



# Characterization of ash in algae and other materials by determination of wet acid indigestible ash and microscopic examination



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## ABSTRACT

Algae are known for high ash content. It is important to properly characterize their ash for value added utilization of algae as food, feed, and feedstock for biofuels. In this study, 12 algae of different sources were measured for proximate composition and mineral profile. Results showed that the relative difference between ash content by dry ashing and total minerals content by wet digestion increased with ash content. A major cause was soon identified: when using a common procedure of strong attacks for sample digestion before mineral analysis, incomplete digestion existed for most algae samples due to the presence of siliceous materials. It was proposed that algae consist of wet acid indigestible ash (WAIA) and wet acid digestible ash, whereas WAIA is siliceous. Methods to measure WAIA content in the 12 algae, along with oat grain, oat forage, defatted soy meal and fine sand, were then developed based on digestion with nitric acid or sulfuric acid-hydrogen peroxide. For the 12 algae, ash ranged 1.9 to 37.4% dry matter while WAIA by nitric acid digestion varied 0.1% to 25.6%. High correlation between WAIA and ash contents indicates WAIA as an important contributor for algae ash. For identifying what constituted the siliceous materials, all samples in three matrixes (original, ash by dry ashing, and WAIA) were microscopically examined. Because wet acid digestion had an ability to concentrate siliceous materials and maintain their original shape and size, WAIA was the best matrix for microscopic examination. Micrographs of WAIA show three types of siliceous materials in algae: non-diatom cellular structures, diatom cell walls, and sandy particles. It was concluded that high ash content of algae resulted partly from contamination of diatoms and/or sandy particles of geologic origin and that WAIA should be an important quality parameter for algae. Subsequently, several measures are proposed to produce algae with low ash content.

## 1. Introduction

Algae have been used as animal feed (including aquafeed), human food, fertilizer, and a source for extracting high value molecules [1–3]. In recent years, there has been increased interest in using microalgae as an alternative resource for biofuel production [4]. Key advantages of microalgae include prolific growth rates, the ability to grow on lands that are marginal for other agricultural purposes, and the ability to clean up water resources with excess nutrients [4].

When evaluating algae for end uses, it is important to assess chemical properties of the algal biomass [5–7]. In addition to proteins, lipids, and carbohydrates, ash content is a very important quality parameter since ash contains inorganic material and is noncombustible. In general, algal biomass is known for high ash content [5]. For example, algae grown in certain locations contain ash as high as 70%

dry matter (dm) [8]. This phenomenon not only reduces the amount of ash-free matter, the valuable portion of algae biomass, but also causes a concern for heavy metal toxicity and diminishes inclusion levels for algae uses as feed, food and fertilizer [9]. The inorganic matter from algae also poses major operational problems in biomass combustion systems for energy conversion. These include slagging, fouling, high temperature corrosion, and bed agglomeration. All of these can disrupt normal combustion and fluidization behaviors and, if severe enough, can lead to expensive system shut-downs [10]. For reducing ash in algae, physical pretreatments, such as particle size reduction, sieving and centrifugation were tried [11] but the effect was questionable due to time, water and energy consumed.

To predict and mitigate ash-related problems effectively, it is crucial to develop a reliable and easily adaptable methodology to properly characterize the ash forming components in algae biomass. The most

*Abbreviations:* AIA, acid insoluble ash; ANOVA, Analysis of variance; AOCS, American Oil Chemists' Society; CHO, carbohydrate; DM, dry matter; HF, hydrofluoric acid; h, hour; ICP-OES, inductively coupled plasma-optical emission spectrometer; PBR, photobioreactor; PTFE, polytetra-fluoro-ethylene; TM, total minerals; WADA, wet acid digestible ash; WAIA, wet acid indigestible ash; WAIA<sub>N</sub>, wet acid indigestible ash by nitric acid digestion; WAIA<sub>S</sub>, wet acid indigestible ash by sulfuric acid digestion

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common method to characterize algae ash has been instrumental analysis for mineral composition [5,12–15]. Realizing the limitation of mineral analysis alone, Lane et al. [16] characterized algae ash by first subjecting algae samples to sequential chemical extractions with selected solvents, analyzing mineral composition of each fraction, and inferring the mode of occurrence of the main inorganic elements from their distribution patterns among the fractions. This method of combining wet fractionation with mineral profiling had been used in coal and other biofuel materials [10,17,18]. Hampel [8] attempted to characterize algal biomass ash by measuring its two main components: silica and heavy metals but found that the method to measure silica directly was time consuming, expensive, difficult, and prone to errors due to interferences.

Although studies on algae biomass involving measurement of ash content and/or analysis of mineral composition of whole algae or its fractions can provide information on occurrence of inorganic elements in algae and how this varies among species of algae and different methods of production and harvesting, none of them can adequately address several important questions regarding the algae ash component. These include: 1) why algae have higher ash contents, some extremely so, compared to other biological materials, 2) why algae have a large variation in ash content, 3) do ash components in algae differ from other biological materials, 4) what is the chemical nature of algae ash, and 5) how to prevent ash built-up during algae production?

