



## 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  $<75\ \mu\text{m}$  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  $\text{H}_2\text{O}$ , 1.0 M ammonium acetate ( $\text{NH}_4\text{Ac}$ ), and 1.0 M hydrochloric ( $\text{HCl}$ ) 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  $\text{Cl} > \text{K} > \text{P} > \text{Mg} > \text{Na} > \text{Ca}$ . 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  $\text{NH}_4\text{Ac}$  solution. As determined via chemical fractionation analysis, lipid extraction leads to the content of water-soluble Ca in the defatted microalga being  $\sim 69.6\%$  higher than that in the raw microalga counterpart, which is accompanied by a reduction in the amount of Ca leached in  $\text{NH}_4\text{Ac}$  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  $\text{CO}_2$  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 [ $\text{KH}_2\text{PO}_4$ ] 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  $80\ ^\circ\text{C}$  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  $80\ ^\circ\text{C}$  overnight to evaporate any residue solvent. Both the raw and defatted microalgal biomass were stored at  $5\ ^\circ\text{C}$  in a vacuum storage container prior to use.

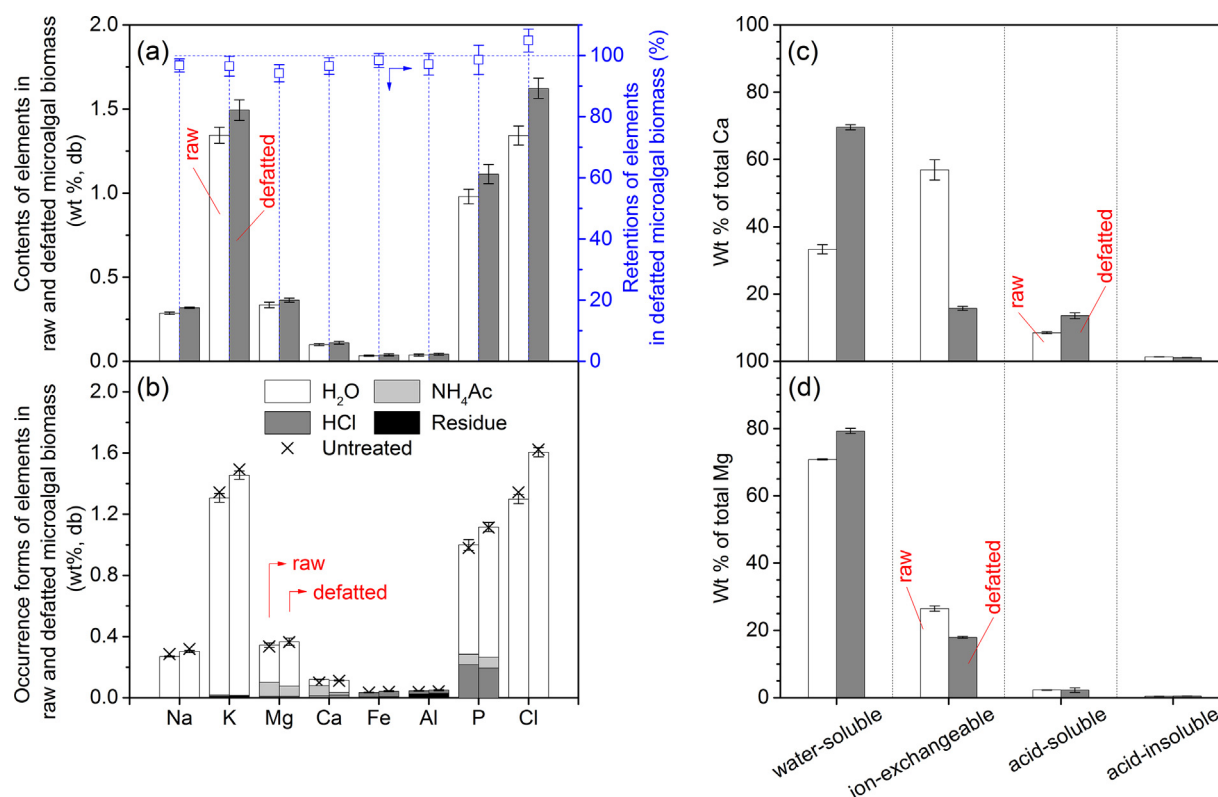
Sequential leaching of the raw and defatted microalgal biomass was conducted following a modified chemical fractionation method, to investigate the occurrence forms of key ash-forming elements. The method was developed based on the procedures reported in prior studies [24,25], the details of which are 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 ( $\text{NH}_4\text{Ac}$ ) and 1.0 M hydrochloric ( $\text{HCl}$ ) 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 ( $\sim 6.5$ ). 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.,  $\text{Cl}^-$ ,  $\text{NO}_3^-$ ,  $\text{PO}_4^{3-}$  and  $\text{SO}_4^{2-}$ ) and ammonium ( $\text{NH}_4^+$ ) 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  $\sim 8.5$ – $20.9\%$  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.

