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Research paper

# Chemometric analysis of composition of bio-crude and aqueous phase from hydrothermal liquefaction of thermally and chemically pretreated *Miscanthus* x *giganteus*

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ABSTRACT

Bio-crude from hydrothermal liquefaction (HTL) of lignocellulosic biomass is a potential alternative to conventional fossil fuels. Continuous HTL of lignocellulosic biomass requires feedstock pretreatment to obtain pumpable slurries. This work extends upon previous evaluations of pretreatment methods for obtaining stable slurries of *Miscanthus* x *giganteus*. In the current study, extensive characterization of the bio-crude and aqueous phase from HTL of thermally and chemically pretreated *M.* x *giganteus* is reported and molecular differences of these samples are evaluated. The bio-crude and aqueous phase is characterized with gas chromatography coupled to mass spectrometry using pre-derivatization with silylating reagent and methyl chloroformate, respectively. Principal component analysis shows that bio-crudes of untreated and slurry stabilized samples have higher concentrations of small organic acids and fatty acids, samples pretreated at mild alkali conditions have higher concentrations of alcohols, polyaromatics, and methylated phenolics, and samples pretreated with strong alkali conditions have higher concentrations of cyclic oxygenates and ketonized aromatics. Aqueous phases are separated based on the addition of slurry stabilizer which has higher concentrations of dicarboxylic acids and phenol. The detailed exploration of both bio-crude and aqueous phase will be of interest for other investigations of the pretreatment effects on biomass for hydrothermal liquefaction.

## 1. Introduction

Increasing demand for fossil fuels and chemicals along with ever depleting amounts of crude oil is facilitating research in sustainable fuel and bio-based chemical platforms. Production methods for bio-fuels include: fermentation, transesterification, pyrolysis, hydrothermal carbonization, and hydrothermal liquefaction (HTL) [1,2]. HTL is carried out at near-critical conditions which are typically 300–400 °C and 20–30 MPa [3]. The main advantages of HTL are that drying or dewatering of the feedstock are typically not necessary and the oxygen content of the bio-crude is reduced to less than 20% [4]. HTL utilizes the unique properties of hot compressed water where the ion dissociation constant of water is increased to $10^{-11}$, the dielectric constant decreases to less than 20 and the density is decreased (based on a pressure of 30 MPa) [5]. The change in physical properties of water leads to acid-base catalyzed reactions, solubility of hydrophobic compounds and stabilization of transition states which are otherwise unfavorable in aqueous solution [6].

The rapidly growing human population of especially developing countries means that demand for food will increase significantly. Large-scale expansion of bio-ethanol and bio-diesel would lead to a decrease in world food supply and significantly increase prices on sugar, corn, and oil seeds [7]. Non-food biomasses, such as lignocellulosics are abundant and certain species can be grown on non-arable land. Certain lignocellulosic biomasses which include several grass species are cultivated as perennial energy crops where costs of nutrients are minimal due to their inherent efficient nutrient recycling and acceptable growth rates leading to annual harvesting [8]. However, pumping of lignocellulosic biomass has been recognized as a major obstacle for commercial HTL [9,10]. Sedimentation and dewatering of biomass slurry means that clogging of reactor systems with higher biomass loadings is likely to occur [11]. Pretreatment of biomass to obtain stable slurries include mechanical, biological, chemical, and thermal treatment and also the addition of slurry stabilizers [12]. Pretreatment methods involving two-stage

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HTL have been proposed as an effective method of isolating polysaccharides and nitrogen containing compounds in the first stage while producing bio-crude of high quality in the second stage. The polysaccharides are potential value-added chemicals while the nitrogen containing fraction can be used for algae cultivation [13,14]. The effect of several of the aforementioned methods has been investigated with regard to loading capability and bio-crude characteristics [15]. However, detailed molecular characteristics of the bio-crude and aqueous phase from different biomass pretreatment methods have not been reported and are the sole aim of this work. Knowledge of the change in molecular characteristics would be of importance for further processing of the bio-crude into fuel or potential value-added chemicals.

Much research in HTL has been invested into possible utilization of the aqueous phase such as recirculation to make biomass slurries [16], its use for recycling to cultivate algae [17], the production of fuel gas or hydrogen through anaerobic digestion or hydrothermal gasification [18,19]. Hence, knowledge of the effect of biomass pretreatment on the aqueous product fraction from HTL is valuable for further processing of the aqueous phase.

The aim of the pretreatment procedure conducted in this work was to increase the dispersability of the biomass without altering the chemical characteristics. Optimization of the pretreatment procedure and evaluation of yields, elemental composition, and gas composition has been described in detail elsewhere [20]. Thus, the sole purpose of the current work is to evaluate the differences in molecular composition of the same samples.

