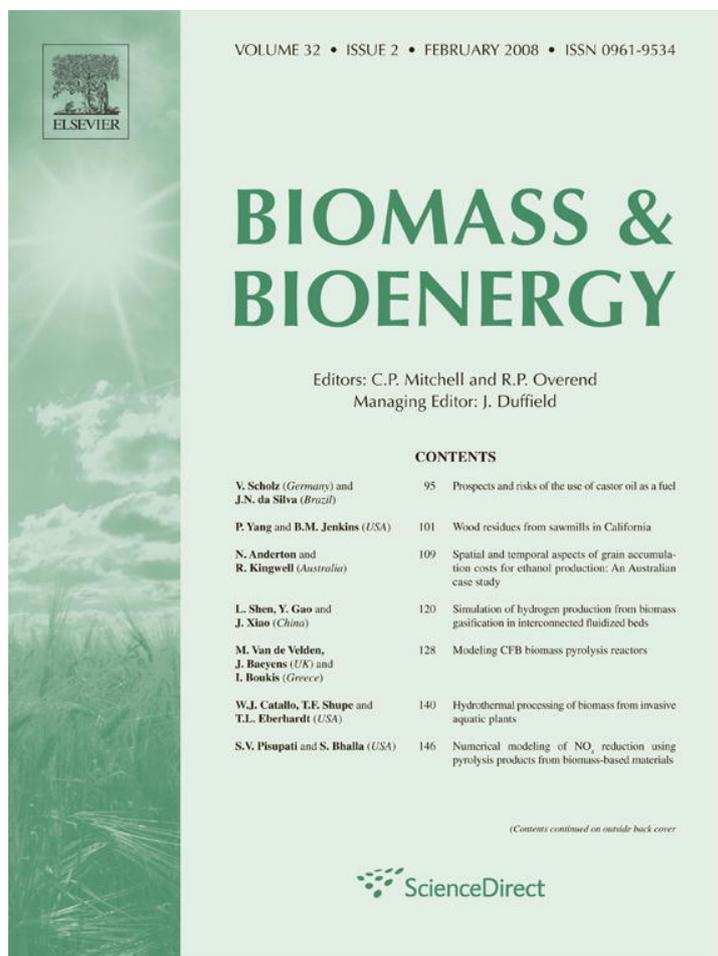


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## Hydrothermal processing of biomass from invasive aquatic plants

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### ABSTRACT

The purpose of this study was to examine the hydrothermal (HT) treatment of three invasive aquatic plants (i.e., *Lemna* sp., *Hydrilla* sp., and *Eichhornia* sp.) with respect to the generation of semi-volatile hydrocarbon product mixtures and biomass volume reduction. Identical HT treatments yielded similar semi-volatile product mixtures for *Hydrilla* sp. and *Eichhornia* sp. versus a significantly different mixture for *Lemna* sp. Pre-treatment (i.e., control) extracts of the plant substrates showed no semi-volatile hydrocarbons. Post-HT treatment product mixtures were comprised of complex mixtures of compounds including branched and unbranched alkanes and alkenes as well as light aromatics including substituted benzenes and phenols. All three plant HT product mixtures were dominated by phenol, C<sub>1</sub> alkyl phenols, and oxygenated cycloalkenes. *Lemna* sp. products showed much more diverse distributions of C<sub>2</sub>–C<sub>5</sub> alkyl benzenes, alkyl indanes, and alkyl naphthalenes at higher relative levels. Other products from the *Lemna* sp. HT treatment included C<sub>2</sub>–C<sub>4</sub> phenols, and alkyl indole and indanol compounds. Results of wet chemical analyses showed that a major difference between *Lemna* sp. and the other two plants was significantly higher extractives levels in the former. It was found that this fraction accounted for much of the complexity in HT product mixture of the *Lemna* sp. biomass. For all HT treatments the substrate mass was reduced by 95% or more.