This study was designed to specifically address these questions. It began with collection of 12 algae samples from different sources, followed by proximate analysis and mineral composition profiling. It was soon found that mineral analysis based on the nitric acid digestion could not adequately characterize the ash components in algae samples. Careful examination of the method for mineral analysis and review of algae chemistry led to a key reason for inadequacy of the mineral analysis: unlike most other biological materials, algae (particularly diatoms) contain substantial amounts of silica or silicates. Because silicates are non-combustible, they remain as ash upon combustion. Silicate is also extremely resistant to acid digestion. Therefore, even with the use of a very common procedure of strong attacks for sample digestion, a substantial portion of ash in algae samples could not be digested before instrumental analysis of minerals [19,20].

Although the problem of incomplete digestion for mineral analysis could be addressed by replacing the strong attack procedure with a total decomposition procedure that involves an additional step of adding hydrofluoric acid (HF) to dissolve silica from algae samples, it is hard to practice in a typical analytical lab due to inherent drawbacks of using HF as a reagent [19,21]. More importantly, the procedure of total decomposition with HF addition cannot measure silica or Si content [19,21]. In order to develop a reliable and easy-to-adapt methodology to quantitatively characterize the ash component of algae, an alternative yet efficient approach was proposed in this study: Following a strong attack procedure, the insoluble siliceous particulates in the digestion tubes, instead of being discarded, were measured gravimetrically upon centrifugation at the end of the wet acid digestion. By doing so, the content of siliceous material could be indirectly measured. Furthermore, the new concept that algae ash consists of wet acid indigestible ash (WAIA) and wet acid digestible ash (WADA) was introduced. The objectives of the study were subsequently expanded to include: 1) developing methods to measure the content of WAIA, 2) determining the relationships among total ash, total minerals (TM), WAIA and WADA contents, and 3) identifying chemical nature of the ash component by microscopic examination of all samples in three forms: original matrix (as is), ash by dry ashing, and WAIA.

## 2. Materials and methods

### 2.1. Materials

Twelve algae samples were collected, in a duplicate set, from

different sources and producers across the USA. They were named according to the order of receiving and thus the names of producers/providers were omitted to avoid any possible negative impact on perception of their products based on the results of this study. Among the 12 algae, only Algae 4 was grown in a photobioreactor (PBR) system, the rest were all grown in open pond systems. For a few algae samples, information on strains was known, but for many others such information was lacking. Information on production details, harvesting methods, and postharvest handling/processing was also lacking. However, this lack of information did not compromise achieving objectives within this study. Upon receiving, all samples had already been dried by the providers, except for Algae 11, which was collected from a nearby waste water treatment facility, centrifuged at  $3000 \times g$  for 10 min to remove extra water, and dried in a forced air oven (Model 1325F, Sheldon Mfg. Inc., Cornelius, OR) at 60 °C overnight.

Non-algae materials included hulled oat grain (cultivar 'Ajay'), oat forage (cultivar 'CDC Dancer'), defatted soy meal, and sand. Oat grain and forage were grown at the University of Idaho Experimental Station farm in Aberdeen, Idaho. Forage was harvested at a pre-flowering stage and dried in the forced air oven at 60 °C overnight. Defatted soy meal was obtained from the Archer Daniel Midland Co. (DeKalb, IL). Sand was collected from the shore of American Falls Reservoir, Idaho.

### 2.2. Sand preparation

The sand sample was dried in the forced air oven at 60 °C overnight, and then 300 g was sieved with a 200-mesh U.S. standard sieve and a pan, fitted into a sieve shaker (DuraTap, Model DT168, Advantech Mfg. Co., New Berlin, WI), with shaking and tapping for 15 min. The sand of larger particle size on the top of the sieve was discarded. The fine sand from the pan (about 40 g) was washed with water several times before drying in the forced air oven at 140 °C for 1 h. It was then stored in a sealed plastic bag.

### 2.3. Analysis for proximate composition

Dried algae samples were analyzed for moisture, protein, oil, and ash contents. The four non-algae materials were measured only for moisture and ash content. Moisture content was measured by drying in the forced air oven at 105 °C for 3 h [22], while the oil content was analyzed by an AOCs Official Procedure [23], using a fat analyzer (Model XT 10, Ankom Technology, Macedon, NY, USA) with hexane as the extracting solvent. The total nitrogen content was measured by a combustion method [22], using a protein analyzer (Model FT528, Leco Corp. St. Joseph, MI, USA). In calculating protein content, a conversion factor of 4.78 for nitrogen-to-protein conversion was used [24]. Ash content was measured, using a furnace heated to 600 °C overnight (about 16 h). Carbohydrate (CHO) was calculated as the difference between 100 and the sum of protein, oil, and ash contents in % dry matter basis. Here, the CHO is defined as a heterogeneous mixture of all those components that were not measurable by the methods for moisture, ash, protein and oil. They include cellulose, lignin and hemicelluloses, as well as starch, pectin, oligosaccharides, sugars, organic acids and pigments.

### 2.4. Acid digestion and mineral analysis

The algae samples were also measured for contents of individual mineral elements, using a Perkin-Elmer Optima 3200 ICP-OES (inductively coupled plasma-optical emission spectrometer) to quantify constituents in an aqueous solution following nitric acid digestion of the samples. For the nitric acid digestion, the procedure of Anderson et al. [20] was used with modification. Briefly, each algal sample was weighed (125–200 mg) and put into a heavy duty 15 mL glass centrifuge tube. Under a fume hood, 3 mL of 70% nitric acid was added into each tube. Tubes were vortexed and left in the fume hood

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