Improve the biodegradability of post-hydrothermal liquefaction wastewater with ozone: conversion of phenols and N-heterocyclic compounds
Libin Yang, Buchun Si, Marcio Arêdes Martins, Jamison Watson, Huaqiang Chu, Yuanhui Zhang, Xiaobo Tan, Xuefei Zhou and Yalei Zhang

ABSTRACT
Hydrothermal liquefaction is a promising technology to convert wet biomass into bio-oil. However, post-hydrothermal liquefaction wastewater (PHWW) is also produced during the process. This wastewater contains a high concentration of organic compounds, including phenols and N-heterocyclic compounds which are two main inhibitors for biological treatment. Thus, proper treatment is required. In this work, ozone was used to convert phenols and N-heterocyclic compounds with a dosage range of 0–4.64 mg O₃/mL PHWW. After ozone treatment, the phenols were fully converted, and acids were produced. However, N-heterocyclic compounds were found to have a low conversion rate (21.7%). The kinetic analysis for the degradation of phenols and N-heterocyclic compounds showed that the substitute played an important role in determining the priority of ozone reactions. The OH moiety in the ring compounds (phenols and pyridinol) may form hydroxyl radical, which lead to an efficient reaction. A substantial improved biodegradability of PHWW was observed after ozone treatment. The ratio of BOD₅/COD was increased by about 32.36%, and reached a maximum of 0.41. The improved biodegradability of PHWW was justified by the conversion of phenols and N-heterocyclic compounds.

Key words | biodegradation, hydrothermal liquefaction, ozone treatment, N-heterocyclic compounds, phenols

INTRODUCTION
Hydrothermal liquefaction (HTL) is a promising technology for biofuels production from wet biomass (Yu et al. 2011; Zhou et al. 2015). However, a wastewater stream is produced after the thermochemical conversion, in which 65–80% of the nutrients and 30–40% of the organic compounds from the feedstock remain (Yu et al. 2011; Zhou et al. 2013). This stream, known as post-hydrothermal liquefaction wastewater (PHWW), must be treated to recycle the nutrients and avoid environmental pollution. Although, a successful separation of high value chemical products (phenolic compounds) in PHWW by a modified adsorption resin was reported (Chen et al. 2015), a further economic evaluation needs be conducted as the high cost of resin and complicated characteristics of the PHWW. Biological treatment, such as anaerobic digestion and microalgae cultivation have shown its potential to recover energy and nutrients from PHWW. However, a high dilution ratio (15-500) was previously required due to the presence of...
inhibitors contained in the PHWW (Biller et al. 2012; Du et al. 2012; Pham et al. 2015). The PHWW contains a high concentration of organic acids, alcohols, amides, esters, ketones, phenols, N-heterocyclic compounds and aromatic compounds (Gai et al. 2015; Tommaso et al. 2015). Most of these chemical compounds are potential inhibitors. Pham et al. confirmed the high toxicity of N-heterocyclic compounds in PHWW, and these compounds lead to a low removal (30%) in the cultivation of algae using PHWW (Pham et al. 2015). Further, aromatic compounds tended to inhibit the readily biodegradable organic fractions and their own biodegradation (Hernandez & Edyvean 2008; Haritash & Kaushik 2009). Moreover, the presence of phenols above 400 mg/L have been shown to limit the degradation of N-heterocyclic compounds by a competitive inhibition pattern (Adav et al. 2007; Sun et al. 2011). Therefore, a pretreatment is required to remove the toxic compounds and improve the biodegradability of PHWW.

Ozone has shown potential to be a reliable pretreatment method because the oxidative properties of ozone previously enhanced the biodegradability of organic chemicals (Hübner et al. 2015). During ozone treatment, various recalcitrant compounds, such as aromatic compounds, furfural and pyridine have been converted to easily biodegradable compounds (Andreozzi et al. 1991; Chedeville et al. 2009; Punzi et al. 2015; Van Aken et al. 2015). Recent studies have reported that ozone pretreatment led to increasing biodegradation, including decreasing the presence of toxic compounds, promoting a higher chemical oxygen demand (COD) removal and reducing the lag-phase (Somensi et al. 2010; Van Aken et al. 2015). However, conflicting results have also shown that ozone pretreatment generated more toxic effluent when compared with the initial wastewater (Souza et al. 2010). In this work, the ozone treatment of PHWW was investigated to reveal: (1) the effect of ozone treatment on the distribution of organic compounds and the biodegradability of PHWW; (2) the kinetic degradation of phenols and N-heterocyclic compounds.

