Enhancement in mineralization of some natural refractory organic compounds by ozonation–aerobic biodegradation

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Abstract: Two schemes, the first involving ozonation followed by final aerobic biodegradation (phase I experiments), and the second involving initial aerobic biodegradation, followed by ozonation and subsequent final aerobic biodegradation (phase II experiments), were examined for enhanced mineralization of refractory model compounds, viz. gallic acid, tannin and lignin. In all cases, and irrespective of the applied scheme, chemical oxygen demand (COD), total organic carbon (TOC), COD/TOC ratio, and specific UV absorbance at 280 nm attributed to the model compounds decreased with application of increasing ozone dose. The residual organic matter remaining after ozonation exhibited enhanced aerobic biodegradability in all cases. Further, in all cases and irrespective of the applied scheme, the overall amount of COD and TOC removed through the combination of ozonation and biodegradation processes increased with increase in ozone dose for all three model compounds, and more than 90% COD removal could be achieved with an ozone dose of 3 mg ozone absorbed per mg initial TOC, as compared with approximately 40% COD removal when no ozone was applied. Treatment by the first scheme resulted in the fraction of starting COD removed through biodegradation decreasing with increase in ozone dose in all cases, while this fraction increased or remained constant during treatment using the second scheme. In the case of tannin and lignin, similar overall COD removal could be achieved at lower ozone doses using scheme II. Due to incorporation of the initial aerobic biodegradation step in scheme II, the ozone requirement for additional mineralization, ie mineralization over and above that achieved by aerobic biodegradation, was also lower than that in scheme I.

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Keywords: gallic acid; tannin; lignin; ozonation; biodegradation

INTRODUCTION

Wastewaters from various industries, eg distillery, tannery, pulp and paper, textile, agro-processing, etc. may contain difficult-to-degrade synthetic compounds or macro bio-molecules of natural origin such as lignin and tannin. Integrated advanced oxidation–biological systems appear to be feasible for treating recalcitrant compounds present in such complex wastewaters.1 Kaindl et al2 reported that with respect to refractory compounds in wastewater, ozone treatment with subsequent bio-filtration can provide a considerable reduction in chemical oxygen demand (COD) and it must be used as the last step in a multi-stage treatment due to the higher specific costs of ozonation compared with biological degradation. Improvement in biodegradability caused by ozonation in such cases can be attributed to removal of the bio-inhibitory characteristics of recalcitrant compounds due to changes in the molecular structure on ozonation.3 Balakrishnan et al4 reported that application of ozone as a pre-treatment to usual biological treatment enhances biodegradability because ozone degrades higher molecular weight organic molecules into lower molecular weight molecules, which even simpler forms of microorganisms can easily digest.

Several researchers have reported enhancement of biodegradability of solutions of specific recalcitrant organic compound on ozonation. These include studies carried out with naphthalene sulfonic acid,5 pentachlorophenol,6,7 chloro- and nitro-phenols,8 pyrene,9 and non-biodegradable substituted aromatic hydrocarbons.10 Many studies have also been reported concerning application of integrated advanced oxidation–biodegradation processes for enhancement of biodegradability of wastewaters from specific industries containing refractory organic compounds. Yeber et al11 reported the degradation of a pulp mill effluent by several advanced oxidation systems such as O3/UV, O3/UV/TiO2, O3/UV/ZnO, O2/UV, O2/UV/TiO2. The combination of O3/UV showed the highest
increase in biodegradability. Helble et al\textsuperscript{12} concluded that a combination of ozone with a fixed bed biofilm reactor is one of the most efficient treatments for elimination of COD, color, and Absorbable Organic Halides (AOX) of waste from the pulp and paper industry. Chemical oxidation with ozone is the preferred process for the treatment of dyes in textile wastewaters in order to achieve high color removal efficiencies and to improve the biodegradability without the production of sludge.\textsuperscript{13} Karahan et al\textsuperscript{14} also showed that ozone applied prior to biological processes is very effective for color removal from soluble dyes in textile wastewaters. Chemical oxidation using ozone or other advanced oxidation technologies resulted in the reduction of COD and poly-phenol content and improved the biodegradability of wastewaters from the processing of table olives.\textsuperscript{15–17} Beltran et al\textsuperscript{18} observed that biological treatment of distillery spent-wash presented problems due to the presence of refractory compounds such as condensed ‘poly-phenols’ with tannin and lignin-like structures, which are toxic to microorganisms in high concentrations. It was concluded that since such ‘poly-phenols’ are aromatic compounds, and thus prone to attack by electrophilic agents such as ozone, ozonation seems to be a suitable option for increasing the biodegradability of such wastewaters. Subsequent studies\textsuperscript{19,20} have shown that limited and controlled oxidation of organic carbon in distillery wastewaters by ozonation results in the modification of persistent organic compounds into biodegradable fractions, resulting in considerable enhancement of biodegradability.

