Effect of temperature on recalcitrant dissolved organic nitrogen (rDON) concentration: Application of thermochemical treatment of biosolids

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Abstract

The hydrothermal liquefaction (HTL) reaction was tested on biosolids at 270, 300, 330, and 345℃ to determine the effect of temperature on the recalcitrant dissolved organic nitrogen (rDON) formation. Total nitrogen (TN) of aqueous co-product of HTL (ACP) slightly decreased with increases in temperature from 5100 ± 690 mg/L to 4200 ± 120 mg/L. The ammonia increased from 2200 ± 40 mg/L to 2500 ± 20 mg/L. The half-maximal response (EC_{50}) decreased from 6.8 %ACP to 1.0 %ACP concentration. The rDON5 concentration of the ACP increased from 1200 ± 30 mg-N/L to 1600 ± 60 mg-N/L for temperatures from 300℃ to 345℃. In this study, a higher concentration of rDON along with more diversity of N-containing heterocyclic compounds were associated with greater toxicity.

Introduction

As utilities work to improve energy efficiency, new biosolids treatment processes are being adopted, including thermal hydrolysis (CAMBI) and hydrothermal liquefaction (Genifuel). A thermochemical process called hydrothermal liquefaction (HTL) utilizes water at high temperatures and pressures to convert the biomass to carbon-rich crude oil (Roberts et al. 2013). Energy recovery through thermochemical methods requires three general processes: feed processing, deconstruction (gasification, pyrolysis, or liquefaction), and upgrading (Sturm 2016). Genifuel Corporation utilizes HTL and catalytic hydrothermal gasification (CHG) to convert wet biomass (as low as 15% solids) into biocrude oil. The products can be processed with their fossil fuel equivalents within existing petrochemical infrastructure.

HTL begin with 10-20% dry weight biosolids, while other thermochemical processes require biosolids to have a low moisture content (>80% dry weight). Also, HTL process has been tested with many feedstocks including wastewater sludge and wastewater-cultivated algal biomass (Roberts et al. 2013, Roberts et al. 2015). HTL converts all macromolecules, i.e., lipid, protein, and carbohydrate, to post HTL products (Maddi et al. 2016). During the temperature ramp and holding time, interactions between macromolecules have characteristics similar to the Maillard reaction (Liu et al. 2008, Peterson et al. 2010). The Maillard reaction occurs between the amine groups present in proteins and the carbonyl groups present in carbohydrates (Peterson et al. 2010) which result in a broad range of brown, high molecular weight polymers referred to as melanoidins (Martins 2003, Peterson et al. 2010, Wang et al. 2011).

Melanoidins are responsible for color and recalcitrant dissolved organic nitrogen (rDON) compounds in the ACP of HTL. In addition to color and recalcitrant nitrogen, a list of 48 N-containing organic heterocyclic compounds that are commonly reported in the ACP are highly toxic to mammalian cells (He et al. 2017, Hu et al. 2017, Jena et al. 2011, Maddi et al. 2017). Jena reported that 7.5% ACP induced a 50% reduction in Chinese hamster ovary cell density (Jena et al. 2011).

For a utility to adopt thermochemical processes to convert biosolids, there must be a clear mass and energy balances or pathway for the conversion of the biosolids to minimize the risk of innovation. This study focuses on the dissolved organic nitrogen (DON) fraction of ACP to determine the biodegradable (bDON) and recalcitrant (rDON) fractions. In the study, HTL was performed at four different temperatures of 270, 300, 330, and 345 °C to determine the effect on the formation of rDON.

Material and methods

Four HTL reactions were performed with 300 mL of 10 wt% algal biomass suspension loaded into a 450 mL Parr reactor. The final temperatures of 270, 300, 330, and 345 °C were tested, corresponding to pressures of 840, 1300, 1900, and 2300 psi. The temperature ramp rate was 5 °C/min, and the holding time was 1 hour. ACP was then recovered by centrifugation at 4000 rpm for 1 minute and filtration through 0.45 µm.