In this study, a comprehensive GC-MS analysis of both bio-crude and aqueous phase is presented along with principal component analysis (PCA) to investigate the impact of thermal and chemical pretreatment pathways with *M.* × *giganteus* when subsequently subjected to HTL. The highly complex sample matrices mean that significant co-elution can occur which would make extensive characterization difficult and would influence the chemometric analysis. Therefore, derivatization procedures were employed to increase/decrease volatility, improve detection limits, and to improve chromatographic performance [21]. Bio-crude was derivatized with *N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide (MSTFA) to enhance detection of compounds containing nucleophilic groups. Aqueous phase was derivatized with methyl chloroformate (MCF) without prior sample manipulation to avoid loss of volatiles, potential condensation, and to enhance extraction in chloroform. Baseline correction and peak alignment was performed on raw chromatographic data [22,23]. PCA was performed on a total of 192 analytes for the bio-crude and on 81 analytes for the aqueous phase. To our knowledge this is the first experiment to extensively evaluate GC-MS data of HTL products from pretreated lignocellulosic biomass.

## 2. Materials and methods

### *2.1. Material and reagents*

All solvents (dichloromethane, chloroform, methanol, pyridine) were obtained in analytical grade (pro analysi – p.a.) from Sigma Aldrich. Carboxymethyl cellulose (CMC) sodium salt, $K_2CO_3$ (p.a., >99%), NaOH (p.a., >99%), 4-bromotoluene (p.a., 98%), MCF (p.a., 99%), and MSTFA (p.a., >98.5%) were also obtained from Sigma Aldrich. *M.* × *giganteus* was provided by Department of Agriculture Sciences, Aarhus University, AU-Foulum, Denmark (56°29′41″N; 9°33′31″E). It was harvested in October 2014 after one year of growth. The grass was separated in stems and leaves, chopped in coarse pieces of 5–10 cm length and oven-dried overnight at 105 °C. The dried feedstock was knife-milled to a particle size of 1 mm and further cyclone milled to fine particles of <80 μm. The milled *M.* x *giganteus* was stored in a closed plastic bucket at 20 °C until further processing. Pretreatment experiments of the milled feedstock were carried out within a month upon milling.

### *2.2. HTL batch experiments*

The following describes the origin of the samples for which product yields have been presented in previous work. A detailed description of the optimized pretreatment and preparation of samples is presented elsewhere [20]. Thus, this work focuses only on the results of GC-MS analysis of the product fractions in an effort to explain the molecular effects of the optimized pretreatment method.

Six pretreatment methods were tested before the HTL experiments (Table 1). Each sample name was abbreviated as listed in Table 1 followed by a number indicating the replicate number. Each pretreatment method was repeated three times. The effect of both strong and mild alkali pretreatment was investigated. The alkali pretreatments were carried out with and without CMC addition. The impact was evaluated against HTL of slurries without alkali salts but with CMC present and finally slurries of untreated biomass. The samples containing CMC, including samples with mild and strong alkali conditions, were not subjected to any further pretreatment. The samples containing only alkali catalyst were subjected to heating at 100 °C for 15 h and subsequently put through a commercial blender.

The HTL reaction was performed in 50 $cm^3$ batch reactors acquired from the High Pressure Equipment Company (HiP). This setup is described in detail elsewhere [24]. The HTL experiment was primed by loading 25 $cm^3$ of the pretreated slurries into the reactor and sealing the reactor by means of a torque wrench set to 200 N m. The reactor was then pre-pressurized to 5 MPa with Argon gas in order to minimize steam reforming and assist post-experimental recovery of gasses formed during the HTL. The reactor was heated using an Omega Engineering FSB-4 fluidized sand bath (FSB) preheated to 360 °C. The reaction conditions were 350 °C, 25 MPa and 15 min residence time. The collected HTL products consisted of a bio-crude, an aqueous phase, a solid residue and a gas phase. The aqueous phase was recovered in a separatory funnel and extracted with dichloromethane. The reactor was washed three times with 10 $cm^3$ of dichloromethane. The dichloromethane fractions were combined and vacuum filtrated to isolate the solid residue. Finally, dichloromethane was evaporated by rotary evaporation to yield the bio-crude.

### *2.3. Analytical methods*

The total organic carbon (TOC) content of the water phase was determined in triplicates by Hach Lange DR2800 spectrophotometer with TOC-kit LCK387.

The aqueous phase was derivatized with MCF prior to GC-MS analysis. The reagent reacts efficiently with carboxylic acids and phenolics (Fig. 1). However, the reactivity towards alcohols is poor. MCF was chosen to avoid sample manipulation such as drying with potential loss of volatiles, and condensation reactions. Derivatization was carried out by mixing 200 $mm^3$ of aqueous phase, 40 $mm^3$ of 5.0% w/w sodium hydroxide solution, 200 $mm^3$ of methanol, and 50 $mm^3$ of pyridine. MCF (25 $mm^3$) was added and the sample was vortexed for 30 s followed by another 25 $mm^3$ of MCF and 30 s of vortexing. The derivatized sample was extracted with 400 $mm^3$ chloroform, and phase separation was facilitated with 400 $mm^3$ of 50 mmol $dm^{-3}$ sodium bicarbonate solution. The top aqueous layer was removed and the chloroform layer was transferred to a vial with insert. Details of the derivatization method have previously been described [25]. Analysis was performed using an Agilent 7890B GC coupled with a quadrupole mass filter MS (Agilent, 5977A). The GC