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## 1. Introduction

In recent years, the authors have documented the transformation of under-utilized biomass (WB) into gas-phase and semi-volatile hydrocarbon mixtures in hydrothermal (HT) reaction systems. HT refers to near- and supercritical water systems (e.g., 300–700 °C, 20–50 MPa) under anoxic (reducing) conditions. HT treatments of all types of biomass (e.g., protein, cellulose, chitin, starch, DNA, bacteria cells, yeast cells, diatoms, decommissioned preservative-treated wood, grass, invasive and noxious terrestrial vegetation, and municipal sewage slurry) have resulted in transformation of the biomolecules to mixtures of gas- and liquid-phase aromatic and aliphatic

chemicals [1–4]. HT transformation data in these studies were generated under simple reaction conditions (i.e., the substrates were treated in heated water under pressure with no added reactants or catalysts) with the intention of (1) estimating gas-phase and semi-volatile hydrocarbon yields of WB resources, (2) identifying the most abundant hydrocarbons generated in this process, and (3) relating where possible the products to specific precursors in the WB.

Currently, there are no data in the public domain on products resulting from HT treatment of vascular aquatic plants, particularly those which pose ecological problems (i.e., invasive or noxious nature). In many temperate aquatic ecosystems, non-woody aquatic pest vegetation can become

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dominant and have profound adverse ecological impacts. In particular, invasive aquatic plants, growing in or near water that are either emergent, submergent, or floating, can interfere with lake biogeochemical processes, fisheries, recreational activities (e.g., swimming and boating), and detract from the aesthetic appeal of the system [5].

Aquatic plants of particular concern in the US include hydrilla (*Hydrilla* sp.), water hyacinth (*Eichhornia* sp.), and small duckweed (*Lemna* sp.). The first two species above are listed on the USDA Federal Noxious Weed List [6]. All three plants are widely distributed in the southeast and southwest US with strongest infestations in the Gulf Coast states [7]. The distribution of the plants is variable each year, depending on numerous biological factors. In general, the populations of each of these species seem to be increasing. For example, in 1988 the Florida Department of Natural Resources estimated that over 200 km<sup>2</sup> of water in Florida contained *Hydrilla* sp. [8]. *Hydrilla* sp. continues to spread in Florida and in 1995 covered 400 km<sup>2</sup> of water in 43% of public lakes [7]. *Hydrilla* sp. and other noxious aquatic plants are distributed by animals (e.g., birds) and are also transported unintentionally when boats and related gear are moved from one water body to another. Of the 26,000 km<sup>2</sup> of aquatic habitat in Louisiana, more than 16,000 km<sup>2</sup> are subject to noxious growth of aquatic vegetation in a given year [9].

A range of efforts have been employed to control these plants but have yielded limited success. Currently, the most common control techniques include dredging of waterways and herbicides (i.e., 2,4-D for hyacinth and fluridone (Sonar) for hydrilla). Both methods can be costly and can adversely affect the ecosystem. After dredging, this material is commonly left to decompose.

There have been, however, attempts to industrially utilize biomass from these plants. For example, biogeneration of methane has been reported for *Eichhornia* sp. with yields of approximately 16% over an extended treatment period [10]. Other work has explored the use of *Eichhornia* sp. for paper products [11], livestock feed, and removal of N and P species in sewage from waterways, and removal of heavy metals from solution [12,13]. In all cases, including bio-methane generation, substantial amounts of unreacted or partially degraded substrate remained after utilization. In some cases, this residue was contaminated with heavy metals and toxic chemicals. As HT approaches have been shown to generate gas-phase and semi-volatile hydrocarbon mixtures (with yields of approximately 30% and 10%, respectively), afford almost complete elimination of the substrate mass, and facilitate the recovery of heavy metals and toxic organic compounds, it was of interest to evaluate the HT treatment of these plants. Therefore, the objective of this research was to evaluate the HT treatment of three major pest aquatic plants with respect to hydrocarbon yields, chemical composition of semi-volatile mixtures, and volume reduction.

## 2. Materials and methods

Representative whole-plant samples of *Lemna* sp., *Eichhornia* sp., and *Hydrilla* sp. were collected in June 2005 in the Atchafalaya River basin, St. Martin Parish, LA. The river basin

is at approximately 0–5 m elevation. The sample location was at 30° 5' 24 N and 91° 18' 36 W. The samples were in a vigorous state of mature growth. The authors maintained possession of the samples from collection to analyses.

Samples were brought to the laboratory in large buckets with water from the river. We attempted to remove inorganic debris (e.g., sediments) before the plant matter was spread out on the bench top in a lab and air-dried with the lights on for 1 month. Other organisms (e.g., algae, water bugs, diatoms, etc.) may have remained in the plant material after reasonable efforts were made to remove foreign materials. Dried plant material was ground using a Wiley Mill to particles that could pass through a 2 mm mesh screen. The resulting material was representative of the whole plants. Moisture contents were determined using an oven at 100 °C; ash contents were determined using a muffle furnace at 450 °C.

