account was kept of all samples removed from the reactors, so that any water loss by evaporation was replaced with distilled water prior to sampling. Experiments were continued and data were collected until the observation of a stable soluble COD plateau where the threshold level was established as less than 3–5% variation between consecutive samples. In the batch tests the samples were adjusted to a pH of 7–8, a range suitable for biological activity. Nitrification inhibitor (Formula 2533TM, Hach Company) was added to all bioreactors in order to prevent any possible induced interference.

COD-based molecular size fractionation

Raw and 40 minute ozonated (applied ozone dose = 1,000 mg/h, initial ozonation pH = 3.5) NT and ST formulations were subjected to mass distribution experiments by the use of vacuum and molecular filtration. First, a vacuum filtration by glass fibre and membrane filter papers and afterwards molecular filtration were applied in a Millipore stirred ultrafiltration cell. The ultrafiltration cell operating under positive pressure (0.4–2.5 bar; N2 as the inert gas) has a volumetric capacity of 400 mL (Amicon, Model 8400). In ultrafiltration experiments, the samples were filtered on membrane discs that have molecular weight cut-off values ranging from 100 to 1 kDa (PL series, Millipore).
Sample aliquots collected after each filtration step were subsequently subjected to COD measurements.

Results and discussion

Effect of ozone application on inert COD content

Unlike the outdated approach that mainly uses the increase of BOD₅ alone or together with BOD₅/COD ratio as a tool to show the improved biodegradability of an effluent, current understanding of environmental biotechnology concentrates on the concept of “biochemically inert” (recalcitrant) COD content of discharges either initially present in the effluent itself or generated during the course of biochemical reactions. Thus, the initially inert soluble COD content, SI, and soluble residual microbial products, SP, (both expressed as mg/L COD) are the key parameters to be addressed while evaluating the biodegradability of such effluents. Initially, inert soluble COD and soluble residual metabolic products together compose the outlet COD of a well-operated biological treatment plant as all of the particulate components of discharges will leave the biological treatment plant by sludge disposal. The results devoted to the assessment of SI and SP in raw and ozonated NT and ST are presented in Table 2. It should be noted that CT indicates the COD concentration of the samples.

By only checking the COD contents (CT) of the raw and ozonated tannin formulations given in Table 2, it can be concluded that application of ST as an auxiliary chemical must be preferred as ST introduced less COD in to the segregated dyebath discharge. It is noteworthy that even subjecting the effluent of NT to ozone was not adequate to improve the related discharge quality to a level associated with untreated ST. Owing to very limited COD removal efficiency obtained with ozone it is evident that other treatment alternatives must be examined for NT.

The delusive nature of the previous immature conclusion that addressed ST as the preferable auxiliary was apparent from the presented data both on initially inert soluble COD (SI) and residual COD (SR). Although NT formulation exerted a COD contribution more than two-fold of the one related to ST, only 2% of this organic input yielding solely 25 mg/L COD can be considered as initially bio-recalcitrant. Obviously the segregated effluent containing NT formulation did not require any pretreatment to improve its biodegradability. In the case of ST a relatively high initially inert soluble COD of 135 mg/L

<table>
<thead>
<tr>
<th>Test Substance</th>
<th>CT (mg/l)</th>
<th>Initially inert soluble COD SI (mg/l)</th>
<th>Residual COD SR (mg/l)</th>
<th>SI/CT (%)</th>
<th>SR/CT (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NT</td>
<td>1,100</td>
<td>25</td>
<td>100</td>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td>ST</td>
<td>465</td>
<td>135</td>
<td>190</td>
<td>29</td>
<td>41</td>
</tr>
<tr>
<td>Pretreated* NT</td>
<td>925</td>
<td>10</td>
<td>40</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Pretreated* ST</td>
<td>280</td>
<td>40</td>
<td>95</td>
<td>14</td>
<td>34</td>
</tr>
</tbody>
</table>

*By applying 40 minute ozonation at a dose of 1,000 mg/h and an initial pH of 3.5

Results and discussion

Effect of ozone application on inert COD content

Unlike the outdated approach that mainly uses the increase of BOD₅ alone or together with BOD₅/COD ratio as a tool to show the improved biodegradability of an effluent, current understanding of environmental biotechnology concentrates on the concept of “biochemically inert” (recalcitrant) COD content of discharges either initially present in the effluent itself or generated during the course of biochemical reactions. Thus, the initially inert soluble COD content, SI, and soluble residual microbial products, SP, (both expressed as mg/L COD) are the key parameters to be addressed while evaluating the biodegradability of such effluents. Initially, inert soluble COD and soluble residual metabolic products together compose the outlet COD of a well-operated biological treatment plant as all of the particulate components of discharges will leave the biological treatment plant by sludge disposal. The results devoted to the assessment of SI and SP in raw and ozonated NT and ST are presented in Table 2. It should be noted that CT indicates the COD concentration of the samples.

By only checking the COD contents (CT) of the raw and ozonated tannin formulations given in Table 2, it can be concluded that application of ST as an auxiliary chemical must be preferred as ST introduced less COD in to the segregated dyebath discharge. It is noteworthy that even subjecting the effluent of NT to ozone was not adequate to improve the related discharge quality to a level associated with untreated ST. Owing to very limited COD removal efficiency obtained with ozone it is evident that other treatment alternatives must be examined for NT.