Other studies which relate ozonation with improvement in subsequent biodegradability include a reported increase in anaerobic biodegradability of domestic sewage sludge through ozonation,\textsuperscript{21} reports regarding ozone treatment being extremely efficient for conversion of dissolved organic carbon (DOC) to biodegradable dissolved organic carbon (BDOC) during treatment of drinking water,\textsuperscript{22} reported increase in biodegradability with the reduction in apparent molar size of dissolved organic matter (DOM) on ozonation, which suggests the connection of the breakdown of DOM and improved biodegradation,\textsuperscript{23} and reports that ozonation products of hydrophobic organic molecules are often more polar and more bio-available than the original contaminants.\textsuperscript{10} Taken in totality, these observations seem to indicate that after ozonation, wastewaters containing refractory compounds may be further mineralized through a subsequent biodegradation step.

Additional support for enhanced biodegradation of refractory compounds on ozonation may be found in an article by Boethling and Sabijic,\textsuperscript{24} which suggested that the approximate order in which various functional groups in an organic matrix may be viewed as contributing to aerobic biodegradability is as follows, hydrolyzable group (ester, amide, anhydride) > hydroxyl > carboxylic acid group, sites of un-saturation > benzene ring, methyl group, or methylene groups. The destruction of aromatic rings and unsaturated sites in organic molecules, and the resultant formation of acidic and alcoholic groups by ozonation may thus suggest an enhancement in biodegradability. It was further inferred\textsuperscript{25} that compounds that are already partially oxidized are generally considered to be more easily attacked by microorganisms than those that are not, and all other things being equal, hydrolyzable molecules are considered to be the most readily attacked. Also, molecular mass, alkyl branching, halogenation, and nitrogen hetero-cycles are viewed as negative factors in biodegradability.\textsuperscript{25}

Based on the literature review presented here it is clear that, generally, ozonation of wastewaters containing recalcitrant organic compounds results in enhancement of subsequent biodegradability. However, considering the higher specific costs of ozonation compared with biological degradation, ozone must be used in a controlled and efficient manner for enhancement of biodegradability. This is only possible when data regarding most efficient treatment configurations for ozone application and corresponding ozone requirements for enhancement of mineralization of refractory organic matter are available. This study is a continuation of a related study\textsuperscript{26} on the mineralization of three refractory organic compounds of natural origin, viz. tannin, lignin and gallic acid by (1) ozonation only, and (2) initial biodegradation followed by ozonation. The objective of the present study was to determine the extent of mineralization of the above compounds by (1) ozonation–final biodegradation, and (2) initial biodegradation–ozonation–final biodegradation. In both cases, the effect of variable ozone dose on the extent of mineralization was also examined.

**MATERIALS AND METHODS**

**Model compounds**

The three model compounds used were, gallic acid, AR Grade (3,4,5-trihydroxybenzoic acid; molecular formula: C\textsubscript{6}H\textsubscript{6}O\textsubscript{5}; molecular weight: 170 g; minimum assay: 98.0%); supplier: Fine-Chem. Ltd, Mumbai, India); tannin, AR Grade (gallotannic acid; molecular formula: C\textsubscript{76}H\textsubscript{52}O\textsubscript{46}; molecular weight: 1701.23 g; minimum assay: 90.0%); supplier: Fine-Chem. Ltd); and lignin, LR Grade (supplier: National Chemicals, Mumbai, India). The carbon, hydrogen, nitrogen and oxygen content of these compounds were estimated using an elemental analyzer and are presented elsewhere.\textsuperscript{26}

**Experimental protocol**

The experiments were carried out in two phases. Phase I experiments involved ozonation of aliquots of pure model compounds, ie gallic acid, tannin and lignin at various ozone doses, followed by final aerobic biodegradation. Phase II experiments consisted of an initial biodegradation step for each model compound, followed by ozonation of the effluents from the aerobic bioreactors at various ozone doses, followed...
by a final biodegradation step. The parameters monitored in both cases were ozone consumption, specific UV absorbance at 280 nm, COD and total organic carbon (TOC).

