



Short communication

Occurrence forms of key ash-forming elements in defatted microalgal biomass

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ABSTRACT

This study reports occurrence forms of key ash-forming elements in a defatted microalga, and for comparison, its corresponding raw microalga. Freeze-dried powders of a marine microalga (*Nannochloropsis oceanica*) were sieved to a size fraction of <75 μm and used as raw microalga. The raw microalga was then extracted with hexane to remove crude lipids and prepare a defatted microalga. The raw and defatted microalgae were subjected to chemical fractionation analysis, i.e., sequential leaching in H₂O, 1.0 M ammonium acetate (NH₄Ac), and 1.0 M hydrochloric (HCl) acid. The results demonstrate that, whereas the contents of Fe and Al in the raw and defatted microalgae are extremely low, those of other ash-forming elements follow a sequence of Cl > K > P > Mg > Na > Ca. Chemical fractionation results suggest that virtually all of the Na, K, and Cl in the raw and defatted microalgae are water-soluble. While majority of P in the two fuels are water-soluble and acid-soluble, most of Mg and Ca are leached in water and NH₄Ac solution. As determined via chemical fractionation analysis, lipid extraction leads to the content of water-soluble Ca in the defatted microalga being ~69.6% higher than that in the raw microalga counterpart, which is accompanied by a reduction in the amount of Ca leached in NH₄Ac solution. Similar trend is also observed for Mg, but to a lesser extent.

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1. Introduction

Microalgal biomass is a promising feedstock for the production of liquid transport biofuels because of some key advantages over terrestrial biomass [1]. These advantages include high oil productivity [2], ability to grow on nonarable land with saline water [3], potential application in wastewater management [4], and efficient utilization of CO₂ in flue gas from power stations [5]. Currently, most commercial efforts have been focused on the extraction of lipids for biodiesel production [6], leaving defatted microalgae that contain substantial energy as byproducts [7].

Effective recovery of energy chemically stored in defatted microalgae is necessary to improve the techno-economic performance and lower environmental burdens of microalgal biodiesel production [7,8]. This can be achieved via conventional thermochemical conversions, including pyrolysis [6], gasification [9], combustion [10], and hydrothermal liquefaction [11]. Similar to those in other solid fuels [12–20], inorganic species (e.g., alkali metals) inherently presented in defatted microalgae may act as catalysts

for thermochemical reactions [21] and are responsible for notorious ash-related issues during gasification/combustion [1]. Thus, a thorough understanding on the transformation of these inorganic species during these thermochemical conversion processes is essential for the development of corresponding technologies. This requires knowledge on the occurrence forms of inorganic elements in defatted microalgae because the transformation of these elements during thermochemical conversions is strongly dependent on their speciation, similar to those reported for other solid fuels (e.g., coal and terrestrial biomass) [22,23]. However, to the best of our knowledge, occurrence forms of key ash-forming elements (e.g., Na, K, Mg, Ca, Fe, Al, Si, P, S, and Cl), which contribute most to the ash-related issues during biomass gasification/combustion [23], in defatted microalgae have not been reported in open literature.

Consequently, this study aims to systematically investigate occurrence forms of key ash-forming elements in a defatted microalga, and for comparison, its corresponding raw microalga, using chemical fractionation method. Effects of lipid extraction on the occurrence forms of these inorganic elements in the raw and defatted microalgae, as determined via chemical fractionation analysis, were also discussed.

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2. Experimental section

The freeze-dried powders of a marine microalga (*Nannochloropsis oceanica*, termed as “*N. oceanica*” hereafter) were purchased from Yantai Hairong Microalgae Breeding Co., Ltd (Penglai, China). The microalga was cultivated in seawater, using monopotassium phosphate [KH_2PO_4] and ammonium sulfate [$(\text{NH}_4)_2\text{SO}_4$] as fertilizers. The as-received microalga was sieved to a size fraction of $<75 \mu\text{m}$ and used as raw biomass. The raw microalgal biomass was then extracted with hexane at 80°C for $\sim 48 \text{ h}$ until the recirculated solvent run clear, using a Soxhlet extraction apparatus, to remove crude lipids and prepare defatted biomass. After lipid extraction, the defatted biomass was dried at 80°C overnight to evaporate any residue solvent. Both the raw and defatted microalgal biomass were stored at 5°C in a vacuum storage container prior to use.

Sequential leaching of the raw and defatted microalgal biomass was conducted following a modified chemical fractionation method, to investigate the occurrence forms of key ash-forming elements. The method was developed based on the procedures reported in prior studies [24,25], the details of which are illustrated in Section 1 of the [Supplementary Material](#). Briefly, it is a consecutive leaching process using increasingly aggressive solvents [i.e., ultrapure water, 1.0 M ammonium acetate (NH_4Ac) and 1.0 M hydrochloric (HCl) acid] under batch condition. The method quantifies key ash-forming elements of four occurrence forms, including water-soluble, ion-exchangeable, acid-soluble, and acid-insoluble residue [25]. It should be noted that, in a pioneering study by Wu’s group, semi-continuous (rather than batch) water leaching was recommended to eliminate the effects of acidic leachate and thereby quantify real water-soluble elements in lignocellulosic biomass [26]. However, pH values of the leachate from washing the raw and defatted microalgae with water

under batch condition, as measured by a pH meter (PB-10, Sartorius, Germany), are close to neutral (~ 6.5). This indicates that chemical fractionation is capable of quantifying real water-soluble inorganic species in the microalgal biomass studied.

