Ozonation within an Activated Sludge System for Azo Dye Removal by Partial Oxidation and Biodegradation

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Abstract
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Keywords
Ozonation, Biodegradation, Ozone, Activated Sludge, Orange II By-products, Gc-Ms, Color Removal, Biosorption, Azo Dye, Solid Phase Microextraction, Biodegradability

Disciplines
Biochemical and Biomolecular Engineering | Bioresource and Agricultural Engineering | Environmental Sciences

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Abstract

Pre-ozoneation is often uneconomical for typical wastewaters with varied mixtures of organic compounds as more biodegradables than non-biodegradables are oxidized, all requiring ozone. The concept developed in this paper is ozonation within an activated sludge system to oxidize recalcitrant substances to more degradable forms and byproducts and to immediately assimilate or biodegrade these within the biological system. The focus was on a novel method of combining ozonation and biological treatment in one integrated unit without adversely affecting the bacterial population responsible for the biological degradation.

An azo dye, spiked into the wastewater feed was used to study removal of a recalcitrant compound in a biological system. Orange II was introduced in synthetic wastewater and fed into two 6-L activated sludge units operated in parallel, one of which was ozonated and the other one was the control unit. The ozone dosage, directly into the aeration basin, was studied at 45 and 30 mg/L based on feed rate of the wastewater. The color and COD removal were measured. The ozonolysis intermediates of the dye were identified using solid phase microextraction and gas chromatography-mass spectrometry and were verified to be biodegradable. The color removal in the ozonated unit was much higher than in the control unit. The role of biosorption in color removal was quantified by determining adsorption isotherms of Orange II on biomass and found to be the main removal mechanism in the non-ozonated control. Ozone caused some biological changes within the microbial system, but did not prevent the normal biological degradation of organic compounds measured as COD.
Introduction

Organic dyes are complex, typically polyaromatic compounds. Dye house wastewaters are highly colored with such residual dyes. Wastewater from the textile industry is characterized by presence of color, high total organic carbon content, low biodegradability, e.g. BOD₅:COD ratio less than 0.1, some toxicity, and some suspended solids content (Wu et al., 2001; Liakou et al., 1997a; Liakou et al., 1997b).

Azo dyes are a particular class of dyes which are widely used in the textile industry. They also find numerous uses in other fields. Orange II is a representative of the azo-class of dyes. It is mono-azoic, having a single N=N double bond to which the aromatic heterocyclic rings are attached. The chromophore part of the dye is responsible for the color and the auxochromic part enhances the color and the dye-ability. In this case, the azo group is responsible for the color while the sulfonyl group renders the dye capable of coloring wool and silk (Zissi and Lyberatos et al., 1996; Waring and Hallas, 1990; Venkataraman, 1977, 1952). The structural formula of Orange II is shown in Figure 1.

![Figure 1: Structural formula of Orange II (4 – (2 – hydroxyl – 1 – naphthylazobenzene-sulfonic acid)](image)

Several studies on advanced oxidation processes (AOPs) have been conducted to observe the treatability of textile wastewater since conventional biological methods are generally unsatisfactory for the degradation of dyes. It was established that although the AOPs proved to be effective individually as well as in various combinations, ozonation coupled with biological treatment stood out as a significant candidate to be considered for full-scale operations (Ledakowicz et al., 2001; Turan-ertas, 2000; Perkowski et al., 2000). Ozone, with its high oxidation potential, is safer and a less toxic alternative to chlorine and is less dependent on pH than other oxidants like permanganate, peroxide and others. Ozonation also improves sludge settlability, wastewater biodegradability, and achieves good color removal (van Leeuwen, 1988; Churchley, 1994; Saayman et al., 1996; Beltran et al., 1997; Lopez et al., 1998; Ciardelli et al., 2001).

Beltran et al. (1999) conducted a two-part experiment where ozonation and activated sludge were combined to study the effects on domestic wastewater. The first part
consisted of biodegradation followed by ozonation. The order was reversed in the second part. The studies suggest that post-ozonation can help remove persistent compounds that are not degraded in the biological stage. The prior treatment with activated sludge improved the performance by removing easily biodegradable compounds that would otherwise compete for ozone. Pre-ozonation improved the general overall biodegradability of the wastewater. Other researchers (Qian et al., 1994; Scott and Ollis, 1995) concur.