**Initial aerobic biodegradation**

Reagent bottles of 1000 cm$^3$ capacity were used as aerobic reactors for initial aerobic biodegradation experiments. An air compressor (Model: Comair NF264, New Delhi, India) was employed for a continuous supply of air to the reactors through an aquarium diffuser. All reactors were kept at temperatures between 30 and 35°C, and operated at a hydraulic retention time of 2 days. Three aerobic reactors were operated, with pure gallic acid, tannin and lignin, prepared in Biochemical Oxygen Demand (BOD) dilution water (as described in Method No 5210 B, in Ref. 27), as feed. All reactors were initially started with sewage from the oxidation pond at IIT Kanpur, and operated in semi-batch mode. Continuous and sufficient air bubbling was provided in order to keep the microbial population in suspension, and to ensure that oxygen was not rate-limiting in the reactors. Aeration was stopped for 30 min each day, and biomass in the reactor was settled. Five hundred cm$^3$ of the supernatant was then extracted from each reactor and replaced with an equal volume of the desired feed. This ensured negligible washout of biomass and a very high Biological Solids Retention Time ($\theta_c$) in the reactor. For the first 15 days, reactors were fed only with sewage to ensure rapid biomass growth. From the 16th day onwards, 25% of the COD influent to the reactor was replaced with model compound. This proportion was increased to 50, 75 and 100% from the 21st, 26th and 31st days onwards. From the 31st day onwards, feed solutions of model compounds were prepared in BOD dilution water, which contained essential macro- and micro-nutrients required for biological growth. This was required because pure gallic acid, tannin or lignin was deficient in these nutrients. Effluent COD from each reactor was monitored until the achievement of steady state, followed by gradual stepwise increase in influent COD concentration to the desired value. Reactors were operated at these final influent COD concentrations for more than two months and the steady state condition was assumed on attainment of constant effluent COD values (within ±5%) over an extended period of time. Effluents collected from the reactors were steam-sterilized and stored at 4°C for subsequent final biodegradation experiments.

**Ozonation procedure**

The experimental set-up used for ozonation consisted of a bubble contactor operated in semi-batch mode with arrangements for continuous measurement of gaseous ozone concentration influent and effluent from the reactor (see Saroj et al26 for details). For phase I experiments, aliquots of the model compounds, ie gallic acid, tannin and lignin, were prepared by adding model compounds in BOD dilution water, buffered by addition of orthophosphate to pH 7, and ozonated for various time periods. For phase II experiments, model compounds prepared as before were first aerobically biodegraded through an initial biodegradation step, before ozonation at various doses. Detailed description of the ozonation procedure may be found elsewhere (Saroj et al26). Influent and effluent gaseous ozone concentrations to the reactor were recorded at 5 min intervals during ozonation for determination of adsorbed ozone dose. Ozonated aliquots were stored at 4°C for subsequent final biodegradation experiments.

**Preparation of acclimatized seed for final aerobic biodegradation**

Preparation of acclimatized seed for final aerobic biodegradation experiments was carried out in aerobic reactors similar to those used for the initial biodegradation experiments described earlier, and operated under similar conditions. Three aerobic reactors were operated, with ozonated gallic acid, tannin and lignin (starting COD 1000 mg dm$^{-3}$, ozonated for 60 min, with influent gaseous ozone concentration of 50 mg dm$^{-3}$) as feed. The operating procedure for the reactor was also similar to that described earlier for initial aerobic biodegradation experiments. Settled sludge from these reactors was used as seed for final biodegradation experiments.

**Final aerobic biodegradation**

Aerobic reactors used for final aerobic biodegradation in both phase I and phase II experiments were fabricated from glass columns of 130 cm$^3$ capacity, equipped with a porous bottom plate made of sintered glass (see Fig 1). Oxygen was supplied to each reactor through a manifold system connected to the air compressor (model: Comair NF264). Twenty-five
Table 1. Summarized details of ozonation (phase I) and initial biodegradation–ozonation (phase II) experiments involving gallic acid, tannin and lignin