### 2.1. HT treatment of ground and whole samples

Weighed particle samples for each plant were added to water to afford slurries of 30 mL<sup>-1</sup>. These were charged into custom-made high-pressure/corrosion-resistant titanium autoclaves at pH 7.0. The HT slurry system was sparged by bubbling with argon for 1 h, sealed under an argon blanket, and heated at 400 ± 10 °C for 4 h including heat-up and cool-down. Internal pressures during HT treatment exceeded 30 MPa.

Untreated (control) samples of each plant were weighed into cellulose thimbles (Whatman, Springfield Mill, UK) and Soxhlet extracted with ultrapure dichloromethane (DCM) to determine the presence of any semi-volatile hydrocarbons or related materials in the substrates. The resulting extracts were dried over anhydrous sodium sulfate and analyzed by gas chromatography-mass spectrometry (GC-MS).

The GC-MS used was a Shimadzu QP500 GC-MS; DB-5 capillary column (30 m; 0.25 mm id; 0.25 μm film); injector 250 °C; temperature program 70 °C (4 min) ramp 4 °C min<sup>-1</sup> to 250 °C (10 min); sampling rate 2 Hz and mass acquisition range 50–300 amu in the full-scan mode. Analyte transfer to the mass spectrometer source was at 280 °C and the electron impact source was 70 eV. Target ions spanned the inclusive molecular weight range between C<sub>2</sub> benzenes (106 amu) and coronene (300 amu). Product identification was performed using (a) comparison of experimental data with authentic standards, (b) interpretation of mass spectra (molecular ions, isotopic structures, and logical fragment losses), and (c) comparison of spectra with computerized libraries of mass spectra. Mass spectra were considered acceptable if there was a signal:noise ratio of >3 for the base peak of interest, and minimal background interference with respect to isotopic clusters and fragments. MS tuning (perfluorotributylamine, a standard calibrant) was performed at least once daily, and all MS analyses for comparison were conducted under the same tune. Digital background subtraction and chromatographic overlay algorithms were used for further evaluation of GC-MS data from standards and extracts.

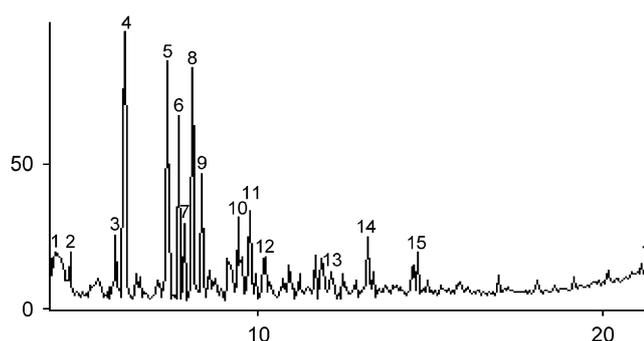
### 2.2. Wet chemistry analyses

Two aliquots of each aquatic plant sample were exhaustively extracted using a method adapted from the ASTM standard

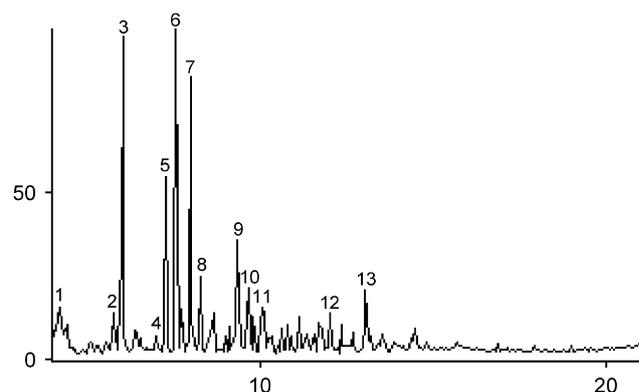
[14]. Each aliquot was extracted with ethanol:toluene (7:3, 6 h) and then ethanol (6 h) using a Soxhlet apparatus. Samples were then extracted twice (4 h and then overnight) with deionized water, dewatered, and then dried in an oven (100 °C). Organic solvent extracts were concentrated by rotary evaporation, transferred to small glass vials, and dried under a stream of N<sub>2</sub> to afford oils that were stored under vacuum (overnight) prior to weighing. Aqueous extracts were freeze-dried, collected in small glass vials, and weighed. Extractive-free aquatic plant samples were ground further in a Wiley Mill equipped with a 40-mesh sieve before lignin content determinations by the Klason [15] and acetyl bromide [16] methods. In the case of the latter, an average extinction coefficient of 17.04 L g<sup>-1</sup> cm<sup>-1</sup>, determined for a collection of herbaceous crop plants [17], was used to calculate the values for lignin content. A sample of extractive-free loblolly pine (*Pinus taeda* L.) wood (40 mesh) was analyzed as a control. An extinction coefficient of 23.30 L g<sup>-1</sup> cm<sup>-1</sup>, determined for a softwood lignin [18], was used to calculate the lignin content for this sample.

