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Quantitative analysis of nitrogen containing compounds in microalgae based bio-oils using comprehensive two-dimensional gas-chromatography coupled to nitrogen chemiluminescence detector and time of flight mass spectrometer☆

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ABSTRACT

Insight in the composition of the algae derived bio-oils is crucial for the development of efficient conversion processes and better upgrading strategies for microalgae. Comprehensive two-dimensional gas chromatography (GC × GC) coupled to nitrogen chemiluminescence detector (NCD) and time-of-flight mass spectrometer (TOF-MS) allows to obtain the detailed quantitative composition of the nitrogen containing compounds in the aqueous and the organic fraction of fast pyrolysis bio-oils from microalgae. Normal phase (apolar × mid-polar) and reverse phase column (polar × apolar) combination are investigated to optimize the separation of the detected nitrogen containing compounds. The reverse phase column combination gives the most detailed information in terms of the nitrogen containing compounds. The combined information from the GC × GC–TOF-MS (qualitative) and GC × GC–NCD (quantitative) with the use of a well-chosen internal standard, i.e. caprolactam, enables the identification and quantification of nitrogen containing compounds belonging to 13 different classes: amines, imidazoles, amides, imides, nitriles, pyrazines, pyridines, indoles, pyrazoles, pyrimidines, quinolines, pyrimidinediones and other nitrogen containing compounds which were not assigned to a specific class. The aqueous fraction mostly consists of amines (4.0 wt%) and imidazoles (2.8 wt%) corresponding to approximately 80 wt% of the total identified nitrogen containing compounds. On the other hand, the organic fraction shows a more diverse distribution of nitrogen containing compounds with the majority of the compounds quantified as amides (3.0 wt%), indoles (2.0 wt%), amines (1.7 wt%) and imides (1.3 wt%) corresponding to approximately 65 wt% of the total identified nitrogen containing compounds.

1. Introduction

Fossil fuels will continue to be accessible at low cost for a considerable amount of time due to the existence of potential reserves, increased exploitation of unconventional reserves such as shale oil and with the recent technological progresses in the recovery of petroleum and gas using advanced production methods such as horizontal drilling and fracturing [1–6]. On the other hand, there are serious concerns regarding the variations in the security of supply arising from geopolitical instabilities, ever-increasing demand for finite fossil resources and, the more important, environmental concerns related with greenhouse gas emissions [1,2,7]. Therefore, there is an urgent need to investigate renewable, abundant and cleaner alternative feedstocks such as biomass to replace fossil resources. Among the renewable biomass feedstocks, microalgae are considered as third generation biomass feedstocks, i.e. not competing with food nor for land. Indeed, microalgae production does not compete with agricultural land used for food crops since they can be cultivated on non-productive, non-arable lands such as coasts, deserts and offshore marine environments or even in waste water [8,9]. Further superior characteristics compared to terrestrial plants include higher growth rate, higher area-specific yield and superior photosynthetic efficiency [1,8,10]. Finally, they can

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also be integrated with fossil-fuel-fired power plants for capture and use of CO_2 through photosynthesis [1].

Microalgae are composed of proteins, carbohydrates, lipids, and other compounds such as ashes and acids [11]. There are two main pathways namely, biochemical and thermochemical pathways to transform microalgae biomass into biofuels. It is very crucial to select the right conversion technology to ensure that the biofuel production is sustainable but also has the potential to become economically viable. Biochemical conversion processes suffer from long reaction times (on the order of days), lengthy reaction steps, high production costs and low conversion efficiency caused by enzymes and microbes [10]. On the other hand, thermochemical conversion is considered as an interesting conversion method which can utilize all the components present in the microalgae without the need of any chemical addition in combination with short conversion times (on the order of seconds to minutes) [8,10–12]. Pyrolysis is viewed as one of the promising thermochemical conversion methods to convert algal biomass to biofuels with high fuel-to-feed ratios [13].

Fast pyrolysis is a thermochemical decomposition process occurring usually at a temperature range of 400 and 600 °C with short residence times (orders of seconds to minutes) in the absence of oxygen or air, to produce a mixture of gases, liquid (bio-oil) and solids residue [14–22]. Bio-oil from microalgae is a complex mixture of aliphatic and aromatic hydrocarbons, phenols, long chain fatty acids (C_{14}-C_{22}) and nitrogen containing compounds which are formed due to the degradation of proteins and chlorophyll [15,18,23]. In contrast to the under-functionalized petroleum, mostly containing C and H, microalgae is over functionalized, and hence contains substantial amounts of O- and N-containing compounds which could require further upgrading of bio-oil via deoxygenation and denitrogenation [1,11] if they cannot be extracted. If bio-oils with large amounts of nitrogen containing compounds would be combusted, nitrogen oxides (NO_x) and soot would be formed, leading to considerable air pollution [1,11]. Detailed compositional analysis of bio-oils is required not only to develop efficient conversion processes but also to optimize the upgrading processes. Note that there is already some information about the characterization of microalgae based bio-oils using gas chromatography coupled to mass spectrometry (GC–MS) [14–17,20–22] and to a lesser extent with gas chromatography coupled to flame ionization detector (GC-FID) [18,19].