<table>
<thead>
<tr>
<th>Type</th>
<th>Starting COD (TOC), mg dm(^{-3})</th>
<th>Initial COD (TOC), mg dm(^{-3}) (after initial aerobic biodegradation)</th>
<th>Ozone dose, mg ozone absorbed per mg initial TOC</th>
<th>Ozone absorbed, mg dm(^{-3}) COD (TOC) after ozonation, mg dm(^{-3})</th>
<th>Dilution factor (dilution before final aerobic biodegradation)</th>
</tr>
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<tbody>
<tr>
<td>Gallic acid, phase I</td>
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<td>3013 (1234)</td>
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<td>0</td>
<td>3013 (1234)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.82</td>
<td>1011</td>
<td>2060 (985)</td>
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<td></td>
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<td>1.63</td>
<td>2017</td>
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<td></td>
<td></td>
<td>2.45</td>
<td>3023</td>
<td>1240 (884)</td>
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<td></td>
<td></td>
<td>3.19</td>
<td>3931</td>
<td>914 (862)</td>
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<td>1006</td>
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<td>1512</td>
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<td></td>
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<td>2.58</td>
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<td></td>
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<td>1058</td>
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<td>2076</td>
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<td></td>
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<td>4.83</td>
<td>2996</td>
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<td>0</td>
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<td>329</td>
<td>983 (381)</td>
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<td>1.48</td>
<td>653</td>
<td>729 (305)</td>
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<tr>
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<td></td>
<td></td>
<td>2.18</td>
<td>958</td>
<td>593 (268)</td>
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Reactors of this type (for details, see Table 1) were kept in a wooden box and operated simultaneously. One hundred cm\(^3\) of feed material, ie ozonated and diluted aliquots of the model compounds (see Table 1 for details), was initially taken in these reactors. Five cm\(^3\) of acclimatized seed of the appropriate type was then added to each of these reactors and aeration started. The reactors were operated in semi-continuous mode, with a hydraulic retention time (HRT) of 2.5 days. During daily feeding of the reactors, the air supply to the reactors was stopped and the sludge accumulated in the reactor was allowed to settle for 30 min before withdrawal of 40 cm\(^3\) of supernatant through the glass tube at the top by using a syringe, and addition of an equal volume of feed. The biological solids retention time in the reactors was very high since no sludge was wasted except for any inadvertent loss when settled effluent was withdrawn each day. Influent and effluent COD values to all reactors were regularly monitored. Steady state condition was assumed on attainment of constant effluent COD values (within ±5%) over an extended period of time. Effluent TOC and BOD values were measured after attainment of steady state.

**Analytical methods**

pH was measured using a combination pH electrode (Toshiwal CL-51, New Delhi, India) connected to a digital pH meter (Toshiwal CL-54). Gaseous ozone was directly measured using an UV absorbance based ozone-monitoring device (ANEROS, Ozomat GM-6000-OEM, Tubingen, Germany). The COD of the samples was analyzed in triplicate by using a closed reflux method as described in Ref. 27 (Method No 5220 C), and the average value reported. For TOC determination, samples were analyzed in triplicate using a total organic carbon analyzer (Model TOC-VCPPN, Shimadzu, Tokyo, Japan), and the average values reported. BOD measurements were carried out in duplicate using an automatic BOD measurement apparatus (Hach BODTrak, Loveland, Colorado, USA). Absorbance measurements were carried out in duplicate using a UV-visible spectrophotometer (Varian CARY 50 Conc, Palo Alto, California, USA), and 1 cm quartz cells and the average values reported. The samples were suitably diluted when absorbance values of samples were greater than one. The repeatability of all analytical procedures mentioned above was within ±10%.

**RESULTS AND DISCUSSION**

Phase I experiments involved ozonation of the model compounds at various ozone doses followed by final aerobic biodegradation. The starting COD and TOC values of the model compound aliquots, absorbed...
ozone doses and the corresponding quantity of ozone absorbed in each case are shown in Table 1. Ozonation resulted in a decline in the COD and TOC values of all aliquots. Such decline is in general agreement with observations of previous researchers.\textsuperscript{17,18,26} The COD and TOC values of various aliquots after ozonation are also shown in the same table. Phase II experiments involved initial aerobic biodegradation, followed by ozonation and final aerobic biodegradation. The starting COD values of the aliquots used for phase II experiments are shown in Table 1. Initial aerobic biodegradation of these aliquots resulted in a decline in COD and TOC values. The amount of COD degradation observed is in agreement with values reported by Bhat \textit{et al.}\textsuperscript{28} who studied COD removal from tannin solutions through biodegradation using acclimatized microorganisms. The COD and TOC values after initial biodegradation, which are referred to as the initial COD and TOC values, are also shown in the same table. Ozonation details of the aliquots after initial aerobic biodegradation, and the resulting decline in COD and TOC in all cases are also presented in Table 1. In both phase I and phase II experiments, ozonated aliquots were diluted before final aerobic biodegradation experiments as per the details presented in Table 1. The measured COD and TOC values after dilution were ±10% of the expected values.