### 3. Results

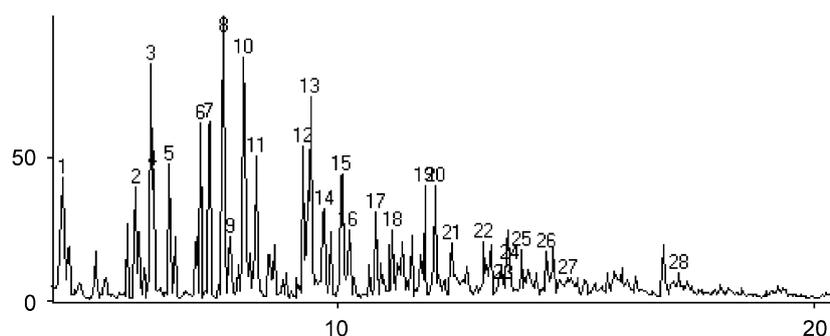
HT treatment of the three aquatic plants, *ceteris paribus*, yielded similar semi-volatile product mixtures for *Hydrilla* sp. and *Eichhornia* sp. versus a significantly different mixture for *Lemna* sp. as determined by GC-MS (Figs. 1–3). In all cases, pre-treatment (i.e., control) extracts of the plant substrates showed no semi-volatile hydrocarbons, even at concentration factors of around 500. Post-treatment product mixtures from all plants were comprised of complex mixtures of hydrocarbons including branched and unbranched alkanes and alkenes as well as light aromatics including substituted benzenes and phenols. All product mixtures were dominated by large amounts of phenol and two of its C<sub>1</sub> alkyl homologs and oxygenated cycloalkenes (e.g., cyclopentenone and C<sub>1</sub>- and C<sub>2</sub>-cyclopentenones). On the other hand, *Lemna* sp. showed a much larger number of alkyl benzenes (C<sub>2</sub> through C<sub>5</sub>), alkyl indanes, and alkyl naphthalenes at high levels. Minor products from *Lemna* sp. HT treatment



**Fig. 1 – *Hydrilla* sp.: major HT semi-volatile products.** 1. Hydrocarbon (C<sub>11</sub>). 2. C<sub>1</sub>–cyclopentenone. 3. C<sub>2</sub>–furan. 4. Phenol. 5. C<sub>2</sub>–cyclopentenone. 6. C<sub>1</sub>–phenol. 7. Acetylcyclohexene. 8. C<sub>1</sub>–phenol. 9. C<sub>3</sub>–benzaldehyde. 10. C<sub>2</sub>–phenol. 11. C<sub>2</sub>–phenol. 12. C<sub>1</sub>–benzenemethanol. 13. C<sub>2</sub>–benzenemethanol. 14. C<sub>1</sub>–indole. 15. C<sub>2</sub>–indole.



**Fig. 2 – *Eichhornia* sp.: major HT semi-volatile products.** 1. C<sub>2</sub>–cyclohexanol. 2. C<sub>1</sub>–cyclopentenone. 3. Phenol. 4. C<sub>2</sub>–cyclohexene. 5. C<sub>2</sub>–cyclopentenone. 6. C<sub>1</sub>–phenol. 7. C<sub>1</sub>–phenol. 8. Acetylcyclohexene. 9. C<sub>2</sub>–phenol (2-isomers). 10. C<sub>2</sub>–phenol (3-isomers). 11. C<sub>2</sub>–phenol. 12. Hydroxyindane. 13. C<sub>1</sub>–indole.