Comprehensive two dimensional gas chromatography (GC × GC) has allowed an enormous step forward for the analysis of bio-oils since it provides a considerably higher chromatographic resolution than one dimensional gas chromatography (GC) [24–31]. In principle, GC × GC–MS is the ultimate tool for the identification of unknown compounds in bio-oils. However, this method has proven to be mainly applicable for the analysis of oxygenated and pure hydrocarbons [30–39]. The quantification of nitrogen containing compounds proves to be more difficult because of the interference of hydrocarbon matrix, making the detection of nitrogen compounds using the standard techniques a challenging task [24,34,40–44]. Therefore, an element selective detector such as nitrogen chemiluminescence detector (NCD) coupled to GC × GC [24,34,40–43] was applied for the detection of nitrogen containing compounds in fossil derived oils such as plastic waste pyrolysis oil [24], shale oil [40] and petroleum fractions [34,41–43]. To the authors' knowledge this has not been done for bio-oils yet. On the other hand, algae based bio-oils are extremely suited for this purpose because of the substantial amount of nitrogen in algae and hence also in the resulting bio-oil.

Therefore, in this study the detailed compositional analysis of microalgae specifically, *Scenedesmus almeriensis* (freshwater microalgae), based fast pyrolysis bio-oils regarding nitrogen containing compounds has been performed using GC × GC coupled to an element selective detector which is NCD (quantification) and a mass selective detector TOF-MS (qualification). The influence of normal and reverse phase column combinations was investigated to further improve and optimize the separation of the detected nitrogen containing compounds. Finally, an optimal method was developed using an internal standard for the quantification of nitrogen containing compounds in microalgae based bio-oils. The information obtained from this study provides new understanding of the composition of bio-oils in general and algae bio-oils in particular, which is essential not only for the evaluation of the fast pyrolysis process but also for the better assessment of possible upgrading strategies.

Table 1
Measured elemental composition of the aqueous and organic fraction of the microalgae based bio-oil by elemental analysis.

	Aqueous fraction	Organic fraction
C (wt%)	6.04 ± 0.35	68.59 ± 2.55
H (wt%)	11.23 ± 0.02	9.50 ± 0.35
N (wt%)	3.68 ± 0.05	8.54 ± 0.99
O (wt%)	79.05 ± 0.35[a]	13.37 ± 2.76[a]

[a] Calculated by subtraction.

2. Materials and methods

2.1. Chemicals and standards

Analytical gases (helium, nitrogen, hydrogen, carbon dioxide and air) were provided at a minimum purity of 99.99% (Air Liquide, Belgium). 2,3-Dimethylindole, propanamide, 5,5-Dimethylimidazolidin-2,4-dione, 5-Ethyl-5-methylimidazolidin-2,4-dione, 1-Methylimidazolidin-2,4-dione and 2-ethylpyridine were purchased from Sigma-Aldrich with a minimum purity of 97%. 2,6-Dimethylquinoline, 2,4-Imidazolidinedione, pyrazole, 2-Methylindole, 2-Pyrrolidone, 2,5-Dimethylpyrrole, hexanitrile, 5-Methyl-2-pyrrolidone, quinoline, 2-Methylpyridine and pyrrole were purchased from Sigma-Aldrich with a minimum purity of 98%. Pyrazine, caprolactam, indole, acetamide, 2-methylpyrazine, benzonitrile, pyridine and 4-Methylquinoline were purchased from Sigma-Aldrich with a minimum purity of 99%. Tetrahydrofuran (THF) was purchased from Chem-Lab with a minimum purity of 99%. The elemental composition of the organic and aqueous fractions of the microalgae based bio-oil was obtained using Flash EA2000 (Thermo Scientific, Interscience, Belgium). The device was equipped with a thermal conductivity detector (TCD) which was used for the determination of the elemental carbon, hydrogen and nitrogen content. The elemental composition was derived based on at least three repeat analyses of the samples. The oxygen amount of both fractions of the bio-oil was determined by subtraction. Table 1 shows the elemental composition of the analyzed organic and aqueous fractions of the microalgae based bio-oil.

2.2. Sample preparation

Organic and aqueous fractions of the microalgae based bio-oil were analyzed in this study. Non-catalytic pyrolysis experiments with microalgae, namely *Scenedesmus almeriensis,* have been performed in a fully controlled, semicontinuously operated lab-scale setup [45] at a temperature of 480 °C. Upon condensation of the pyrolysis vapors, the pyrolysis-oil spontaneously phase-separated, into an aqueous phase and a heavier, organic phase.

The organic fraction was dissolved in THF (2:1, w/w) and the aqueous phase was not diluted in any solvent prior to gas chromatographic analysis. A well-chosen internal standard, specif-