Treatment of wastewater containing toxic/recalcitrant substances with biological methods followed by ozonation would still require another biological stage to degrade the intermediates (formed upon ozonation) or would necessitate recirculation of the ozonated effluent back into the first biological stage for treatment. This method would increase the throughput rate and impact equipment size, particularly solids handling and separation (van Leeuwen et al., 2001, 2009). Additionally, it would also entail increasing the footprint of the conventional activated sludge plant to accommodate the ozone contacting facility followed by another biological treatment option, all adding to the capital and operational costs.

It has also been documented that several intermediates of persistent compounds, such as dyes (dye intermediates include aromatic amines which have toxic potential), may be toxic and require further degradation before final discharge (Brown and DeVito, 1993, as cited in Wallace, 2001). In such cases, it would be prudent to allow a greater contact time between the oxidant and the dye, which would be achieved if the chemical oxidation took place in the same unit as the biological degradation – the hydraulic residence time (HRT) would be the controlling factor (van Leeuwen et al., 2009).

Considering the ideas addressed above, this research focuses on a novel method of combining ozonation and biological treatment in one integrated unit aimed at not only reducing the footprint of the plant but also to effect satisfactory color removal and reduction of COD, without adversely affecting the bacterial population responsible for the biological degradation. This approach was first investigated by Van Leeuwen et al. (2001, 2009).

The objectives of this study are listed below.

- Establish an ozone dosage range for color reduction
- Determine the biodegradability of the azo dye, Orange II
- Identify the ozonolysis byproducts of Orange II
- Determine overall effects of ozonation within activated sludge on the microbes and settling characteristics

**Experimental**

**Materials**

Synthetic wastewater was prepared by the addition of the organic and inorganic compounds as shown in Table 1. The azo dye under consideration, Orange II, was added to nutrients to constitute the wastewater feed to the experimental plants. Orange II was purchased from Acros Organics while most of the other reagents were obtained from Thermo Fisher Scientific. The COD of the wastewater was tested and found to amount to 450 mg/L.
Table 1: Feed composition

<table>
<thead>
<tr>
<th>Synthetic Wastewater Components</th>
<th>Stock Concentrations, mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea</td>
<td>75.2</td>
</tr>
<tr>
<td>Nutrient broth</td>
<td>120</td>
</tr>
<tr>
<td>Potato starch</td>
<td>12</td>
</tr>
<tr>
<td>Non-fat dried milk</td>
<td>120</td>
</tr>
<tr>
<td>Dietary fiber</td>
<td>20</td>
</tr>
<tr>
<td>Sodium acetate</td>
<td>40</td>
</tr>
<tr>
<td>Sodium bicarbonate</td>
<td>20</td>
</tr>
<tr>
<td>Potassium phosphate, $K_3PO_4\times H_2O$</td>
<td>6</td>
</tr>
<tr>
<td>Ferrous sulfate, $FeSO_4\times 7H_2O$</td>
<td>1</td>
</tr>
<tr>
<td>Orange II</td>
<td>5</td>
</tr>
</tbody>
</table>

Procedures for integrated activated sludge treatment with ozonation

The experimental set-up used in this study is shown in Fig. 2. It consisted of two small-scale activated sludge plants having a working volume of 6 L each, operated in parallel.

The biomass in the bench-top reactors was procured from an aeration basin of the Boone, Iowa municipal activated sludge plant. The two plants employed fritted glass porous diffusers to continually aerate the biomass and were operated at a HRT of 10 h by pumping the wastewater at 0.6 L/h. A solids retention time of 10 d and a solids concentration of 3.5 g/L was maintained by wasting 0.6 L mixed liquor once every day. The peristaltic pumps were Masterflex (Cole-Parmer Instrument Company).
Ozone gas was generated from oxygen in an Ozonology lab generator. The ozone was dosed directly into one of the reactors via a porous glass diffuser at 27 and then 18 mg/h, i.e. 45 then 30 mg/L based on the wastewater feed rate, or 1.3 and then 0.85 mg O₃ g⁻¹ biomass h⁻¹ and varied by feed voltage or oxygen flow rate using a variable control valve. The higher dosage seemed to detrimentally affect the biomass, so it was lowered. The other reactor was operated identically, but without ozonation to serve as the control.