The delusive nature of the previous immature conclusion that addressed ST as the preferable auxiliary was apparent from the presented data both on initially inert soluble COD (SI) and residual COD (SR). Although NT formulation exerted a COD contribution more than two-fold of the one related to ST, only 2% of this organic input yielding solely 25 mg/L COD can be considered as initially bio-recalcitrant. Obviously the segregated effluent containing NT formulation did not require any pretreatment to improve its biodegradability. In the case of ST a relatively high initially inert soluble COD of 135 mg/L

<table>
<thead>
<tr>
<th>Tested chemical</th>
<th>EC₅₀</th>
<th>EC₅₀</th>
<th>EC₅₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>NT</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>ST</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Pretreated** ST</td>
<td>2</td>
<td>13</td>
<td>34</td>
</tr>
<tr>
<td>Pretreated** NT</td>
<td>3</td>
<td>6</td>
<td>28</td>
</tr>
</tbody>
</table>

*By applying 40 minute ozonation at a dose of 1,000 mg/h and an initial pH of 3.5

Table 2 Inert COD fractions (mg/L)

Table 3 Percent volumetric EC values obtained for original and pretreated ST and NT after seven days exposure to Phaeodactylum tricornutum
that necessitates pretreatment was encountered. An initially inert COD removal of 70% was monitored by applying ozone to ST. When both of the tannin formulations were comparatively evaluated, in terms of the lowest achievable effluent COD levels after bio-treatment, NT was observed to yield a residual COD of 100 mg/L, of which 25 mg/L comes from initially inert soluble COD (S_I) and the rest from inert metabolic products (S_P). The inert COD test conducted on the pretreated ST sample showed that the initially inert soluble COD content of 40 mg/L could be increased to 95 mg/L due to soluble microbial product generation.

From all the evaluation presented on inert COD data it can be inferred that the outcomes of a feasibility study comparing the usage of NT without applying any pretreatment to the segregated effluent, versus employing ST by subjecting the segregated discharges to ozone pretreatment, must be adopted.

**Acute toxicity tests**

The results related to the acute toxicity (in % v/v EC values) of raw and ozonated NT and ST samples are tabulated in Table 3. The effective dilutions causing 20, 50 and 80%
inhibition of algal growth showed that both of the original tannin samples exerted high toxicity towards *Phaeodactylum tricornutum* indicating the necessity of a suitable pretreatment. The literature data obtained on the same tannin formulations dealing with inhibition of activated sludge culture stated that although ST exhibited more toxicity than NT, the inhibitory effect of ST was completely eliminated by applying 40 minute ozonation at the same experimental conditions (Koyunluoglu *et al.*, 2006). However the same source pointed out that no significant changes in activated sludge inhibition were observed for NT after applying ozonation. According to the outcomes of the present study moderate improvements in terms of toxicity exerted by NT and ST samples towards *Phaeodactylum tricornutum* were obtained after ozonation.

The effect of ozonation on the COD distribution of different molecular weight cut-offs

Ozonation is known to cleave polyaromatic, complex molecules to phenols and ultimately to carboxylic acids. The distribution of COD for raw and ozonated (for 40 minute at a dose of 1,000 mg/h and an initial pH of 3.5) NT and ST samples in terms of molecular weight cut-offs was also investigated. For this purpose, different COD fractions were determined for both tannin formulations. The COD values of different molecular weight cut-offs are presented in Figure 1.

As shown in Figure 1, a rather fluctuating COD distribution was obtained for the original NT formulation known to have a higher molecular weight and more complex structure when compared with ST. The data on COD distribution revealed that 46% of the COD originated from the <1 kDa range for the untreated NT sample. After exposure to ozonation the COD content of the same fraction was elevated to 93% indicating fragmentation of the originally higher molecular weight chemicals to low molecular weight partially degraded substances. The original ST solution had its highest COD (89%) at the molecular cut-off of <1 kDa due to its relatively low molecular weight as compared to NT. Hence, as expected, no significant changes were observed on its COD in terms of molecular weight fractionation after being subjected to ozonation.

Conclusions

Textile dyeing and finishing mills usually adopt a batch mode of production that in turn generates intermittent discharges containing various chemicals. Through the application of in-plant control practices such as the substitution of auxiliary chemicals by more ecological ones (for instance replacing conventional and synthetic carriers and tannins with ecological or natural ones, respectively), practising a specific treatment scheme on segregated effluent streams, etc.; to the textile industry many environmental and financial benefits can be obtained. On the other hand, it is necessary to attribute special emphasis to biorecalcitrance and toxicity while defining a sound in-plant pollution control strategy among various alternatives. This study was devoted to examining two alternative dye auxiliary chemicals used commonly in textile dyeing operations, namely, natural tannin (NT) and synthetic tannin (ST), in terms of their toxicity towards *Phaeodactylum tricornutum* and inert COD levels. Both tannins exerted high toxicities towards *Phaeodactylum tricornutum* and their toxicity could not be reduced significantly after ozonation at moderate doses. The inert COD content of the natural tannin appeared to be already quite low. Hence without any partial chemical pretreatment, biological treatment could directly be applied to natural tannin. From another point of view the relatively high inert COD of the synthetic tannin could be removed by 70% upon ozonation. Thus, the same ultimate COD level can be achieved after passing the natural tannin and ozonated synthetic tannin through a biological treatment system. Ozonation resulted in a shift of the molecular size distribution revealing that the main function of ozonation on the natural tannin
formulation is to rupture the relatively high molecular weight aromatic groupings of this dye auxiliary chemical. However, respectively high complete mineralisation, reflecting itself with a higher COD removal efficiency, can be considered as the main outcome of the ozonation applied on synthetic tannin.

Acknowledgements
The authors wish to thank Dr Izzet Alaton for supplying the tannin samples. This study was conducted as part of research activities of The Environmental Biotechnology Centre of The Scientific and Technical Research Council of Turkey. It was jointly supported by The Scientific and Technical Research Council of Turkey within the scope of COST Action Nr. 628 and The Research and Development Fund of Istanbul Technical University.

References