Solid samples, i.e., the raw and defatted microalgae as well as the solid residues after sequential leaching, were subjected to a range of comprehensive analyses. These included proximate and ultimate analyses, quantification of key ash-forming elements (i.e., Na, K, Mg, Ca, Fe, Al, P, and Cl), as well as morphology and chemistry analysis. The leachates from sequential leaching were analysed for the concentrations of aforementioned ash-forming elements. For the leachates from water extraction, the concentrations of anions (i.e., Cl^- , NO_3^- , PO_4^{3-} and SO_4^{2-}) and ammonium (NH_4^+) were also determined. Detailed analytical procedures are given in Section 2 of the [Supplementary Material](#).

3. Results and discussion

The weight loss of the raw *N. oceanica* after lipid extraction is $\sim 13.2 \text{ wt}\%$ (dry basis). This leads to slight increases in the contents of ash and fixed carbon in the defatted microalga, accompanied by a reduction in the content of volatile matter, compared to those in the raw microalga (see [Table S1](#)). [Fig. 1a](#) illustrates the contents of key ash-forming elements in the raw and defatted microalgae, along with their retentions in the defatted microalga after lipid extraction. Clearly, Cl, K, and P are dominant elements in the raw and defatted microalgae, followed by Mg, Na, Ca, Al and Fe. Consistent with the increase in the ash content, the contents of these ash-forming elements in the defatted microalga are $\sim 8.5\text{--}20.9\%$ higher than those in the raw microalga, as a result of the extraction of crude lipids and $\sim 100\%$ retention of all these elements in the defatted microalga (see [Fig. 1a](#)).

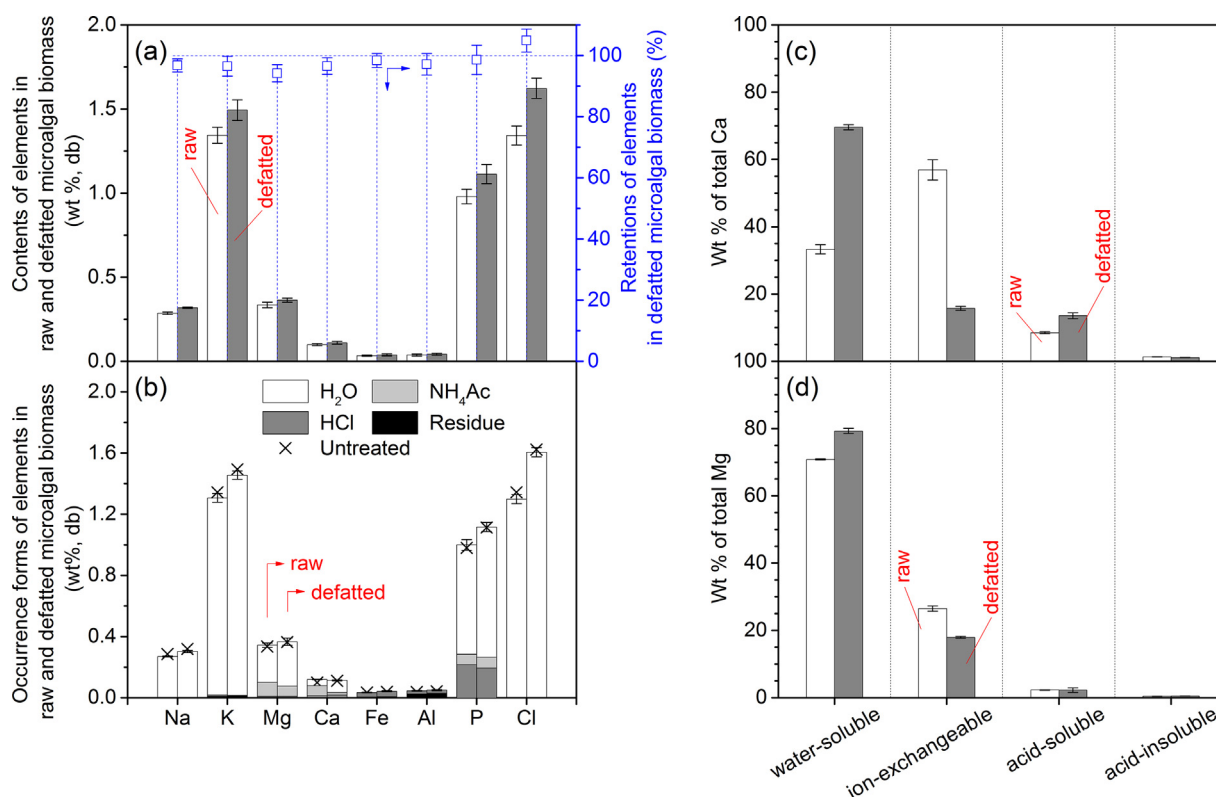


Fig. 1. Panel (a) contents of key ash-forming elements in the raw and defatted microalgal biomass and their retention in the defatted biomass. Panel (b) chemical fractionation results for the raw and defatted microalgal biomass. Panels (c) and (d) occurrence forms of Ca and Mg in the raw and defatted microalgal biomass, expressed as wt% of total Mg or Ca in these samples, respectively.

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