CHARACTERIZATION OF RESIDUAL *Nanochloropsis oculata* MICROALGAE AND ITS POTENTIAL FOR BIOFUEL PRODUCTION

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Abstract

Biofuel production from microalgae has been explored nowadays to substitute declining fossil fuel. Conversion of microalgae (e.g., *Nanochloropsis oculata*) to biofuel usually used fresh biomass and not the residual ones. In this study, the characterization of residual *N. oculata* microalgae and its potential for biofuel production was investigated. Results of proximate and ultimate analyses and high heating value were compared to its original values gathered and recorded during the characterization of fresh *N. oculata* samples. Particles of its residual being characterized exhibited high uniformity. Result of characterization showed that volatile matter of *N. oculata* drastically reduced from 81.27% (fresh sample) to 62.00% (residual sample), implying a possible drop of extractable bio-oil within *N. oculata*. Its ash content increased from 13.57% to 36% following a decline of fixed carbon from 5.17% to 2.00%, suggesting quality deterioration of residual *N. oculata*. Additionally, the increase of ash content considerably reduced the elemental compositions: carbon (48.31% to 33.44%), hydrogen (7.66% to 4.52%), oxygen (24.85% to 21.62%), nitrogen (4.80% to 3.55%). The high heating value has lowered from 17.4 MJ/kg to 14.94 MJ/kg. These apparent declined of *N. oculata* characteristics for biofuel production, however, are still within the range of values of most algal species (macro, micro, green, blue-green, brown, red, diatom, marine, and freshwater) suggesting comparability and acceptability in microalgal-based biofuel industry in general.

Keywords: biofuel production, *Nanochloropsis oculata*, residual microalgae

1.0 Introduction

The declining fossil fuel resources result in increasing oil prices and continually triggers environmental problems which necessitate the world to look for sustainable and renewable energy sources (Bahadar and Bilal Khan, 2013). One promising alternative energy-rich feedstock candidate identified is microalgae (Klein et al., 2018; Maeda et al., 2018). It is an advantageous feedstock for biofuel production over those conventional ones (e.g., corn, sugarcane, cassava, palm oil, jatropha, etc.) because of its distinctive characteristics such as fast growth rates; high oil contents; non-reliance to agricultural land and less water requirement for cultivation (Brennan and Owende, 2010);and the positive impact on food supply, biodiversity and environment (Bennion et al., 2015; Biagini et al., 2006; Keris-Sen et al., 2014; Zhao et al., 2015). Additionally, microalgae can be grown in ponds of wastewater treatment plant (lvarez-Daz et al., 2015; Ruiz et al., 2014) primarily to remove NH_{4+}, NO_3, and PO_4; to sequester CO_2 (Wang et al., 2008); and to reduce concentrations of other industrial greenhouse gases.

Growing microalgae in wastewater ponds are believed to be a cost-efficient and environmentally sustainable strategy (Ji et al., 2015). Besides, the produced biofuel per se is non-toxic, renewable, biodegradable, low or non-sulfur bearing, and cleaner-burning fuel with excellent lubricity and high flash point properties (De Luna et al., 2017). These are prime reasons why microalgae to biofuel production have been explored nowadays. Conversion of microalgae to biofuel, when optimized and established, could bring significant positive economic and environmental impact in the local and global arena. In the Philippines, for instance, biofuel technology optimization and establishment could address the prolonged dependence of its production to coconut and palm oils, which are supposedly intended for the food industry.

In converting microalgae to biofuel, feedstocks could either be sourced out from fresh harvested microalgal biomass or those from properly stored ones. It is essential that those stored microalgae are used before it reached significant degradation stage. This ideal practice supposedly strictly followed because the length of time between harvest and processing as well as meteorological conditions impacts the microalgal metabolism leading to biomass and lipid degradation (Napan et al., 2015). In all cases, a fundamental characterization of biomass as a feedstock (Biagini et al., 2006), regardless of fresh or old samples, is required for biofuel production.