**Final aerobic biodegradation: phase I experiments**

Aliquots of the model compounds, after ozonation at various ozone doses, were diluted and used as feed in aerobic reactors used for phase I final biodegradation experiments. The average influent and average steady-state effluent COD from such reactors are shown as a function of the absorbed ozone dose in Fig 2(A) (for gallic acid), Fig 2(B) (for tannin) and Fig 2(C) (for lignin). Corresponding TOC values are presented in Fig 2(D) (for gallic acid), Fig 2(E) (for tannin) and Fig 2(F) (for lignin). The percentage of influent COD or TOC that was removed through final aerobic biodegradation is also shown in each figure as a function of the absorbed ozone dose. The results (Fig 2(A–C)) indicate that for all model compounds, COD removal increased progressively with ozone dose, from less than 45% in the unozonated case to 70% or more at the higher ozone doses. A similar trend was also observed for TOC removal (Fig 2(D–F)). These results prove that ozonation of gallic acid, tannin and lignin result in enhancement of subsequent aerobic biodegradability, resulting in progressively enhanced COD and TOC removals with increase in ozone dose. Other researchers, based on studies involving post-ozonation biodegradation of several specific refractory organic compounds\textsuperscript{5–10} and wastewaters containing refractory organic compounds,\textsuperscript{11–20} have reported similar enhancement in biodegradability.

COD/TOC ratios measured prior to final aerobic biodegradation show a rapidly decreasing trend with increasing ozone dose for gallic acid (Fig 3(A)) and tannin (Fig 3(B)), while a similar decline is noticed only at higher ozone doses in the case of lignin (Fig 3(C)). The decline in COD/TOC ratio with an increase in the ozone dose probably indicates partial oxidation of organic matter remaining after ozonation.\textsuperscript{23,26} This partially oxidized organic matter is presumably more amenable to aerobic biodegradation, thus accounting for the observed enhancement of aerobic biodegradability.\textsuperscript{25} Moreover, at all ozone doses the observed COD/TOC ratio increased after final aerobic biodegradation for gallic acid (Fig 3(A)) and tannin (Fig 3(B)), and remained approximately the same in the case of lignin (Fig 3(C)), except for an increase noticed at the highest ozone dose. This suggests selective biodegradation of easily biodegradable organic matter formed due to ozonation, and the continued presence of some residual reduced organic matter with high COD/TOC ratio, presumably of a refractory nature, in the effluent after final aerobic biodegradation.

Specific UV absorbance at 280nm, which is an indicator of the aromatic content,\textsuperscript{29} was measured prior to final aerobic biodegradation in all cases. This value was also observed to decrease with an increase in the ozone dose for gallic acid (Fig 3(D)), tannin (Fig 3(E)) and lignin (Fig 3(F)). This suggested formation of aliphatic and presumably more biodegradable organic entities on ozonation.\textsuperscript{30} Specific UV absorbance values measured after final aerobic biodegradation show considerable increase in most cases, especially at higher ozone doses (see Figs 3(D–F)). This again suggests selective removal of biodegradable organic matter, and the presence of residual organic matter, possibly aromatic and refractory in nature, in the effluent after final aerobic biodegradation.

The five day BOD\textsubscript{5}/COD ratio of the effluent after final aerobic biodegradation during phase I experiments was less than 0.20 for gallic acid (Fig 3(G)), tannin (Fig 3(H)) and lignin (Fig 3(I)), again suggesting the presence of mostly refractory organic matter in the effluent after final aerobic biodegradation.

**Final aerobic biodegradation: phase II experiments**

In addition to a decline in COD of 40–45% for all model compounds through initial aerobic biodegradation in phase II experiments (see Table 1 for details), a substantial decline in specific UV absorbance at 280nm for gallic acid (from 0.16 to 0.06), tannin (from 0.16 to 0.07) and lignin (from 0.17 to 0.09), was also observed, suggesting partial destruction of aromaticity through the initial aerobic biodegradation step. This is supported by a study by Chowdhury \textit{et al.}\textsuperscript{31} who showed a considerable decline in the aromatic content of tannin solutions through aerobic degradation experiments.
Figure 2. Enhancement of aerobic biodegradation of gallic acid, tannin and lignin through ozonation: phase I experiments. Starting COD values (same as initial COD values): gallic acid: 3013 mg dm$^{-3}$; tannin: 1648 mg dm$^{-3}$; lignin: 1768 mg dm$^{-3}$. Starting TOC values (same as initial TOC values): gallic acid: 1234 mg dm$^{-3}$; tannin: 608 mg dm$^{-3}$; lignin: 620 mg dm$^{-3}$. All samples were diluted before final aerobic biodegradation. Dilution factors are given in Table 1.