**METHODS AND MATERIALS**

**HTL process**

The PHWW was collected from a pilot-scale HTL experiment. The HTL reactor was operated using swine manure at a reaction temperature of 270 ± 10 °C and with a total solid content of 13%. The retention time of the HTL reaction was 1 h. The PHWW was stored in a cold chamber at 4 °C and filtered by a 0.45 μm filter just before ozone treatment. The COD and BOD₅ concentration of the PHWW was 39,825 ± 884 mg/L, and 12,200 ± 346 mg/L respectively. The PHWW had a high concentration of acids, including lactic acid (7,597 ± 873 mg/L), acetic acid (2,415 ± 170 mg/L), propionic acid (2,176 ± 31 mg/L), i-butyric acid (456 ± 76 mg/L), n-butyric acid (2,464 ± 86 mg/L) and valeric acid (489 ± 10 mg/L). The total nitrogen content of the PHWW was 1,685 ± 55 mg/L, and the NH₄⁺-N was 555 ± 7 mg/L.

**Experimental setup**

The setup for PHWW ozone treatment is shown in Figure 1. The ozone gas (2.32 mg/min) was produced by a portable ozone producer (SATA 03601) fed with ambient air. The
flow rate of the air was maintained at 0.1 L/min by a rotameter. A sample of 100 mL of PHWW was put in a 250-mL glass column reactor. To improve the mass transfer of ozone in the PHWW, a magnetic stirrer was used in the glass column reactor. A trap of 200 mL of 20 g/L KI solution was used to capture the excess ozone. The excess ozone was quantified by titration using 5 mM sodium thiosulfate standard solutions (Byers & Saltzman 1958). The 5 g/L starch aqueous solution was used as an indicator. The pH was not controlled during ozone treatment. Samples of ozone-treated PHWW were collected at 0, 30, 60, 90, 120, and 200 min, respectively. All the experiments were conducted in duplicate at room temperature.

**Analytical methods**

To evaluate the changes in biodegradation during ozone treatment, the COD and biological oxygen demand for 5 days (BOD₅) were measured. The COD was evaluated by Hach method 8000, the BOD₅ was measured by Hach method 8043, and the aerobic sludge was used as the bacterial seeds which were collected from the Urbana Sanitary District (Urbana, IL, USA). To better understand the nitrogen distribution during ozone treatment, the ammonia and total nitrogen (TN) content were analyzed. The ammonia content was measured by Hach method 8058. TN analysis was conducted according to Hach method 10071. The pH changes were measured by a pH meter. The organic compounds composition was analyzed by gas chromatography–mass spectrometry (GC-MS) (7890A, Agilent Technologies, Santa Clara, CA, USA) as previously described (Tommaso et al. 2015). The mass spectra were interpreted according to the NIST Mass Spectral Database (NIST08) and W8N08 library (John Wiley & Sons, Inc.). An internal standard (3-methyl butanoic acid, 0.1 μM) was used to normalize all data before comparing between samples. The quantification of acids was conducted by a high-performance liquid chromatograph (Shimadzu Scientific Instruments, USA) equipped with a refractive index detector and an Aminex HPX-87H column (Bio-RAD, CA, USA).

