Fall 2019

Application of a Biodegradable and Recyclable Chelating Agent for Ash Removal from Algae

Temitope George Daramola
Old Dominion University, tdarm001@odu.edu

APPLICATION OF A BIODEGRADABLE AND RECYCLABLE CHELATING AGENT FOR ASH REMOVAL FROM ALGAE

by

Temitope George Daramola

A Thesis Submitted to the faculty

Old Dominion University in Partial Fulfilment of the

Requirements for the Degree of

MASTER OF SCIENCE

ENVIRONMENTAL ENGINEERING

OLD DOMINION UNIVERSITY

December 2019

Approved by:

Supervisor: Dr. Sandeep Kumar

Dr.Mujde Etern-Unal

Dr. James W Lee

ABSTRACT

APPLICATION OF A BIODEGRADABLE AND RECYCLABLE CHELATING AGENT FOR ASH REMOVAL FROM ALGAE

Temitope George Daramola

Old Dominion University, 2019

Supervisor: Dr Sandeep Kumar

Ash is inherent in algae biomass and it causes operational difficulties, equipment failure, and disposal issues during biomass conversion to biofuels and bioproducts. The objectives of this study are to (i) investigate the use of the biodegradable chelating agent for reducing ash content of algae and (ii) evaluate the potential of regeneration and recycle of the chelating agent for multiple uses. Conventionally, ethylenediamine-tetraacetic acid (EDTA) has been studied extensively to remove ash from biomass. However, EDTA is non-biodegradable and causes environmental issues at the disposal.

Nitrilotriacetic acid (NTA) is a biodegradable chelating agent and was used in this study for removing ash from algae. The algae samples were first treated with deionized water (DI) followed by NTA and DI wash at 130℃ for 2 hours. Ash analyses were conducted on the raw and treated algae samples. It was observed that the use of NTA reduced ash content of algae from 15.2 wt.% to 3.8 wt% which implies to 85.1wt% of ash removal both NTA and DI wash. The NTA liquor saturated with ash was regenerated by the use of Na_2S with $Ca(OH)_2$. The proposed regeneration method caused inorganic (ash) contents of the saturated NTA liquor to precipitate. The top/clear portion of the solution was decanted and recycled for the next cycle of ash removal from algae. The precipitate/bottom portion was stored for composition analysis. The results of this study provide an environmentally benign method of ash removal from algae biomass feedstock.

ACKNOWLEDGMENT

Firstly, praises and thanks to God almighty, for His continuous blessings throughout my research, and for the completion of my program.

I would like to express my deepest gratitude to my committee chair, Professor Sandeep Kumar, for his support, guidance, advice, and vast knowledge. His counsel and direction helped me through my research work, without his persistent help this thesis would not have been possible.

I would like to say big thank you to my committee members, Dr. James Lee and Dr. Mujde Ertern-Unal, for their encouragement and insightful suggestions. I would also like to say a big thank you to all my lab mates in the Biomass Research Lab (BRL): Mason, Kam, Ashani, Pushpita, Sagar, Anuj, Tina, and Oumar Sacko from chemistry department, for their support, encouragement, and assistance. Additionally, I would like to say a big thank you to the CEE department of Old Dominion University.

Lastly, my deepest appreciation to our collaborators on this project, Sandia National Lab. There are many people who have contributed to the successful completion of this thesis. I extend many, many thanks to the Old Dominion University community. God bless.

Table of Contents

List of Tables

List of Figures

CHAPTER 1

1. INTRODUCTION

1.1 Alternative source of energy

Biomass serves as a renewable source of energy, which can thermochemically be converted into liquid fuel (biofuel). This conversion can be done by various methods which include pyrolysis, liquefaction, and gasification. In addition to this, substituting fossil fuel with biofuel generated from biomass will ultimately lead to the reduction of greenhouse gases. The slight shift of attention noticed of recent in biofuel is generally due to climate change and the global warming problem [1]. Biofuel, in general, is more environmentally friendly than fossil fuel. Sizeable explorations have been done on the study of biomass to further its development for the production of energy as an alternative to fossil fuel. Some of the frequently examined biomass include woody, terrestrial, agricultural and algal biomass have all been investigated extensively for its conversion into various forms of energy. Some of the early discovered biomass also known as first-generation biomass were gotten from edible feedstock; such as palm oil, sugar cane, potato, soybean, and other digestible feedstock [2]. The first-generation biomass was unsustainable because of its direct competition with food and feed crops in general, which could lead to the increment in prices of food. Non- edible biomass such as lignocellulosic feedstock, provided a more viable option. It is obtained from both agricultural and forest remnant. Another advantage of the lignocellulose feedstock over the first generation biomass is its high yield; however, the major limitation attached to the biomass for the production of biofuel is the threat it poses to the global ecological system and also the amount of land availability it requires to meet both the needs and demands globally of biofuel.[3]