To date, no characterization study conducted on residual microalgal biomass that had been left unattended or exposed to ambient environmental conditions for so long. Such physical and chemical characterizations are necessary as these have significant impacts in the economics of its conversion process (Naik et al., 2010) and so with the design and operation of associated conversion processing facilities (Cai et al., 2017). Hence, this characterization study of residual microalgae, particularly the *N. oculata*, which have been left unattended and exposed to environmental conditions for two years. The said microalgal biomass was kept in a container after a previous study of a fresh N. oculata has been completed (Maguyon and Capareda, 2013).

This work mainly aimed at determining the physical and chemical characteristics of residual *N. oculata* and its suitability to biofuel production. These *N. oculata* microalgae species have lipid productivity of 84.0 142.0 mg/L/day during cultivation with available lipid content of 22.7-29.7 wt% relative to its dry weight biomass (Martins et al., 2010) making it potential for biofuel production.

2.0 Materials and Method

2.1 Residual *N. oculata* sample preparation

The residual *N. oculata* samples were obtained from leftovers of the pyrolysis study conducted at Bio-Energy Testing and Analysis (BETA) Laboratory, TAMU, Texas, USA (Maguyon and Capareda, 2013). The collected residual *N. oculata* were oven dried at 105°C for 24h (Yuan et al., 2011), ground using a laboratory mill (Model 4 Wiley Mill, Thomas

Scientific) with 2mm mesh. Ground samples were then sieved in a Fischer standard brass test sieve (Fischer Scientific Company, Massachusetts, USA) to determine its particle size distribution using ASTM E-11 specification, Fisher Scientific Company, USA. The sieved samples were then kept in an air-tight container ready to use for proximate analysis, ultimate analysis, and heating value determination.

2.2 Analytical Methods

The prepared samples underwent characterization according to standard procedures of proximate analysis, ultimate analysis, and heating value determination. The proximate analysis determined the moisture content, volatile matter, ash content, and fixed carbon following ASTM ASTM E1755 and ASTM D3172. The fixed carbon content of the samples was calculated by difference. On the other hand, the ultimate analysis comprising content determination (wt%) of carbon, hydrogen, oxygen, nitrogen, and sulfur (CHONS), was performed using Vario MICRO Elemental Analyzer (Elementar Analysemsysteme GmbH, Germany) following ASTM D5373. The heating value was determined according to ASTM D5865 using Parr Isoperibol Bomb Calorimeter (Model 6200, Parr Instrument Company, Moline II).

2.3 Biofuel potential determination by data comparison

The results of the proximate analysis, ultimate analysis, and heating value determination for residual *N. oculata* was then compared to the data of previous studies with similar sample source and species. In other words, characteristics of residual *N. oculata* was compared to its characteristics the first time it was studied in its original and fresh form. A comparison against similar *N. oculata* microalgal species from the different study was also undertaken. Finally, characteristics of the residual *N. oculata* was compared to the characteristics of the whole algal species (macro, micro, green, blue-green, brown, red, diatom, marine, and freshwater) to have a more in-depth look in evaluating its potential against the entirety of algae to biofuel production. Data used in the comparison are mean, maximum, and minimum values which are taken from a published extensive peer-reviewed work (Vassilev and Vassileva, 2016).