To quantify rDON in the ACP, a 5-day biological nitrogen uptake (rDON5) experiment was conducted with 1% or 0.5% ACP as the sole N source of growth media using *E. coli* and *P. putida* and grown for 5 days at 37 °C, shaking at 120 rpm. At the end of the 5-day period, the samples were centrifuged at 10,000 rpm for 2 minutes and filtered through a 0.22 µm filter. The rDON5 and bDON part of ACP was measured using equation (1) and (2).

$$rDON5 = (TN_{PG} - Ammonia_{PG}) * ACP\ dilution \qquad \text{Equation (1)}$$

$$TN = Ammonia + rDON5 + bDON \qquad \text{Equation (2)}$$

Where TN and ammonia are the concentrations of total nitrogen and ammonia in the ACP, respectively; and TN_{PG} and $Ammonia_{PG}$ are the concentrations of total nitrogen and ammonia in the post-growth supernatant. The color of HTL-ACP was analyzed for absorbance over the range of 390-700 nm at a scan rate of -300 nm/min with a data interval of 0.5 nm by a BioTek™ Eon™ Microplate Spectrophotometer (Peterson et al. 2010). Also, the effective concentration of rDON compounds at a half-maximal response (EC_{50}) was evaluated for 1% to 10% dilution of ACP using *B. subtilis*. The EC_{50} reflects the concentration of rDON that produced a 50% reduced maximum growth rate of tested bacteria when incubated for 24 hours at 37 °C. The MOPS media was modified by adding different concentrations of ammonia for each ACP, as ACP contains ammonia, to make a constant ammonia concentration of 2300 mg-N/L in all % ACP samples. Using a microplate reader, density measurements were taken at 650 nm every 15 minutes. The optical density data was then analyzed by logistic regression (Eq. 3) to find the maximum rate of growth

(r_{max}) for each ACP concentration. Then, the Hill equation (Eq. 4) (Goutelle et al. 2008), was numerically solved based on r_{max} and maximum %Fit (Eq. 5) for a range of possible EC_{50} values, and the Hill equation coefficient (n) was reported.

$$\frac{dN}{dt} = r_{max}\left(\frac{K-N}{K}\right) * N \qquad \textit{Equation (3)}$$

$$r_{max} = r_{bottom} + \frac{(r_{top}-r_{bottom})}{1+(\frac{\%ACP}{EC_{50}})^{-n}} \qquad \textit{Equation (4)}$$

$$Fit\% = \frac{1-\sum(r_{measured}-r_{modeled})^2/n^{0.5}}{maximum\ of\ r_{measured}} \qquad \textit{Equation (5)}$$

Where r_{bottom} and r_{top} are the maximum and minimum growth rate for the set of %ACP. And, dN/dt is per capita growth rate, K is carrying capacity, and N is population size.

Results and Discussion

Figure 1 shows the trend between ammonia and DON concentrations of ACP at varying temperature. By increasing the temperature from 270 ℃ to 345 ℃, the ammonia increased from 2200 ± 40 mg/L to 2500 ± 20 mg/L, and DON decreased from 2800 ± 350 mg/L to 1700 ± 60 mg/L (Table 1). Ammonia starts being released to the solution at temperatures lower than 180 ℃ (Yu et al. 2011) as a result of hydrolysis of macromolecular content of biosolids. In addition, decomposition of melanoidins at temperatures higher than 200 ℃ releases more ammonia. Consequently, DON concentration decrease since melanoidins are consider as a part of DON. Nevertheless, decomposition of the melanoidins begins at 200 ℃ and continues to temperatures close to 350 ℃, and decomposition increases the formation of water-insoluble compounds (Minowa et al. 2004).

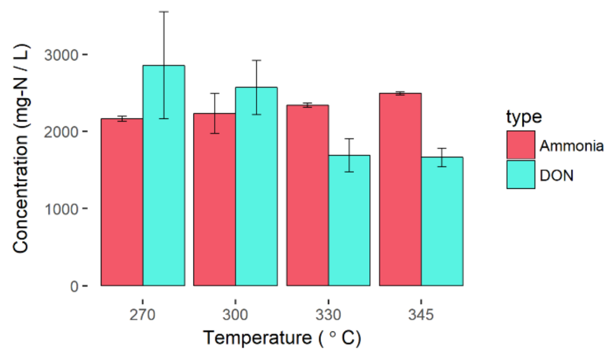

Figure 1. Concentration of ammonia and dissolved organic nitrogen from total nitrogen (TN) of aqueous co-product of hydrothermal liquefaction HTL-ACP for temperatures of 270, 300, 330, and 345 ℃.

Table 1. Properties of aqueous co-product of hydrothermal liquefaction HTL-ACP for temperatures of 270, 300, 330, and 345 ℃.