In recent years, microalgae have been getting a lot of attention over other biomass because it does not stand a direct competition to food or feed production. Algae possess a much higher photosynthetic efficiency than both first and second-generation biomass; hence, leading to a much better diminution of CO_2, and also a higher growth rate. Thus, algae pose as a more sustainable biofuel source, that reduces the greenhouse gas emissions and also doesn't require any land quality for it to grow. [4], [5]. A vital limitation associated with the use of algae to generate an alternate source of energy is its production on a large commercial scale which can be ascribed to the high cost of production. Algae can be described as a unicellular or multicellular organism; they can be grown in both marine and freshwater [6]. Algae can be grown in wastewater and also in any other poor water quality source, the chemical composition of algae is one of the main features that distinguish it from other biomass, and it can be affected by the conditions surrounding the environmental habitat of the algal biomass. Such conditions include light, temperature, pH, nutrients, pollution, and salinity in the water used for culturing the algae. Light is the primary donor of all the listed conditions, and as such, it's also used to classify algae into different zones or layers, depending on the distance of the algae from the surface of the sea or pond. Algae absorb light differently, depending on the species of the algae, and some algae are more adaptive in nature than others. Some precise algae can condone not so favorable environmental conditions, this type of algae can be produced all through the year, they do not need fresh water for their cultivation [7].

Algae undergo a rapid growth rate, and reproduction for algae occurs quite rapidly, it only takes hours for algae to be reproduced.[1]. Algae provide a higher growth rate, and higher energy conversion efficiency by photosynthesis than terrestrial biomass, it's considered as the most renewable, environmentally friendly, and sustainable source of biofuel.

Even though algae present a lot of advantages when compared to other biomass, several obstacles still limit the wide utilization of algae, a significant challenge is the high cost of production. The overall process of converting algae into liquid fuel or any form of energy is highly expensive when compared to fossil fuel from the cultivation of algae to its harvesting, separation and finally processing it into biofuel or bioenergy. [8]. Fossil fuel also as to be utilized for the transportation of algae during its processing, which may also add to the total cost of processing algae. Due to its high cost of production, algae at the moment is not a commercially viable option to replace fossil fuel.

1.2 Limitations of Algae

Immense use of nutrients and fertilizers are added to the water used for culturing algae. This makes the water rich in metals such as aluminum, calcium, potassium, sodium, phosphorus, iron, magnesium, zinc, and silica [9]. The presence of these metals limits the use of algae for direct combustion and gasification, it also reduces the efficiency of the bioproducts to be obtained from algae during conversion. Ash has an exceedingly harmful effect on the machine or equipment used for the conversion of algae into liquid fuel, chemicals, and other bioproducts. The ash produced in thermochemical conversions such as gasification or pyrolysis is due to the rapid high temperature that the algae is subjected to during the process. The presence of ash when this takes place leads to low efficiency in the biofuel, slagging of the reactor used, and permanent damage to the equipment used. [10]. The ash content in algae varies, depending on the species of algae, how the algae were cultured, and the means of storage of the algae can also be a factor that adds to the overall ash content or percentage of the ash. High ash content can lead to a significant increase in the cost of conversion, high inorganic component during biochemical conversion directly corresponds to depletion of some of the organic component (carbohydrate) of the biomass.

[11].Furthermore, conversion technologies such as catalytic upgrade into liquid fuel can be hindered by the ash content in algae. The initial removal of ash from algae before the conversion will amount to total capital cost savings, higher efficiency in the biofuel or bioproduct produced, and will also reduce the damage to technological equipment and machines.