Ozonolysis to determine byproducts

A solution of 5 mg/L Orange II was ozonated to 30 mg/L, i.e. about 50 mol O₃:mol Orange II. Dye samples were introduced in a 1 L batch reactor provided with continuous magnetic stirrer and two gas diffusers to improve the contact between the ozone and the target solution. The ozone was produced from oxygen in a Sander Labor ozone generator. Ozone at the inlet of the reactor was measured every second by means of an Ozone Analyzer BMT 963 Vent (BMT Messtechnik, Berlin) and at the outlet using an Ozone Analyzer BMT 964 BT (BMT Messtechnik, Berlin). Dissolved ozone in the solution was measured with a Dissolved O₃ Analyzer (ATI model Q45H). All this information was collected using a DaqPro 5300 data-logger. The ozonated gas flow was maintained at 30 L/h and the dye solutions were led to react until color was visually removed.

Analytical Methods

Water Analyses  Effluent dye concentrations were determined using a Milton Roy Spectronic 501 spectrophotometer in the visible region. The maximum absorbance is at 484 nm for Orange II, as found with a Spectronic Genesys 2 spectrophotometer. The COD of the effluent samples were measured according to Standard Methods (APHA et al., 1995).

Ozone Determination  Ozone dosages were determined by measuring the flow rates and the gas phase concentrations into and out of the reactor by the standard iodometric method described in Standard Methods (1995). Ozone demand tests of feed and effluent were also based on Standard Method Methodology, but with an endpoint determined by establishing a measurable residual or for 60% color removal of the Orange II.

Physical Biomass Characterization  The MLVSS (Mixed Liquor Volatile Suspended Solids) content and the sludge volume indices for the biomass from both reactors were measured according to Standard Methods (1995).

Biosorption  The extent of the biosorption of Orange II onto the biomass was also considered. The procedure for testing the sorption characteristics of the biomass consisted of adding different quantities of biomass, inactivated by adding 10 mg/L of mercuric sulfate, in 300 mL bottles to a fixed volume of 5 mg/L Orange II-laden feed. These containers were placed in a shaker for 2 h and the concentration of the remaining dye was measured spectrophotometrically at 484 nm.

Biodegradability  The biodegradability of the dye was ascertained by conducting extant respirometric tests. The tests were conducted according to the procedure described by
Ellis and Eliosov (2002). Samples of biomass were continuously oxygenated to equilibrium upon which the dye was introduced and the endogenous respiration rate was observed. An increase or decrease in the respirometric rate, measured as a normalized dissolved oxygen level, would be indicative of the biodegradability of the dye. A decrease in dissolved oxygen would indicate higher respiration due to degradation of additional substrate and an increase inhibition of respiration.

**Solid phase microextraction coupled with analyses of ozonation by-products on GC-MS**

Ozonated samples were analyzed for identification of volatile and semi-volatile by-product formation. Headspace samples of treated waste were sampled with solid phase microextraction (SPME) and analyzed on a gas chromatograph–mass spectrometer (GC-MS). Solid phase microextraction combines sampling and sampling preparation to one step (Pawliszyn, 1997). SPME has been used for identification of ozonation by-products in air (Xiong et al., 2004) and also for analysis of headspace above very complex waste such as swine or poultry manure (Lo et al., 2008; Cai et al., 2007). Water samples with Orange II were ozonated and 25 mL of the treated samples were transferred to 40 mL glass vials (from Supelco, Bellefonte, PA) with hole screw caps and PTFE-faced septa.