Using tannin-degrading microorganisms isolated from tannery soils.

Aliquots obtained after initial aerobic biodegradation were ozonated at various ozone doses, diluted and then used as feed in aerobic reactors used for phase II final aerobic biodegradation experiments. The average influent and average steady-state effluent COD from such reactors are shown as a function of the absorbed ozone dose in Fig 4(A) (for gallic acid), Fig 4(B) (for tannin) and Fig 4(C) (for lignin). Corresponding TOC values are presented in Fig 4(D) (for gallic acid), Fig 4(E) (for tannin) and Fig 4(F) (for lignin). The percentage of influent COD or TOC that was removed through final aerobic biodegradation is also shown in each figure as a function of the absorbed ozone dose. In all cases (Fig 4(A–C)), COD removal increased progressively with ozone dose, from less than 10% in the unozonated case to 60% or more at the higher ozone doses. A similar trend was also observed for TOC removal (Fig 4(D–F)). As shown in Fig 5(A) (for gallic acid), Fig 5(B) (for tannin) and Fig 5(C) (for lignin), ozonation of the residual organic matter after the initial biodegradation step also resulted in a progressive decline in the COD/TOC ratio. Comparison of COD/TOC ratio measured before the final aerobic biodegradation step with the corresponding value measured after final aerobic biodegradation (see Fig 5(A–C)) indicates that in all cases the latter value is larger. As shown in Fig 5(D) (for gallic acid), Fig 5(E) (for
Mineralization of some natural refractory organic compounds

After Final Aerobic Biodegradation

Before Final Aerobic Biodegradation

Gallic Acid, Phase I

COD/TOC Ratio

0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5

0 1 2 3 4

UV Absorbance (280 nm) per mg dm$^{-3}$ TOC

0.00 0.05 0.10 0.15 0.20 0.25 0.30

0 1 2 3 4

BOD$_5$/COD Ratio

0.06 0.08 0.10 0.12 0.14 0.16

0 1 2 3 4

Figure 3. Impact of final aerobic biodegradation experiments on various properties of gallic acid, tannin and lignin: phase I experiments. Starting COD and TOC values are the same as in Fig 2. Starting COD:TOC ratio: gallic acid: 2.33; tannin: 2.71; lignin: 2.85. Starting specific UV absorbance: gallic acid: 0.16; tannin: 0.16; lignin: 0.18. All samples were diluted before final aerobic biodegradation. Dilution factors are given in Table 1.

tannin) and Fig 5(F) (for lignin), ozonation of the initial aerobic biodegradation effluent at various ozone doses resulted in a progressive decline in specific UV absorbance at 280nm. However, corresponding specific UV absorbance values measured subsequent to final aerobic biodegradation were more in all cases (see Fig 5(D–F)). The BOD$_5$/COD ratio of the residual organic matter after final aerobic biodegradation in phase II experiments was less than 0.15 in all cases (Fig 5(G–I)). The general conclusions drawn from Figs 4 and 5 were similar to those for the corresponding phase I experiments (Figs 2 and 3) described earlier.

However, the main distinguishing characteristics of phase II experiments were, first, the initial aerobic biodegradation step was effective in removing 40–45% COD in all cases, and second, even after the initial biodegradation step the residual refractory fraction could be further biodegraded through provision of an ozonation step preceding final aerobic biodegradation.

COD and TOC removals: ozonation versus biodegradation

In both phase I and phase II experiments, overall COD and TOC removals were obtained through a combination of ozonation and aerobic biodegradation processes. Comparative analysis of the relative importance of ozonation and aerobic biodegradation in overall COD and TOC removal during phase I and phase II experiments is presented in Fig 6. In all cases, and both in phase I and phase II experiments, the percent of starting COD and TOC removed
through ozonation increased with an increase in ozone dose (see Fig 6, A and B for gallic acid, C and D for tannin, and E and F for lignin). However, this increase in phase II experiments is either less than or comparable to the corresponding increase in phase I experiments. In contrast, the percent of starting COD removed through final aerobic biodegradation in phase I experiments showed a decline in all cases with an increase in ozone dose, while in phase II experiments percent COD removal through biodegradation (initial and final combined) showed no change with an increase in ozone dose for gallic acid, while an increase was observed with an increase in ozone dose for tannin and lignin (see Fig 6, G for gallic acid, I for tannin and K for lignin). Corresponding TOC removal trends (see Fig 6, H for gallic acid, J for tannin, L for lignin) were, on the whole, similar to the trends in COD removal.