Various treatments have been investigated for the removal of both physiological and non-physiological ash. Jenkins et al. [12] investigated the removal of inorganic components from rice straw and wheat straw using water. Washing of biomass with De-ionized water (DI) as proven effective for the reduction of ash, but most especially non-physiological ash (soil). They are easier to remove when compared to the physiological ash because they are not directly attached to the structure of the algae.[13]. Additionally, Turn et al. [14] detailed the use of distilled water to generate ash removal of about 40% from switchgrass. Several factors such as ratio of the biomass to water, temperature of the water added, and the amount of time the biomass was exposed to the water, were considered for the effective removal of ash from the treated biomass. There is a limit to the amount of the inorganics that water can effectively remove, the majority of the ash content is tied to the structure of the algae.

Several studies have been investigated using acid for the removal of ash, especially physiological ash, which is closer to the inner molecular structure of the algae. In such cases, use of acid has been reported to have an effective removal of ash from the biomass. Stylianos et al. [15] described how the use of nitric acid yielded a notable reduction in the ash percentage of the biomass. The addition of both weak and strong acid as also been investigated and reported to successfully remove the ash content of the biomass [16]. Although acid was reported to be effective in the removal of inorganics, however, the use of acid has an unfavorable effect on the biomass and the environment. Some of the drawbacks experienced include; the disintegration of some of the

organic component of the biomass, most importantly the carbon content of the algae, and a waste stream which may need to be neutralized. Hence, a better alternative is the use of chelating agents, which has recently been getting much more attention because they interact very actively with metal ions and make an alteration to their solubility in the algae. Chelating agents are usually used to remove inorganic metals in soil, this is because chelating agents can accommodate about two or more electron donor atoms, which can then form co-ordinate bonds to just a single atom. The most commonly used chelating agents are ethylenediaminetetraacetic acid (EDTA), citric acid, and nitrilotriacetic acid (NTA). Edmunds et al [17] investigated the use of EDTA to generate a low ash in a bioenergy feedstock, however, they also pointed out some of the of nitrilotriacetic of EDTA. The major restriction that was pointed out was the effect EDTA will have on the environment after being frequently used, EDTA is a non-biodegradable chelant. In contrast to EDTA is NTA, which is a biodegradable chelating agent, and this makes NTA industrially applicable as any form of treatment. Furthermore, it also preserves the hydrolysis of cellulose and hemicellulose, which are important organic components of biomass.

Nitrilotriacetic (NTA) was chosen to be investigated because it does not create any threat to the environment, NTA is also used to isolate trace metals as water-soluble complexes, which are the major constituents of inorganics present in algae.[18]. NTA has been applied and distributed in detergents in developed countries. It is used in paper processing to prevent the decomposition of peroxide and hydrosulfite. Lastly, NTA is used industrially for the treatment of boiler water to avert any buildup of mineral scale in the boiler. [18]. NTA is an amino tricarboxylic acid which has been approved by the Food and Drug Administration in food plants.[19]. Edmunds et al. [16] investigated the use of three acids (acetic, citric and EDTA) for the effective removal of inorganics from wood. EDTA used in this study was employed for the removal of Cr, Cu, As, and Pb. NTA

is comparatively expensive in its synthesized form, this will ultimately lead to an imprudent total cost, for this reason, our focus is on being able to recycle the NTA adequately so that the NTA treatment can be more economical.

Various studies in the past have reported the recycling of EDTA for the removal of inorganics (metal ion) from soil. Zeng et al. [20], Was able to recycle the EDTA a chelant, seven times for the effective treatment of the soil. The main purpose of this research is to be able to generate a cost-effective treatment for the removal of ash from algae, without jeopardizing or deteriorating the organic components of the algae. The ash percentage of the algae will be studied for all the treatments applied, along with the organic constituents of the algae sample. The recycling process will also be evaluated, just to know the number of times the NTA can be recovered and reused, NTA will be recycled by adding Na_2S with $Ca(OH)_2$ to it. Since NTA, and the method that will be applied for recycling will contain some of the metals that are to be removed, Deionized water (DI) will be used to rinse the samples after each extraction process is complete.

CHAPTER 2

METHODS

2.1 Materials

Three different species of algae were treated and analyzed initially to identify the ash content of the different algae samples. Two benthic algal polyculture planktonic species were received from Sandia National Laboratories and accompanied by a Scenedesmus algae. Table 1 below shows the ash content for the sample before and after treatment.

Table 1: Ash analysis for 3 different algae sample.