**RESULTS AND DISCUSSION**

**Organic compounds distribution during ozone treatment**

Ozone treatment was conducted from 0 to 200 min, corresponding to an ozone dosage of 0 to 4.64 mg O₃/mL PHWW, respectively. After 130 min, an outflow of ozone from the PHWW reactor was observed (Figure 2), suggesting the low effective reaction of ozone with chemicals in the PHWW. In order to reveal the effect of ozone treatment on PHWW, the compounds distribution during ozone treatment was analyzed based on the GC-MS results. As shown in Figure 3, the PHWW contains organic acids, N-heterocycles, phenols, and straight and branched amides derivatives. Similar distributions of the composition of PHWW were also reported by previous studies (Gai et al. 2015; Tommaso et al. 2015). The N-heterocycles and phenols that represented 30.6% and 9.2% of the total peak area, respectively, were two main inhibitors for biological treatment. The biological treatment of PHWW showed that a high toxicity of these inhibitors would lead to a long lag phase and low conversion rate, though most of the acids...
in the PHWW could be converted (Tommaso et al. 2015; Zhou et al. 2015).

The conversion of N-heterocyclic compounds and phenols were observed during ozone treatment (Figure 3). After 200 min of ozone treatment, the percentage of the relative peak area of N-heterocycles decreased from 30.6 to 24.6%. This indicated that N-heterocyclic compounds were converted by ozone. The pyridine ring is one kind of N-heterocycle which has been previously reported to be oxidized by ozone (Stern et al. 1997). Andreozzi et al. observed that pyridine oxidation occurred with a strong cleavage of the heterocyclic ring even in the initial ozonation stage, and as a result ammonia, nitrate and amide compounds were produced (Andreozzi et al. 1999). In our work, an increase in the presence of straight and branched amides was observed (Figure 3). This proved the oxidation of N-heterocyclic compounds occurred during ozone treatment. The phenols were also effectively removed during ozone treatment. The percentage of the relative peak area of phenols decreased from 9.2% to 0%. According to previous studies, organic acids were produced during the ozone treatment of phenolic compounds (De Los Santos Ramos et al. 2009). As shown in Figure 3, an increase in the content of organic acids from 51.0 to 67.0% was observed, which coincided with the decrease in the presence of phenols. The increase of the content of acids corresponded with the pH changes, which resulted in a decrease of pH from 5.1 to 4.3 (Figure 3). These produced organic acids tended to be stable, because the mineralization of organic acids can occur by the hydroxyl radical rather than by ozonolysis conducted in this study (Gamal El-Din et al. 2006). Although a low conversion of N-heterocyclic compounds was observed, ozone pretreatment could significantly improve the inhibitory effect due to the presence of competitive inhibition between phenols and N-heterocycles (Yao et al. 2011). The decrease of benzoic acid derivatives, cyclic hydrocarbons, furan and alcohols was also observed. The low percentages of these compounds in the resulting effluent signified that the increase of the biodegradability of PHWW was mainly contributed to the conversion of N-heterocycles and phenols.

**Kinetic analysis of the ozone degradation of phenols and N-heterocyclic compounds**

The values of the pseudo-first order kinetic constant (k, min⁻¹) were obtained by fitting the experimental data shown in Figure 4(a) and 4(c) to straight lines (Figure 4(b) and 4(d)). Furthermore, the values of the half-life (t₁/₂), which represented the time for reducing the concentration of phenols and N-heterocycles to half of the initial concentration before ozonation, were also calculated (Esplugas et al. 2002). As summarized in Table 1, there’s a close relationship between the functional groups attached to the phenols and the compound’s kinetic degradation. Although all phenols were converted after ozone treatment, selective reactions of phenols were observed. Two compounds in particular, 2,6-dimethoxy phenol and 4-ethyl-2-methoxy-phenol, were much more readily converted compared with other phenolic compounds. The lower t₁/₂ values for these two compounds was partly caused by their higher degradation rate, which were about 10 times that for phenol. In addition, the lag-phase also contributed to this difference, and a lag-phase of over 30 min was observed during the degradation of other phenolic compounds (Figure 4(a)). This indicated that these groups (methyl, ethyl, and methoxy) favored ozone treatment. However, these groups may not be the site of initial ozone attack, as the ozone attack mostly occurs at the aromatic rings (Tekle-R Ttering et al. 2016) followed by ring-opening and consecutive double bond cleavages (Valsania et al. 2012).