3.0 Results and Discussion

3.1 Particle size distribution

The particle size distribution of a biomass sample is important in its conversion to biodiesel. Figure 1 shows the highest distribution of ground residual *N. oculata* particles in 630.5μm with a total percentage share of 35%. On the other hand, the lowest percentage share of the particles is those collected in 88.5μm constituting only to 4% in the total distribution. Others having relative high percentage share are those particles gathered in 841μm, 213.5μm and 335μm with a recorded percentage particle distribution of 23%, 23%, and 15%, respectively. The particle size distribution result reveals that ground residual *N. oculata* samples have relatively high uniformity of particles. Though it was observed that there were the little amount (0.40%) gathered in 88.5μm, its presence is too small to affect the uniformity of particles of samples and so with the experimental yields during the experiments. In totality, the prepared ground samples of residual *N. oculata* has a good uniformity of particle size. This desirable result is advantageous in microalgal biomass to biofuel production as uniformity of particle size is a vital consideration in selecting and designing appropriate processes (Loo and Koppejan, 2008). Furthermore, uniformity of particles is necessary as this affects the mixing and fluidization, surface area for mass and heat transfer, and the movement of biomass particles (Cai et al., 2017). This finding supports the claim of previous work published in the literature (Tumuluru et al., 2011) that consistency of physical properties such as size and shape of biomass sample for biofuel production significantly influence its conversion, storage, transportation and handling. Uniformity of particle sizes observed in the ground *N. oculata* would then become the basis for energy conversion technologies in the future (Cai et al., 2017). In fact, uniform particle distribution is one consideration is targeting large-scale power production from biomass (Nhuchhen et al., 2014).

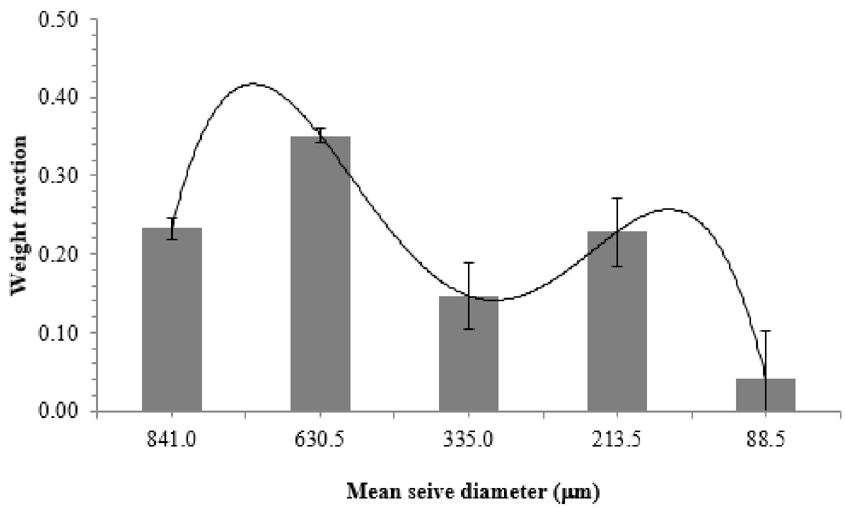

Figure 1: The particle size distribution of ground N. oculata samples used in the study

The proximate analysis of the residual *N. oculata* (Table 1) shows a volatile matter value of 62.00%, which is way lower than its original volatile matter (81.27%) two years ago. The result shows a significant decrease of volatile matter, which is unfavorable for the conversion of residual *N. oculata* to biofuel. This is because the volatile matter is a determinant to the quantity of bio-oil that can be extracted from the biomass. It is a fact that the higher volatile matter the biomass possess, the higher amount of bio-oil it can generate (Pratap and Chouhan, 2013). This volatile matter refers to condensable vapor and permanent gases that are produced when biomass is heated (Cai et al., 2017). The volatile matter value of the residual *N. oculata* implies that the biomass has decreased its potential for bio-oil production over time. A similar trend that is in decreased value, was observed in another study (Sukarni et al., 2014) when it is compared to the volatile matter value of fresh *N. oculata*.

3.2 Characteristics of *N. oculata*

Table 1. Comparison of the characteristics of the residual *N. oculata* with previous studies using *N. oculata*

Characteristics	Reference This study (2014)	Maguyon et al. (2013)	Sukarni et al. (2014)
Proximate analysis (%w/w)			
Volatile matter[a]	62.00	81.27	67.45
Ash content[a]	36.00	13.57	24.47
Fixed carbon[b]	2.00	5.17	8.08
Ultimate analysis (%w/w)			
Carbon	33.44	48.31	28.32
Hydrogen	4.52	7.66	-
Oxygen[b]	21.62	24.85	43.80
Nitrogen	3.55	4.80	-
Sulfur	0.87	0.81	-
Heating value (MJ/kg)[a]	14.94	24.7	16.80