**Fig. 3 – *Lemna* sp.: major HT semi-volatile products.** 1. C<sub>2</sub>–benzene. 2. C<sub>3</sub>–benzene. 3. Phenol. 4. C<sub>3</sub>–benzene. 5. C<sub>3</sub>–benzene. 6. C<sub>4</sub>–benzene. 7. Indane. 8. C<sub>1</sub>–phenol. 9. C<sub>4</sub>–benzene. 10. C<sub>1</sub>–phenol. 11. C<sub>1</sub>–indane. 12. C<sub>1</sub>–indane. 13. C<sub>1</sub>–indane. 14. C<sub>2</sub>–phenol. 15. C<sub>5</sub>–benzene. 16. C<sub>2</sub>–indane. 17. C<sub>5</sub>–benzene. 18. C<sub>3</sub>–phenol. 19. C<sub>1</sub>–naphthalene. 20. C<sub>1</sub>–naphthalene. 21. Indanol. 22. C<sub>4</sub>–phenol. 23. C<sub>4</sub>–phenol. 24. C<sub>4</sub>–phenol. 25. C<sub>4</sub>–phenol. 26. C<sub>2</sub>–indole. 27. Naphthenol. 28. C<sub>2</sub>–biphenyl.

included alkyl phenols C<sub>2</sub>–C<sub>4</sub>, and alkyl indole and indanol compounds.

Questions arose as to the provenance of the significantly more elaborated and abundant semi-volatile product mixture in *Lemna* sp. HT treatment versus the other two plants, particularly with respect to the relatively high levels and extended alkyl homologous series of the benzenes and phenols. Exploratory wet chemistry analyses were performed on samples of each plant (Table 1). It was found that the three plants were comparable in terms of Klason lignin and ash contents. The acetyl bromide lignin contents for the *Lemna* sp. and *Hydrilla* sp. were also similar. Substantially

higher levels of extractives were present in the *Lemna* sp. versus the other two plants. To examine the relationship between extractives and HT products, extractives were HT treated and analyzed. Fig. 4 shows results of control and post-HT treated toluene–ethanol extractives from *Lemna* sp. Fig. 4 shows that HT treatment of the *Lemna* sp. extractives yielded highly extended alkyl series of benzene (up to C<sub>6</sub>) and phenol up to C<sub>5</sub> along with a suite of substituted and oxidized cycloalkanes and alkenes including acetylcyclohexene isomers and substituted benzaldehyde. Comparison of Figs. 3 and 4 confirmed that the extractives of *Lemna* sp. accounted for much of the complexity in the semi-volatile

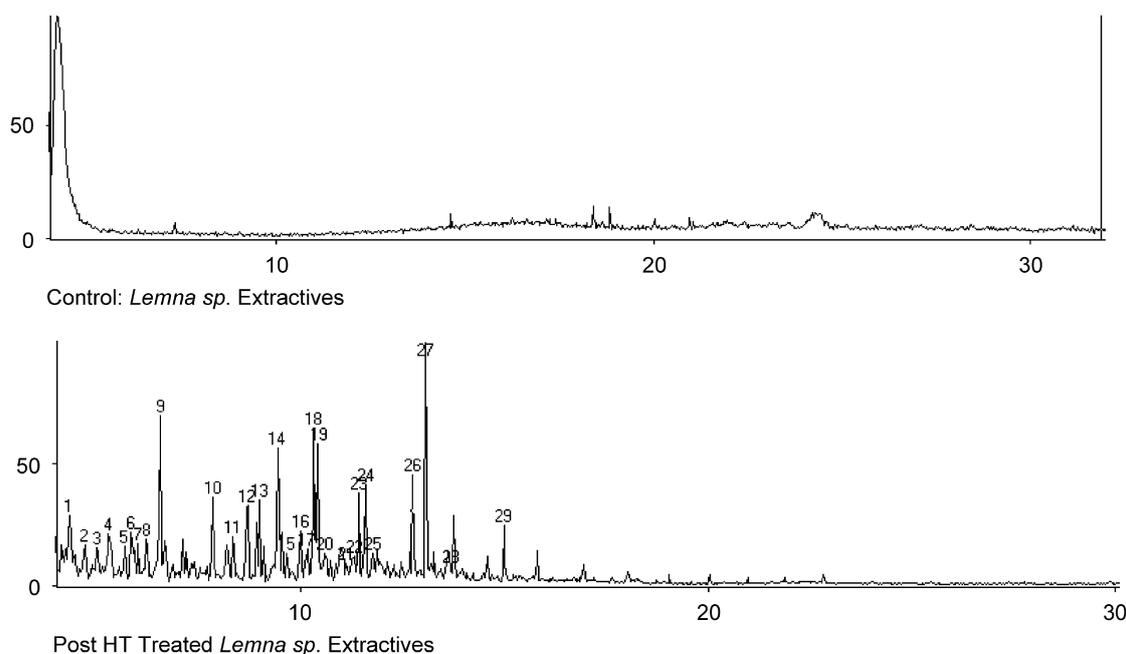
**Table 1 – Selected wet chemical analyses of three North American invasive aquatic plants**