Another interesting finding was that 3-pyridinol showed the most efficient conversion when compared to other N-heterocycles (Figure 4(c) and 4(d)). This may have resulted from the OH moiety in 3-pyridinol. According to Nöthe et al., hydroxyl radicals could be generated by the reaction of ozone with dissolved organic matter (Nöthe et al. 2009). Similar to phenols, the OH moiety present in pyridinol may also form a hydroxyl radical. Hence, a powerful reaction could occur. With a lower t₁/₂ and higher k than phenol (Table 1), the presence of 3-pyridinol suggested that asymmetric N-heterocycles may be easier to break as both the C and N atom in the ring could be attacked. The other N-heterocyclic compounds, as indicated in Figure 4(c) and 4(d), showed a relatively low conversion (15.7–23.2%, based on area changes) (Figure 4(c) and 4(d)). There is limited information on the mechanisms of ozone on N-heterocycles found in previous studies. The major ozone reactive site in N-heterocyclic compounds is the nitrogen atom in the ring (Andreozzi et al. 1991; Dodd et al. 2006). Although ozone can react with heterocyclic ring structures, the slow reaction rate after 130 min indicated the limitation of ozonolysis on N-heterocycles. The sequence for the removal of pyrazine compounds was: 2,6-dimethyl-pyrazine, 2,3-dimethyl-pyrazine, 2,5-dimethyl-pyrazine, ethyl-pyrazine, methyl-pyrazine and pyrazine (from high to low), indicating
that the presence of substituents has an impact on the effective treatment of N-heterocyclic compounds. The more complicated substituent the compounds have, the easier they are to be attacked. 2-pyrrolidinone achieved a removal of 29.8%, which was higher than that of the pyrazine compounds. The asymmetric structure of the pyrrolidinone ring may make it easy to be converted by ozone, even though the pyrrolidinone ring is a saturated heterocycle. The low conversion of N-heterocycles may be caused by the acidic pH during ozone treatment, which has been reported to limit the ozone attack at nitrogen (Sonntag & Von Gunten 2017). Increasing the pH may be a method to accelerate the rate of the reaction, because alkaline conditions are favorable to the formation of hydroxyl radicals, which have a strong reaction rate. Dodd et al. reported N-heterocyclic compounds exhibited pH-dependent variation in their apparent O3 reaction rates, which could increase by up to 100 times when the pH was increased from 4 to 8 (Dodd et al. 2006). However, the nonselective reaction of hydroxyl radicals would oxidize the acids, which could be converted through further biological treatment. This nonselective reaction would also lead to a huge consumption of ozone because of the high concentration of organic compounds in the PHWW. Therefore, this process may not be economically feasible.

Table 1 | Pseudo-first-order kinetics and half-life values for the conversion of phenols and N-heterocyclic compounds

<table>
<thead>
<tr>
<th>Compound</th>
<th>k (min⁻¹)</th>
<th>t_{1/2} (min)</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenol, 2,6-dimethoxy</td>
<td>0.0454</td>
<td>15</td>
<td>0.778</td>
</tr>
<tr>
<td>Phenol, 4-ethyl-2-methoxy</td>
<td>0.0339</td>
<td>20</td>
<td>0.933</td>
</tr>
<tr>
<td>Phenol, 4-methyl-</td>
<td>0.0167</td>
<td>71</td>
<td>0.828</td>
</tr>
<tr>
<td>Phenol, 2-methoxy-</td>
<td>0.0128</td>
<td>84</td>
<td>0.987</td>
</tr>
<tr>
<td>Phenol, 4-ethyl-</td>
<td>0.0066</td>
<td>105</td>
<td>0.864</td>
</tr>
<tr>
<td>Phenol</td>
<td>0.0042</td>
<td>195</td>
<td>0.966</td>
</tr>
<tr>
<td>3-pyridinol</td>
<td>0.0206</td>
<td>34</td>
<td>0.999</td>
</tr>
</tbody>
</table>

Figure 4 | Kinetic degradation of phenols (a), (b) and N-heterocycles compounds (c), (d). A₀: the initial GC-MS area for organics; A: the GC-MS area for organics after ozone treatment.
COD, BOD$_5$, ammonia, and TN biodegradation changes