The occurrence forms of these ash-forming elements, as determined via chemical fractionation, are presented in Fig. 1b, with five important findings observed. First, for all the elements investigated, the sum of the four fractions (i.e., water-soluble, ion-exchangeable, acid-soluble, and acid-insoluble residue, denoted as “H<sub>2</sub>O”, “NH<sub>4</sub>Ac”, “HCl”, and “Residual”, respectively) agrees well with the corresponding value of the two untreated microalgal fuels (shown as crosses, termed as “Untreated”), indicating a ~100% mass balance being achieved for nearly all the elements.

Second, virtually all of the Na, K, and Cl are water-soluble in the raw and defatted microalgae. This is as expected because alkali metals and chlorine in microalgae are known to be highly mobile [27]. While majority of K originates from the fertilizer KH<sub>2</sub>PO<sub>4</sub>, Na and Cl are most likely from the seawater used for microalga cultivation.

Third, whereas majority (~82.4–84.6%) of Fe in the raw and defatted microalgae is acid-soluble, possibly in the form of FeS [28], Al in the two fuels can be found in both acid-soluble fraction (~39.6–43.2%) and acid-insoluble fraction (~56.5–59.8%). The acid-insoluble Al indicates the presence of aluminosilicates, possibly due to clay contamination.

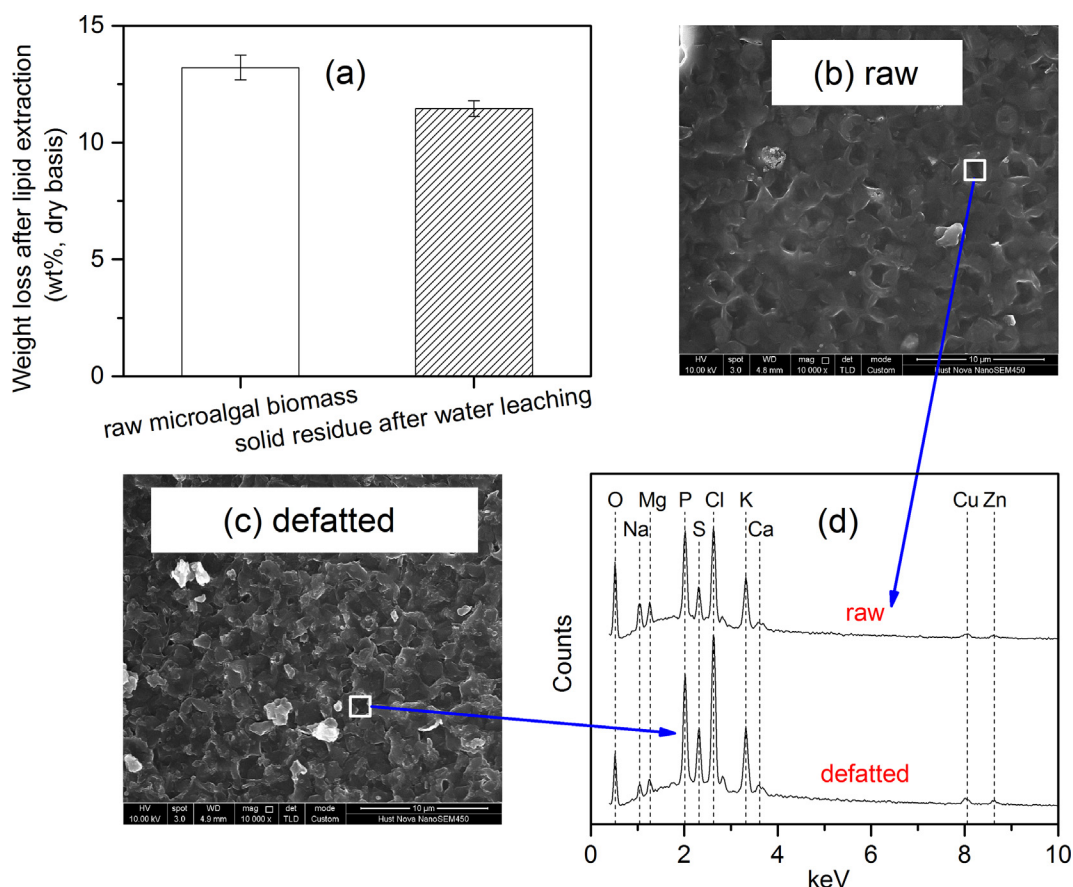
Fourth, P in the raw and defatted microalgae is mainly presented as water-soluble fraction, followed by acid-soluble fraction and ion-exchangeable fraction, with virtually no acid-insoluble fraction observed. The dominance of water-soluble P can be attributed to the use of KH<sub>2</sub>PO<sub>4</sub> as fertilizer. The P leached in NH<sub>4</sub>Ac and HCl acid solutions could be in the form of phospholipids and nucleic acids [1].