	<i>Hydrilla</i> sp. (%)	<i>Eichhornia</i> sp. (%)	<i>Lemna</i> sp. (%)
<b>Extractives</b>			
Toluene:ethanol	4.96 <sup>a</sup> (0.30) <sup>b</sup>	4.91 (0.77)	14.77 (2.12)
Ethanol	0.95 (0.06)	0.93 (0.17)	1.93 (0.35)
Water	12.06 (0.30)	9.93 (0.98)	14.72 (0.38)
Total extractives	17.97 (0.66)	15.77 (1.58)	31.42 (2.84)
<b>Lignin</b>			
Klason method	12.24 (0.97)	15.63 (0.79)	15.22 (0.91)
Acetyl bromide method	6.96 (0.90)	20.56 (0.90)	7.71 (0.66)
Ash (total)	21.66 (0.97)	19.30 (0.15)	18.90 (0.35)

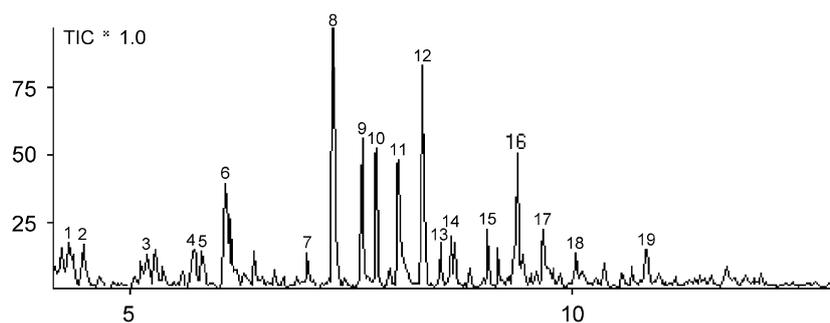
All results are based on the weight of the moisture-free unextracted plant material.

<sup>a</sup> Means based on  $n = 2$  except for Klason ( $n = 3$ ) and acetyl bromide ( $n = 4$ ) methods.

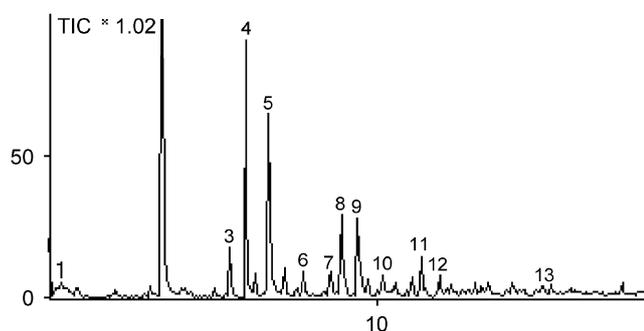
<sup>b</sup> Numbers in parentheses denote standard deviations.



**Fig. 4 – Major semi-volatile products from *Lemna* sp. extractives. Top: control (no HT treatment). Bottom: after HT treatment.**  
 1. C<sub>2</sub>–benzene. 2. Cycloalkane substituted (C<sub>10</sub>H<sub>8</sub>). 3. C<sub>3</sub>–benzene. 4. Cycloalkene (C<sub>8</sub>H<sub>14</sub>). 5–9. C<sub>3</sub>–benzene.  
 10. Acetylcyclohexene. 11. C<sub>4</sub>–benzene. 12. C<sub>5</sub>–cyclopentane. 13. C<sub>4</sub>–benzene. 14. C<sub>5</sub>–benzene. 15. C<sub>5</sub>–cyclopentane.  
 16. C<sub>3</sub>–benzaldehyde. 17. C<sub>5</sub>–benzene. 18. Allethrolone (C<sub>9</sub>H<sub>12</sub>O<sub>2</sub>). 19. C<sub>3</sub>–phenol. 20. C<sub>5</sub>–benzene. 21. C<sub>6</sub>–benzene.  
 22. C<sub>5</sub>–cyclohexane. 23. C<sub>5</sub>–benzene. 24. C<sub>4</sub>–phenol. 25–26. C<sub>4</sub>–phenol. 27. Biphenyl. 28. Diphenylmethane. 29. C<sub>5</sub>–Phenol.