[a] on dry basis, [b] determined by difference

The inferior volatile matter value of residual *N. oculata*, when compared to those of two fresh *N. oculata*, suggests that some microalgal components constituting volatile matter had escaped from the biomass. It can be deduced that leftover microalgae like that of residual *N. oculata* will lead to the decline of its volatile matter so with its potential for bio-oil production when not kept or stored properly. The diminishing potential for bio-oil production of the residual N. oculata is supported by its increasing ash content. Its 36% ash content from the original of 13.57% reveals accumulation of inorganics from the biomass that is not convertible to bio-oil. The long exposure of the residual *N. oculata* without having been properly kept or stored removes volatile matters in the biomass. Intuitively, this situation led to the reduction of the amount of volatiles which tended to the increase of non-volatile fraction or inorganic component such as ash (Maguyon and Capareda, 2013; Sukarni et al., 2014). The residual *N. oculata* nearly tripled the amount of ash content of the original fresh *N. oculata* sample. The high ash content of biomass like algae is less desirable to biomass to energy conversion, as this contributes largely to poorer fuel quality (Vassilev and Vassileva, 2016). Another equally important observation was the declined of fixed carbon content (2.0%) against the original *N. oculata* sample (5.17%). By further comparison of the fixed carbon and ash content of the residual *N. oculata* to that *N. oculata* of Sukarni and co-workers, it shows the inferior quality of the former over the latter.

In terms of the result of ultimate analysis, the residual *N. oculata* has 33.44% carbon, 4.52% hydrogen, 21.62% oxygen 3.55% nitrogen, and 0.87% sulfur. The result reveals that elemental composition of the residual *N. oculata* generally lowered its values when compared to the original *N. oculata* sample (48.31% C, 7.66% H, 24.85% O, 4.80% N, and 0.81% S). The carbon content (48.31%), for instance, became 33.44% after two years. This carbon content, which indicated the abundance of carbohydrates, fatty acids, and lipids (Syazwani et al., 2015) has recorded a decline of 31.48%. This loss is big enough to affect the amount of volatile matter of the residual *N. oculata*. Similarly, hydrogen content that indicated the presence of fatty acids has diminished after two years. The hydrogen content in original *N. oculata*, which is 7.66%, became 4.52% in the residual *N. oculata*. Meanwhile, there is no significant changes observed in terms of sulfur content. The 0.06 difference between the two samples is too small to affect the property of the bio-oil that can be generated from the *N. oculata*. Intuitively, the sulfur content of the original *N. oculata* and the residual *N. oculata* was not affected after two years as sulfur is insusceptible against changing environmental conditions.

Generally, comparing the values between the two *N. oculata* samples (the original and residual), the result shows a decline characteristics of the residual *N. oculata*. When compared to the available elemental composition data of published literature (Sukarni et al., 2014), residual *N. oculata* has a slightly higher carbon content but lower in oxygen content. Its lower oxygen content than that of Sukarni and co-workers is favorable since higher oxygen content causes several disadvantages such as low energy density, immiscibility with hydrocarbons and instability of the bio-oil (Czernik and Bridgwater, 2004).

The declined characteristics of the residual *N. oculata* based on the values of proximate and ultimate analyses is further displayed in the heating value. The 24.7MJ/kg heating value of the original *N. oculata* became 14.94MJ/kg in the residual *N. oculata* which declined by almost 40%. It can be deduced that the decline of heating value is owing to the declined chemical composition values of the residual *N. oculata*. It is a fact that heating value is a function of the biomass or fuels chemical composition (Singh et al., 2013). Thus, the observed declined characteristics of residual *N. oculata* based on its proximate and ultimate analyses follow to have a lower heating value than that of the original *N. oculata*. The heating value of the residual *N. oculata* still lower than the *N. oculata* of Sukarni and co-workers (Sukarni et al., 2014) suggesting that characteristics of microalgae samples indeed deteriorate when exposed to the environment.

To have a broader look at the potential of the residual *N. oculata* in the field of microalgae to biofuel production, Table 2 depicted comparisons using mean, minimum, and maximum values of various algal species.