<table>
<thead>
<tr>
<th>Table 2: GC-MS program and conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Column dimensions</strong></td>
</tr>
<tr>
<td><strong>GC-MS model</strong></td>
</tr>
<tr>
<td><strong>GC Injector temperature</strong></td>
</tr>
<tr>
<td><strong>Initial oven temperature</strong></td>
</tr>
<tr>
<td><strong>Ramping rate</strong></td>
</tr>
<tr>
<td><strong>Final oven temperature</strong></td>
</tr>
<tr>
<td><strong>Constant column pressure and carrier</strong></td>
</tr>
<tr>
<td><strong>Mass-spectrometer (model)</strong></td>
</tr>
<tr>
<td><strong>Scan mode, m/z range</strong></td>
</tr>
<tr>
<td><strong>MS source/MS quad temperature</strong></td>
</tr>
<tr>
<td><strong>Ionization energy</strong></td>
</tr>
<tr>
<td><strong>Compound identification library</strong></td>
</tr>
</tbody>
</table>

SPME fiber 85 µm Carboxen/polydimethylsiloxane (from Supelco, Bellefonte, PA) was used for the extraction of headspace samples of the treated water samples with Orange II for 60 min at 40 °C with 850 rpm agitation with PTFE-coated stir bar (from Fisher Scientific, Pittsburgh, PA). After extraction, the SPME fiber was then immediately inserted into the injection port of GC-MS (Agilent, Wilmington, NC). Further GC-MS conditions for analysis are summarized in Table 2.

**Microbial Investigation** Samples of activated sludge from both the experimental as well as the control unit were subjected to light microscopy to study the floc structure. Characteristic groups of micro-organisms present were noted.
Results & Discussion

Effluent Analysis

The COD and the color removal of the ozonated and control effluent were analyzed and the results are presented below.

COD Removal It is observed from Fig. 3 that the COD removal in both the ozonated and the non-ozonated reactors was variable. The variability, particularly when comparing the first four days to the remainder of the run, can be attributed to adjustment of the ozone dosage and losses and replacement of the mixed liquor in both the activated sludge units. The initial ozone dosage of 45 mg/L (up to day 5) caused a steep decrease in COD removal in the experimental plant when compared to the control plant. This indicated that the ozone dosage was too high and inactivated beneficial bacteria. The synthetic wastewater lacked the greater variety of compounds found in real industrial wastewater. Lack of complexity of the wastewater provided less shielding of the biomass from the high ozone dosage, thereby causing lyses of the cells that are responsible for increasing the final COD of the effluent. Consequently, the average COD removal for the 45 mg ozone/L phase was 81 % for the experimental unit and 88 % for the control activated sludge unit.

Twenty percent of the biomass in the ozonated reactor was discarded and replaced with fresh biomass after 5 days of operation. The same was done with the control unit to maintain microbial diversity. The ozone dosage was subsequently reduced to 30 mg/L and the experiment was continued. In the post-adjustment period it can be seen that the average COD removal in both the experimental and control unit was around 80 % and 82 %. The overall removal for the entire range was 80 % and 84 % for both units. The difference in removal was not found to be statistically significant. The gains in biodegradability through ozonation may have equaled possible inhibition by the presence of ozone.

Color Removal Fig. 4 shows that the color reduction, averaged over time, in the ozonated activated sludge unit was ca. 80 % while that of the control reactor was ca. 40 %. The maximum color removal during the run was 96 % and 67 % for the experimental and the control unit and it occurred during the first couple of days. This large initial color removal may be attributed to more available biosorption area on the fresh biomass. This biosorption can also explain the sudden increase in the color removal in the post-adjustment stage for ozone dose (45 mg/L to 30 mg/L) where some fresh biomass was introduced into the system. It was to be expected that fresh biomass adsorbed color more effectively than biomass that had already been exposed to the dye and achieved a state of equilibrium. While a steady-state was not quite achieved, the trend suggested that the removal of Orange II would level off at 60 % in the integrated process and at 20 % in the control. These values were also compared with the ozone requirements to obtain 60% color removal in the raw feed by ozonation only.
Biosorption tests were conducted to explain the adsorption characteristics of the biomass with the help of a modified isotherm. The sorption test, using different quantities of freshly inactivated biomass in contact with the dye solution, was used to construct a modified Freundlich isotherm (Fig. 5). Any adsorption under steady-state conditions would be due to adsorption on new biomass growth only, as older biomass is already in equilibrium with the remaining color concentration. The mixed liquor suspended solids averaged about 3.5 g/L, i.e., 21 g of biomass per 6-L activated sludge unit. Since the solids retention time was maintained at 10 d, 10% of biomass production amounted to 2.1 g/L per day. The equilibrium dye concentration on the biomass in the control reactor, with an effluent concentration of 4 mg/L of Orange II after 20% total removal, would be approximately 5 mg/g biomass according to the isotherm. This would result in a daily removal of 10.5 mg dye based on a total feed of 72 mg/day, i.e., 14% removal. Even though the actual removal amounted to 20%, adsorption was still the main Orange II removal mechanism in the non-
ozonated control. Similarly, for the ozonated unit, where the effluent had a concentration of 2 mg/L of dye after 60% removal, the equilibrium concentration on the biomass would be 2.2 mg/g biomass and the daily adsorptive removal could be predicted to amount to 4.6 mg from 72 mg/day of Orange II or 8.3%, which is only \( \frac{1}{7} \)th of the actual removal. Biosorption is therefore but a minor factor in the integrated process. Biomass replacement contributed to a larger fraction of the dye removed by biosorption than the typical values used in the calculation above.