Fifth and last, the distribution of Ca over the four fractions in the raw microalga is considerably different from that of Ca in the defatted microalga. Whereas the small amounts of acid-soluble

Ca in the two fuels are largely similar (~0.010–0.015 wt%, db), the quantity of water-soluble Ca in the defatted microalga (~0.079 wt%, db) is ~69.6% higher than that of the water-soluble Ca in the raw microalga. Such increase in the quantity of water-soluble Ca is accompanied by a reduction in the amount of Ca leached via NH<sub>4</sub>Ac solution. This trend is more straightforwardly depicted in Fig. 1c, which presents data based on the total Ca in the raw microalga or the defatted microalgae. Similar finding is also observed for Mg, but to a lesser extent (see Figs. 1b and d). Repeated experiments have confirmed these trends observed for Ca and Mg.

The discrepancies on the distribution of Ca and Mg over the four fractions in the raw and defatted microalgae are somewhat surprising since extracting lipid via hexane is unlikely to cause changes in the occurrence forms of these two elements. While exact mechanisms responsible for this phenomenon remain unclear, one possible mechanism is deduced as follows. In the raw microalga, the presence of lipids in the cells may prevent at least some Ca and Mg, which are inherently water-soluble, being accessed by water molecules during water extraction. Once the lipids are extracted, these elements may become water-accessible, leading to the increases in the amount of water-soluble Ca and Mg in the defatted microalga, compared to the raw microalga.

This hypothesis is indirectly supported by two facts. On one hand, the cells of the raw microalga are largely undestroyed after water extraction. To demonstrate this, the solid residues after leaching the raw microalga with water were collected and dried, before being subjected to lipid extraction with hexane using the Soxhlet extraction apparatus. As illustrated in Fig. 2a, the weight losses of both the solid residues and the raw microalga are similar,



**Fig. 2.** Panel (a) weight loss of the raw microalgal biomass and its derived solid residue from water leaching after extracting lipids with hexane, using a Soxhlet extraction apparatus. Panels (b) and (c) SEM images of the raw and defatted microalgal biomass, respectively. Panel (d) EDS spectra of the raw and defatted microalgal biomass.

indicating that most of lipids are still retained in the solid residues after water extraction. These lipids are likely to prevent some Ca and Mg in the raw microalga, which are inherently water-soluble, being accessed by water, leading to the underestimation on the quantity of the real water-soluble Ca and Mg in the raw microalga.

On the other hand, as show in Fig. 2b, the cells of the raw microalga are compactly clustered, with a diameter of  $\sim 2\ \mu\text{m}$ , which are similar to those described in a prior study [29]. It is evidenced in Fig. 2c that, after lipid extraction with hexane, most of the cell walls undergo disruption and destruction. Most importantly, Ca and Mg are found in the cell walls, as demonstrated by the EDS spectra for the raw and defatted microalgae (see Fig. 2d). Therefore, it is reasonable to speculate that, after lipid extraction, some Ca and Mg presented in the cell walls become more water-accessible due to the disruption of cell walls, and thereby partitioned into the water-soluble fraction during chemical fractionation.

The results presented in Figs. 1 and 2 have a significant implication on the accurate determination of the real occurrence forms of Ca and Mg in raw microalgal biomass. Specifically, there is a possibility of underestimating the real water-soluble Ca and Mg in raw microalgae (at least for the studied microalga), most likely due to the presence of lipids that prevent these elements, which are inherently water-soluble, being accessed by water molecules. It is highly recommended that chemical fractionation analysis should be conducted for both raw microalgae and their defatted microalgae, in order to provide full insights into the occurrence forms of key ash-forming elements (in particular, Ca, and to a lesser extent, Mg) in these microalgal biomass fuels.

#### 4. Conclusions

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  $\text{Cl} > \text{K} > \text{P} > \text{Mg} > \text{Na} > \text{Ca}$ . 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  $\text{NH}_4\text{-Ac}$  solution. The content of water-soluble Ca in the defatted microalga, as determined by chemical fractionation analysis, is  $\sim 69.6\%$  higher than that in the raw microalga counterpart, which is accompanied by a reduction in the amount of Ca leached in  $\text{NH}_4\text{-Ac}$  solution. Mg follows similar trend but to a lesser extent.

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#### Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.fuel.2017.03.044>.

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