These results suggest that in phase I experiments, some organic matter that would be removed by aerobic biodegradation in the absence of ozonation, is instead removed through ozonation on application of ozone. This results in the overall percent COD removal through biodegradation decreasing with an

Figure 4. Enhancement of aerobic biodegradation of gallic acid, tannin and lignin through ozonation: phase II experiments. Starting COD values: gallic acid: 2000 mg dm$^{-3}$; tannin: 2002 mg dm$^{-3}$; lignin: 1995 mg dm$^{-3}$. Initial COD values: gallic acid: 1286 mg dm$^{-3}$; tannin: 1280 mg dm$^{-3}$; lignin: 1300 mg dm$^{-3}$. Starting TOC values: gallic acid: 859 mg dm$^{-3}$; tannin: 738 mg dm$^{-3}$; lignin: 675 mg dm$^{-3}$. Initial TOC values: gallic acid: 438 mg dm$^{-3}$; tannin: 586 mg dm$^{-3}$; lignin: 440 mg dm$^{-3}$. All samples were diluted before final aerobic biodegradation. Dilution factors are given in Table 1.
Comparison of overall COD and TOC removals
During phase I experiments with gallic acid, overall COD removal progressively increased from 40% when no ozone was applied to 94% at an applied ozone dose of 3.19 mg ozone absorbed per mg initial TOC, while the corresponding increase was from 42% to 92% at an applied ozone dose of 3.45 mg ozone absorbed per mg initial TOC during phase II experiments (see Fig 7(A)). The corresponding increase in TOC removal was from 51 to 88% in phase I, and from 54 to 92% in phase II experiments (see Fig 7(B)).
Similar progressive increase in overall COD and TOC removals was also observed for tannin (Fig 7, C for COD and D for TOC) and lignin (Fig 7, E for COD and F for TOC) in both phase I and phase II experiments.

Overall COD and TOC removal was superior through phase II experiments, ie the same percent COD or TOC removal could be obtained at lower ozone doses, for tannin (Fig 7, C and D for COD and TOC respectively) and lignin (Fig 7, E and F for COD and TOC respectively). In the case of gallic acid, COD removal through phase I and phase II experiments was comparable (Fig 7(A)), however, superior TOC removal was observed through phase II experiments (Fig 7(B)). The reason for generally superior performance of phase II experiments vis-à-vis COD and TOC removal, is probably the targeted application of ozone in such
experiments exclusively to the refractory organic fraction,\textsuperscript{2,22,32} which is thus rendered biodegradable more efficiently.

**Comparison of ozone requirements**

In addition to superior COD and TOC removals at comparable ozone doses, phase II experiments have another crucial advantage. Since organic matter is partly mineralized through an initial biodegradation step in phase II experiments, ozonation of two aliquots of same compound with same starting TOC to the same ozone dose will involve ozonating less organic matter in phase II experiments, resulting in a lower ozone requirement. To further elucidate this advantage, the specific ozone requirement, ie ozone requirement per unit additional COD and TOC degradation (ie COD or TOC degradation over and above that achieved through simple aerobic biodegradation), is plotted against overall removal percentages in Fig 7(G–L)). Considering the case

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**Figure 7.** Impact of absorbed ozone dose on overall COD and TOC removal from gallic acid, tannin and lignin aliquots: comparison of phase I and phase II results. A and B (gallic acid), C and D (tannin), and E and F (lignin) pertain to impact of ozone dose on overall COD and TOC removal percentages. G and H (gallic acid), I and J (tannin), and K and L (lignin) pertain to ozone requirement for unit additional (compared with unozonated controls) COD or TOC degradation at various overall COD and TOC removal percentages. Starting COD and TOC values for phase I experiments, and starting and initial COD and TOC Values for phase II experiments are as given in Table 1. All samples were diluted before final aerobic biodegradation. Dilution factors are given in Table 1.
of overall COD removal for gallic acid (Fig 7(G)), removal was approximately 40% in both phase I and phase II experiments when no ozone was applied. In both cases, enhanced COD removals required application of ozone, with specific ozone requirement increasing progressively with increase in percent overall removal. However, the specific ozone requirements in phase II experiments were always less than half of that in phase I experiments at same overall removal percentages. This advantage is even more apparent when overall TOC removal for gallic acid is considered (Fig 7(H)). The same advantage of phase II experiments is also apparent in experiments involving the other model compounds (Fig 7, I and J for tannin, K and L for lignin).