**Figure 5:** Modified isotherm for adsorption of Orange II dye on activated sludge

**Physical Biomass Characterization**

The biomass concentration averaged around 3.5 g/L for both control and ozonated activated sludge plants. There was some variation due to the adjustment of the ozone as well as other factors such as an unusual infestation of worms, which are natural predators of the bacterial colonies. The sludge volume index (SVI) was measured for both reactors using Standard Methods (1995). From Fig. 6, it was observed that the ozonated unit had a lower SVI (averaging around 70 mL/g) than in non-ozonated control unit (averaging around 90 mL/g). However, the sludge settleability in either unit was satisfactory at these SVI values. The gradual decrease in SVI is commonly seen in bench-scale activated sludge systems, due to overspill losses of poorly settleable flocs.

**Figure 6:** Sludge volume index of the biomass from ozonated and non-ozonated units
Biodegradability of Orange II  The extant respirometric technique indicated no biodegradability of the Orange II dye. The tests showed that the respiration rate of the biomass decreased upon introduction of the dye, which is indicative of the recalcitrant/toxic nature of the dye. If the compound were easily biodegradable, the normalized respirometric curve would tend downwards; if the compound has no tangible effect on the biomass, the dissolved oxygen level would not be affected. Figure 7 shows the normalized curves moving upwards, which indicates that Orange II is toxic to the unacclimatized biomass. The fact that the control activated sludge performed as well as the ozonated unit may indicate that the biomass could adapt to the relatively low concentrations as used in these continuous experiments.

Microbial Investigation  Microscope studies indicated that the ozonated biomass flocs were more compact and tight than the non-ozonated biomass. The non-ozonated sludge was darker than the ozonated sludge.

Analysis of byproducts of ozonolysis  The total ion chromatogram of headspace sample of ozonated Orange II solution of Orange II is provided in Fig. 8, showing the ozonolysis by-products resulting in a number of compounds, which have been numbered in this figure.
Figure 8. Total ion chromatogram of headspace sample collected with SPME and analyzed with GC-EI-MS. Numbered compounds are listed in Table 3.

Preliminary identification of ozonolysis by-products of Orange II is provided in Table 3. Most of these substances are readily biodegradable.

The concept of selective oxidation within microbial cultivation is being patented (Van Leeuwen, 2009). This and other work has shown some benefits to using ozonation with different biological treatment systems for the removal of undesirable substances.

Table 3. Preliminary identification of ozonolysis by-products for Orange II.