	N-containing compounds	Toxicity (EC_{50})		TN	TOC	Ammonia	DON
Temperature ℃	Measured GC-MS (number #)	Measured (%ACP)	Measured Hill coef. (n)	Measured (mg/l)	Measured (mg/l)	Measured (mg/l)	Calculated (mg/l)
270	7*	6.8 (91.6%**)	-0.30	5100 (690***)	16000 (710)	2200 (40)	2800 (350)
300	8	5.3 (93.9%)	-1.5	4800 (240)	9500 (190)	2300 (70)	2500 (120)
330	10	4.4 (81.8%)	-0.12	4000 (200)	7600 (130)	2300 (30)	1700 (60)
345	11	1.0 (90.5%)	-0.36	4200 (120)	8300 (150)	2500 (20)	1700 (60)

**Number of dominant groups of N-containing heterocyclic compounds*
***percent model fit*
****Standard deviation inside of parentheses.*

Formation of water-insoluble compounds can be interpreted as a decrease in total organic carbon (TOC) of ACP. Table 1 shows a decreasing trend for total organic carbon (TOC) concentration with increasing temperature. Melanoidins has been criticized for color production during thermal hydrolysis process (THP) which reduce UV transmittance for disinfection. Researchers in Australia have shown that decreasing the temperature from 165°C to 140°C is effective at decreasing color in the supernatant (Dwyer et al. 2008). The color of ACP was analyzed for absorbance over the range of 390-700 nm (Figure 2). The color became less intense from 270 to 345 °C. The absorption trends show the same decrease in TOC and DON concentration of ACP which can be a clear reason for a drop in color intensity.

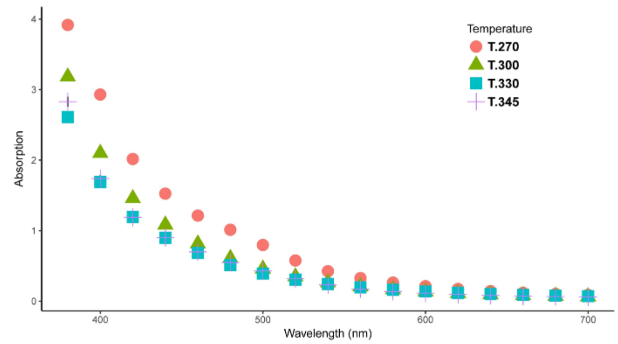

Figure 2. The color of ACP for absorbance over the range of 390-700 nm.

Figure 3 illustrates that when the final reaction temperature was increased from 300 °C to 345 °C, the rDON5 concentration of the ACP increased from 1200 ± 27 mg-N/L to 1600 ± 61 mg-N/L, and bDON decreased from 1300 ± 120 to 70 ± 60 mg-N/L of DON. A list of four N-containing heterocyclic compounds (pyrazine, pyrrolidine, pyrrolidinone, and piperidinone) were consistently present at all temperatures. The diversity of N-containing heterocyclic compounds increased from 7 to 11 with temperature increases from 270 °C to 345 °C based on GC-MS data. The GC-MS data of extracted compounds from ACP have shown increase in two or more methyl groups by increasing the temperature, and they are toxic. Also, the toxicity data illustrated a decrease in EC_{50} from 6.8 %ACP to 1.0 %ACP concentration for temperatures from 270 °C to 345 °C. Therefore, an increase in rDON5 concentration can be interpreted as a result of producing more toxic compounds at higher temperatures.

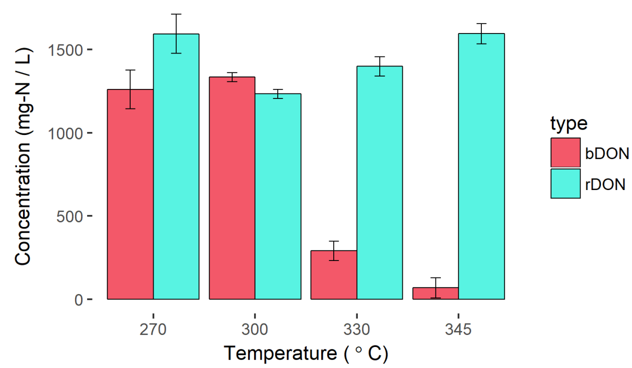

Figure 3. Distribution of recalcitrant dissolved organic nitrogen (rDON) and biodegradable (bDON) of aqueous co-product of hydrothermal liquefaction (HTL-ACP) for temperatures of 270, 300, 330, and 345 ℃.