**Fig. 5** – Major semi-volatile products from starch HT reactions (semi-volatile yield: approximately 4% w/w). 1. C<sub>2</sub>–cyclopentenone. 2. C<sub>2</sub>–hexene (C<sub>8</sub>H<sub>16</sub>). 3. Cyclooctane and C<sub>3</sub>–benzenes (5 isomers). 4,5. C<sub>1</sub>–cyclopentenones (2 isomers). 6. Phenol. 7. C<sub>3</sub>–cyclopentene. 8. C<sub>2</sub>–cyclopentenone. 9. C<sub>1</sub>–phenol. 10. Acetylcyclohexene. 11. C<sub>1</sub>–phenol. 12, 13. Acetylcyclohexenes (2 isomers). 14–17. C<sub>2</sub>–phenols (5 isomers). 18. C<sub>2</sub>–cyclohexenone. 19. C<sub>3</sub>–phenols (7 isomers).



**Fig. 6** – Major semi-volatile products from lignin HT treatment (semi-volatile yield approximately 55% w/w). 1. C<sub>2</sub>–cyclopentenone. 2. Phenol. 3. C<sub>2</sub>–cyclopentenone. 4, 5. C<sub>1</sub>–phenols. 6–10. C<sub>2</sub>–phenols (7 isomers). 11–13. C<sub>3</sub>–phenols (12 isomers).

products observed in the HT treatment of the whole-plant sample.

Further examination of biological precursors of HT semi-volatile compounds in the three aquatic plants included polysaccharide (starch) and lignin standards. HT product analyses for pure starch and lignin are presented in Figs. 5 and 6. These data show that these precursors give rise to relatively simple post-HT mixtures. The cell wall polysaccharides, but not lignin, were the source of the acetylcyclohexene isomers seen in the HT products of all plants and also the extractives from *Lemna* sp. On the other hand, lignin gave rise overwhelmingly to phenol and C<sub>1</sub>–C<sub>3</sub> alkyl phenols. It was concluded that the extractives are the major determinants of the semi-volatile mixtures obtained from HT treatment of the *Lemna* sp.

#### 4. Discussion

The objective of this study was to examine HT treatment of three aquatic plant species (i.e., *Lemna* sp., *Hydrilla* sp., and *Eichhornia* sp.) with respect to the generation of hydrocarbon product mixtures and biomass volume reduction. HT treatment of *Eichhornia* sp. and *Hydrilla* sp. yielded similar semi-volatile product mixtures, with *Lemna* sp. showing substantial

products with no semi-volatiles detected even in highly concentrated control extracts. HT treatment produced a substrate volume reduction of around 95% (i.e., carbonaceous and inorganic particulate residues were equal to or less than 5% by weight) for each plant species leaving particulate residues that had some fuel value (i.e., dried particles were combustible).

The semi-volatile chemical mixtures reflected HT transformation mainly of extractives with contributions from polysaccharides and smaller amounts of lignin. It was found that HT yields for starch were far less than the yields from lignin (approximately 4% and 55%, respectively). This is because the oxygen in starch and other polysaccharides (e.g., cellulose; 55% mass) is reactive and can compete with desirable HT reduction reactions. Conversely, lignin is a polyphenolic macromolecule which yields phenol and its C<sub>1</sub>–C<sub>3</sub> alkyl homologs and methoxylated phenols. Except for the methoxylated species, the substituted phenols and phenol are stable in HT conditions. Therefore, the lignin HT product mixture reflected breakdown (e.g., hydrolysis) of the polymer with minimal chemical reaction of the substrate and minimal competition from reactive oxygen in the substrate. Conversely, the subunit of starch (cellulose) can yield a phenol only after substantial chemical modification (e.g., dehydroxylation, reduction). Furthermore, the product mixture from HT treatment of starch is more complicated than that of the lignin, the latter of which is dominated by phenols.

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