Upon looking at the volatile matter, residual *N. oculata* (62.0%) is higher than the mean volatile matter value (52.4%) of different algal species. It is even very close or equal to the maximum volatile matter value of different algal species. This suggests that though residual *N. oculata* is inferior compared to *N. oculata* separately studied by Maguyon (Maguyon and Capareda, 2013) and Sukarni (Sukarni et al., 2014), its potential in terms of volatile matter for biofuel production is still within the values normally found in different algal species. However, its fixed carbon and ash content is not that so attractive when compared to the usual values of different algal species. Its fixed carbon that is affected in the release of volatile matter (Sukarni et al., 2014) after left for two years is too low compared to the mean value and even lower to the minimum fixed carbon value of different algal species.

Also, its ash content (36.0%) is higher than the mean ash content (26.0%) of different algal species. This value is relatively within the range of the ash content of different algal species with a maximum value of 42.8%. But it is worth mentioning that high ash content is

Table 2. Comparison of the characteristics of residual *N. oculata* and different species of algae (macro, micro, green, blue-green, brown, red, diatom, marine, and freshwater).

Characteristics	Reference: This study (2014)	Vassilev and Vassileva (2016) Mean	Minimum	Maximum
Proximate analysis (%w/w)				
Volatile matter[a]	62.0	52.4	37.2	61.7
Ash content[a]	36.0	26.6	13.1	42.8
Fixed carbon[b]	2.0	21.0	9.0	29.2
Ultimate analysis(%w/w)[c]				
Carbon	52.25	45.1	38.6	54.4
Hydrogen	7.06	7.3	4.6	12.7
Oxygen[b]	33.78	40.4	26.3	53.1
Nitrogen	5.55	5.6	1.1	12.4
Sulfur	1.36	1.62	0.47	3.30
Heating value (MJ/kg)[a]	14.94	17.4	7.9	24.0

[a] on drybasis, [b] determined by difference, [c]values for ultimate analysis are in dry ash-free basis

undesirable in biofuel production. In general, values in the proximate analysis of the residual *N. oculata* is acceptable when compared to that of different algal species.

In terms of its values gathered in the ultimate analysis, it can be observed that the residual *N. oculata* generally within or comparable to that of different algal species. In particular, the carbon (52.25%) is better than the mean value of different microalgae though a bit lower against the maximum value (54.4%). The hydrogen (7.06%), oxygen (33.78%), nitrogen (5.55%) and sulfur (1.36%), on the other hand, are within or even slightly better than the values recorded for different algal species with hydrogen, oxygen, nitrogen, and sulfur mean values of 7.30%, 40.4%, 5.60%, and 1.62%, respectively. Similar to the result of proximate analysis comparison, it can then be inferred that values of residual *N. oculata* are acceptable based on the values of different algal species.

When it comes to heating value, the residual *N. oculata* has 14.94MJ/kg energy, a bit lower than that the mean value (17.4MJ/kg) but within the range of the minimum (7.9MJ/kg) and maximum (24.0MJ/kg) values of different algal species. This implies that the residual *N. oculata* holds potential for biofuel production as its heating value is still suitable for such purpose.

4.0 Conclusion

This work revealed the characterization results of residual *N. oculata* microalgae through proximate, ultimate analyses, and heating value determination. It evaluated its potential for biofuel production. The residual *N. oculata* contains 62.0% volatile matter, 36.0% ash content and 2.0% fixed carbon. Furthermore, residual *N. oculata* has carbon, hydrogen, oxygen, nitrogen, and sulfur contents of 52.25%, 7.06%, 33.78% 5.55% and

1.36%, respectively. Lastly, the residual *N. oculata* has a heating value of 14.94 MJ/kg. These all values making up a relatively inferior characteristic of residual *N. oculata* against its original or fresh form two years back. It is then concluded that the abandonment of the samples without having been appropriately stored have affected its quality for biofuel production. The residual *N. oculata*, however, are still valuable for biofuel production when considering the potential of entire algal species.

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