<table>
<thead>
<tr>
<th>Peak</th>
<th>Compound</th>
<th>CAS</th>
<th>Significant Ions</th>
<th>Retention Time (min)</th>
<th>Spectral Match (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Methylloxirane</td>
<td>75-56-9</td>
<td>58,57,39,68,42</td>
<td>1.80</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>Butanal</td>
<td>123-72-8</td>
<td>72,44,43,41,39</td>
<td>2.50</td>
<td>76</td>
</tr>
<tr>
<td>3</td>
<td>Borate(1-), hydroxytri phenyl-sodium</td>
<td>12113-07-4</td>
<td>78,79,52,51,50</td>
<td>3.42</td>
<td>76</td>
</tr>
<tr>
<td>4</td>
<td>3-Methylbutanal</td>
<td>590-86-3</td>
<td>44,58,41,43,57</td>
<td>4.11</td>
<td>86</td>
</tr>
<tr>
<td>5</td>
<td>Methylbenzene</td>
<td>168-188-3</td>
<td>91,92,65,56,39</td>
<td>5.58</td>
<td>71</td>
</tr>
<tr>
<td>6</td>
<td>Hexanal</td>
<td>66-25-1</td>
<td>56,44,43,57,43</td>
<td>6.56</td>
<td>88</td>
</tr>
<tr>
<td>7</td>
<td>3-heptanone</td>
<td>106-35-4</td>
<td>57,85,72,114,41</td>
<td>8.80</td>
<td>86</td>
</tr>
<tr>
<td>8</td>
<td>N-Heptanal</td>
<td>111-71-7</td>
<td>70,43,44,41,55</td>
<td>9.38</td>
<td>79</td>
</tr>
<tr>
<td>9</td>
<td>3-Hexanone,2,5,-dimethyl</td>
<td>1888-57-9</td>
<td>57,43,41,86,70</td>
<td>9.60</td>
<td>55</td>
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<tr>
<td>10</td>
<td>2-Ethylhexanal</td>
<td>123-05-7</td>
<td>72,57,43,41,39</td>
<td>10.31</td>
<td>88</td>
</tr>
<tr>
<td>11</td>
<td>Octanal</td>
<td>124-13-0</td>
<td>43,41,57,84,56</td>
<td>12.00</td>
<td>95</td>
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<tr>
<td>12</td>
<td>5-Methyl-1-Heptene</td>
<td>1351-04-7</td>
<td>57,70,55,41,83</td>
<td>13.63</td>
<td>68</td>
</tr>
</tbody>
</table>
### Environmental and operational issues

No ozone could be detected in the air exiting the activated sludge units. The ozone demand was too large for any ozone not to be utilized. This finding was not surprising as ozone losses or environmental discharges were not considered to be of any concern in full-scale operations for bulking control either (Saayman et al., 1996).

The activated sludge units were plagued by unexpected mishaps, such as growth of worms and poor control during night hours, so it was difficult to keep the system operating long enough to achieve a definite steady state. However, as a proof of concept, it was clear that the partial removal of a specific compound by ozonation and subsequent biodegradation was possible within an activated sludge unit, without major disruption caused by ozone toxicity.

The COD of the feed was 450 mg/L and that of the effluent was about 80 mg/L. The ozone demand of the feed was shown to be around 300 mg/L before any measurable residual was established lasting for 10 seconds. An ozone dosage of 145 mg/L was required to obtain a 60% color removal. Similarly, the ozone demand of the effluent was 21-25 mg/L to establish a residual. This would indicate that the ozone demand for color removal would be much lower during activated sludge treatment than before treatment.

### Conclusions

This research explored the novel concept of integrating chemical oxidation with ozone and biological treatment into one single unit. Previous research focused on biological treatment with ozone pre- or post-treatment, but this research is unique because ozone was directly injected into an activated sludge system. Conclusions drawn from a laboratory-scale investigation were:

- Removal of the azo dye Orange II was much more effective in a combined process of activated sludge with direct ozonation than just biological or just ozonation.
- Biosorption was a minor mechanism in Orange II removal in the integrated process, but the main mechanism in the non-ozonated control.
- Ozonation improved sludge settleability.
- The ozonolysis products of Orange II were identified as mainly biodegradable substances. These products were expected to be removed by biodegradation in the integrated process. Chemical oxidation works synergistically with the biological process to attain more complete removal of the dye and its byproducts.
• The dye Orange II was inhibitory to bacterial biomass and biodegradation is therefore not considered to be a contributing factor in its removal.
• Headspace sampling with SPME and analysis on a GC-MS was a useful and convenient approach for preliminary identification of ozonolysis by-products.

This process has many advantages over pre- or post-ozonation on large scale mainly with respect to cost and size. The footprint of the plant would not only be reduced, but the amount of ozone too can be minimized as any biodegradable byproducts are removed biologically and would not be available for reacting with more ozone. Also, no ozone would be wasted on oxidizing other biodegradable material as would be the case with pre-ozonation.

References