The nitrogen content of biomass is distributed between the products of the HTL reaction, depending on the reaction condition and biomass characterization (LIN et al. 2016). The N content of gases is negligible (Van Doren et al. 2017) and in all cases, we assumed it as 0.1% of total nitrogen input. The N content of biochar and biocrude products are reported together by difference from total input nitrogen and ACP-TN. Yu et al. reported increases from 0.4% to 23.4% for N content of biocrude and decreases from 98.6% to 3.1% for the N content of biochar when the reaction temperature increased from 100 °C to 300 °C, respectively (Yu et al. 2011). The Sankey diagrams for four different reactions illustrates the effect of temperature for total N distribution. The crude and biochar N content for 270, 300, 330, and 345 °C were 37.6%, 40.2%, 49.8%, and 48.2%, respectively (Figure 4). The percent ammonia increased from 27.3% to 31.0% by increasing temperature from 270 to 345 °C (Figure 4). The bDON drastically decreased from 15.2% to 0.8% of total input nitrogen.

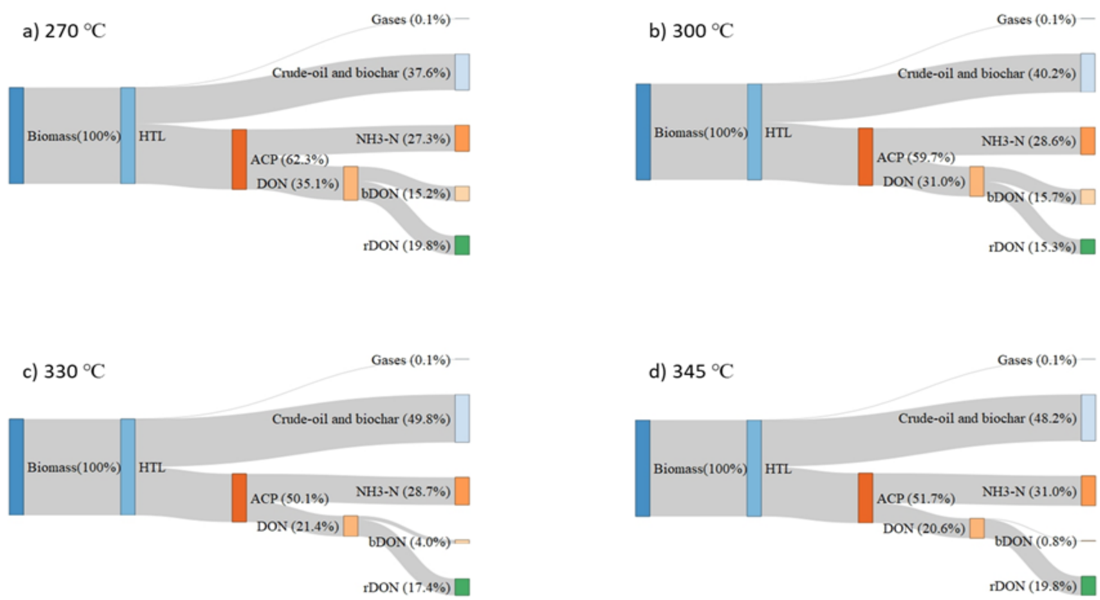

Figure 4. Sankey diagrams showing the mass flows (%) for each of the four reactions: HTL reaction temperature a) 270 ℃; b) 300 ℃; c) 330 ℃; and d) 345 ℃. HTL, hydrothermal liquefaction; ACP, aqueous co-product; DON, dissolved organic nitrogen; rDON, recalcitrant

Conclusion

This study provided an overview of the formation of rDON under thermal biosolids treatment. It reviewed laboratory methods to measure rDON, which cannot be measured directly. Experimental results from a 5-day bacterial bioassay have shown that the rDON5 concentration increased from 1200 ± 30 mg-N/L to 1600 ± 60 mg-N/L as the temperature increased from 300 to 345 °C for thermal biosolids treatment. The diversity of N-containing organic heterocyclic compounds also increased from 7 to 11; the ammonia concentrations increased from 2200 ± 40 mg-N/l to 2500 ± 20 mg-N/l, and the toxicity increased three times as temperatures ranged from 270 to 345 °C. Increasing temperature produced more rDON, which likely related to more diverse heterocyclic compounds and also resulted in higher toxicity.

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