CONCLUSIONS
The main objective of this study was to investigate the extent of mineralization of organic carbon in refractory model compounds, ie gallic acid, tannin and lignin, through a combination of ozonation and aerobic biodegradation processes. Two schemes were tested, the first scheme or phase I experiments involved ozonation followed by final aerobic biodegradation, while the second scheme or phase II experiments involved initial aerobic biodegradation, followed by ozonation and subsequent final aerobic biodegradation. The main conclusions of this study are as follows:

1. In both phase I and phase II experiments, ozonation-induced changes in the residual organic matter properties were responsible for enhanced aerobic biodegradability of post-ozonated aliquots of gallic acid, tannin and lignin. In phase I experiments, COD removal through the final aerobic biodegradation step increased from less than 45% in unozonated controls to 70% or more at the higher ozone doses (see Fig 2). In phase II experiments, the final biodegradation step increased COD removals from less than 10% in unozonated controls to 60% or more at higher ozone doses. Similar trends were also observed for TOC removal (see Fig 4).

2. In both phase I and phase II experiments, overall COD and TOC removals were through a combination of ozonation and aerobic biodegradation processes. Percent overall COD and TOC removals through ozonation increased with ozone dose in both cases, with the increase being more in phase I experiments (see Fig 6(A–F)). In phase I experiments, the overall percent COD and TOC removal through biodegradation decreased with increases in the ozone dose. In phase II experiments, where ozonation was only directed towards the refractory portion of organic matter remaining after initial aerobic biodegradation, percent overall COD and TOC removal through the combination of initial and final aerobic biodegradation processes increased with ozone dose (see Fig 6(G–L)).

3. In all cases and in both phase I and phase II experiments, overall COD and TOC removals of 90% or more could be achieved through application of ozone at the highest doses investigated during this study, compared with 40–50% removals when no ozone was applied. Overall COD and TOC removals were either comparable or superior through phase II experiments for all model compounds, ie same percent of starting COD or TOC could be removed at lower ozone doses. The reason for the generally superior performance of phase II experiments vis-à-vis COD and TOC removal is probably the targeted application of ozone in such experiments exclusively to the refractory organic fraction, which is thus rendered biodegradable more efficiently (see Fig 7(A–F)).

4. Since organic matter was partly mineralized through an initial biodegradation step in phase II experiments, ozonation of two identical aliquots to the same ozone dose involved ozonating less organic matter in phase II experiments, resulting in lower ozone requirement. The specific ozone requirement, ie ozone requirement per unit additional COD and TOC degradation (ie COD or TOC degradation over and above that achieved through simple aerobic biodegradation), was always more than 50% less in phase II experiments as compared with phase I experiments (see Fig 7(G–L)).

Based on the results of this study, it is apparent that integration of ozonation with aerobic biodegradation process is effective in achieving considerable enhancement of mineralization of the refractory model compounds, viz. gallic acid, tannin and lignin. It is also observed that for both schemes investigated, the degree of mineralization was comparable for the three model compounds tested. The second treatment scheme investigated in the phase II experiments involving initial aerobic biodegradation, ozonation and subsequent final aerobic biodegradation is clearly superior, both in terms of overall removals achieved at particular ozone doses, and also based on lower specific ozone requirements for effecting enhanced mineralization of organic matter.

Minimizing ozone requirement is an issue of prime importance for an integrated ozonation–aerobic biodegradation process to be an economically feasible treatment option of mineralization of refractory compounds found in wastewater. The second scheme investigated in this study was found to have a lower ozone requirement for comparable degrees of mineralization. Preliminary cost estimates using this scheme are presented below. Considering refractory wastewater of the type investigated in this study, and with starting COD of 1000 mg dm−3, our results indicate that approximately 40% COD reduction is possible with no ozone treatment. If 90% COD removal is desired, ozone requirement per dm3 of wastewater treated using the second scheme is approximately
750 mg, ie at the rate of 1.5 mg ozone per mg additional COD removed (see Fig 7 (G – L)). Considering 80% ozone contactor efficiency, the ozone application required per dm³ of wastewater treated is 937.5 mg.

Thus after incorporating annualized capital costs, the cost of ozone required is US $4.7 per 1000 dm³ of wastewater. The cost of electricity therefore makes up approximately 75% of the cost of ozone treatment. Thus after incorporating annualized capital costs, the total cost of ozone treatment is approximately US $6.25 per 1000 dm³ of refractory wastewater with starting COD 1000 mg dm⁻³ and 90% COD removal efficiency. In comparison, the total annualized cost of secondary conventional biological wastewater treatment is around US $1 – 2 per 1000 dm³ wastewater.

REFERENCES