As shown in Figure 5, the COD of PHWW tended to be stable after 150 min. The COD of PHWW decreased by 6.4% at the end of ozone treatment (200 min). This indicated that a limited mineralization occurred during the reaction period. In this work, the ozone reaction tended to be dominated by ozonolysis because of the pH variation from 5.1 to 4.3 during ozone treatment, in which molecular ozone remains as the main oxidant when the pH was below 5 (Souza et al. 2010). Ozone treatment could be classified as a direct reaction with ozone molecules (ozonolysis) and indirect reactions with hydroxyl radicals (-OH) (Souza et al. 2010). Ozonolysis was a selective reaction, in which the specific groups (C¼C, OH, CH$_3$, OCH$_3$) and aromatic compounds were readily attacked, and carboxylic acids were produced (Alvares et al. 2001). The formation of -OH usually occurs in the presence of alkaline conditions. In alkaline solutions, ozone molecules are decomposed into free radicals in the presence of hydroxyl anions (OH), and subsequently produce -OH (Yong & Lin 2016). Unlike ozonolysis, the radical reactions are nonselective and extremely rapid chain reactions, which can lead to mineralization (Umar et al. 2013). Therefore, from this aspect, the low COD removal during the ozone treatment is evidence of the main reaction type during ozone treatment.

Compared to the slight COD removal, a significant increase (24.6%) of BOD$_5$ after ozone treatment was observed (Figure 5). The initial BOD$_5$/COD of PHWW was over 0.3, which is a reasonable value for biological treatment (Hu & Wang 1999; Umar et al. 2013). The biodegradable part mostly consisted of acids, but the others were not easily converted. After ozone treatment, the ratio of BOD$_5$/COD increased from 0.31 (0 min) to 0.41 (200 min). This suggested that ozone is a feasible pretreatment method to improve the biodegradability of PHWW.

The changes in the TN showed no clear trend (Figure 5). Further, the value remained relatively consistent throughout the process. However, the ammonia content significantly increased from 555 to 727 mg/L in the first 120 min, and then a slight decrease was observed. The slight decrease in the content of ammonia may be due to the conversion of ammonia to nitrate (Singer & Zilli 1975). The presence of a similar trend for both ammonia and COD indicated the
mineralization of organic nitrogen compounds during ozone treatment to inorganic nitrogen. Here, the ammonia could be directly used by microorganisms, such as algae, which suggests that the ozone treatment of PHWW would be favored over biological treatment.

CONCLUSION

In this work, ozonation was found to be an effective technique for further biological treatment of PHWW. The biodegradability of PHWW was improved and the ratio of BOD₅/COD increased from 0.31 to 0.41. The improved biodegradability mainly resulted from the conversion of phenols and N-heterocyclic compounds, which are two main inhibitors for biological treatment in PHWW. The phenols were fully converted. Although a low conversion of N-heterocycles (21.7%) was observed, ozone pretreatment significantly improved the overall inhibitory effect due to the competitive inhibition relationship between phenols and N-heterocyclic compounds. The kinetic analysis showed that chemical substituents played an important role in determining the priority of ozone reactions. The OH moiety present in the ring compounds (phenols and pyridinol) may form hydroxyl radicals, which lead to an efficient reaction. During ozone treatment, a slight COD removal (6%) was observed. Further mineralization could be conducted through biological treatment. After ozone treatment, the produced acids and ammonia would be favorable for further biological treatment. Biological treatments such as algae cultivation and anaerobic fermentation could be introduced in order to further utilize the ozone treated PHWW.

ACKNOWLEDGEMENT

This work was financially supported by the National Natural Science Foundation of China (No. 51625804, No. 51478324) and the China Scholarship Council.

REFERENCES

Nöthe, T., Fahnenkamp, H. & Sonntag, C. v. 2009 Ozonation of wastewater: rate of ozone consumption and hydroxyl radical
yield. Environmental Science and Technology 43 (15), 5990–5995.