	Initial Ash content (%)	Ash content after treatment (%)
High ash (SS ATS)	85.1	82.7
Medium ash (Green ATS)	53.9	49.2
Low ash (Scenedesmus)	15.2	3.8

Due to the high ash content of both the SS ATS and the Green ATS the algae sample (*Scenedesmus*) that was utilized for this research was grown, cultured, and harvested at Livermore, CA in 2017. The algae were processed at Sandia National Laboratories (Livermore, CA), the technique used to culture the algae is referred to as algal turf scrubber (ATS). 1.2 kilograms of the algae sample was dried and delivered to Old Dominion University, where the sample was pulverized, freeze-dried, and stored before any treatment or analysis was carried out on. Additional materials used for this study were commercially purchased and utilized as received.

2.2 Ash treatment.

Deionized water (DI), and 0.05M concentration of nitrilotriacetic acid (NTA) was chosen for this study. About 3g of algae was measured and placed in a 60℃ oven for 24 h, 3 g of the dry algae was measured and added to a 200mL of DI water and stirred on a metal plate for 2 h. The algae sample was first exposed to DI water so that the non-structural ash/inorganics that are not bound with the structure of the algae can be easily removed before the washed sample is exposed to the chelating acid. The treated sample was then centrifuged using a Fisher Scientific accuSpinTM 400

for easy separation, extraction, convenient analysis, and further treatment. The tubes used were vortexed for 30s to improve the extraction of solids. The recovered liquid solution was stored in a temperature-controlled refrigerator for further analysis, and the solid sample was put in a 60℃ oven for further treatment. The chelation of the recovered solid sample of the dry algae was performed in a 200mL Erlenmeyer flask. NTA was used because it serves as a chelating agent that binds metal ions in a covalent bond and it is less destructive for the algae than using a strong acid. Hence, leads to the removal of metal ions from the cross-linked structure of the biomass.

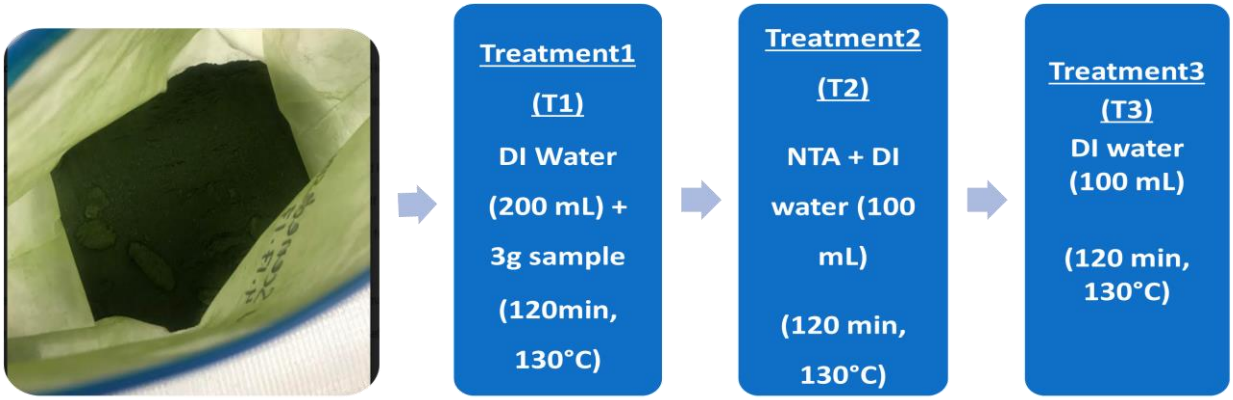

Figure 1

0.05 M of NTA was measured and allowed to stir for 30 min before the algae sample that was dried in a 60℃ oven was added and stirred for 2 h. The sample in the Erlenmeyer flask was then centrifuged again for easy separation between the liquid and the solid sample, recovered algae sample was dried in a 60℃ oven for 24 h for further treatment and analysis. Lastly, at the end of the chelation extraction, the sample was thoroughly washed with 100mL of DI water, centrifuged and dried at 60℃ for 24 h. Five different reaction temperatures were tested (90, 100, 110 120 and 130℃). The degradation temperature for chelating acid is 170℃, this study did not exceed 130℃ to avoid any possible degradation, and also to prevent the loss of the organic components of the algae during the treatment, while still being able to generate an effective removal of the inorganic components present in the algae. After the completion of every treatment on the algae at different

temperatures, algae samples were subjected to ash analysis using the dry oxidation method at 575 ± 25℃ as described by the National Renewable Energy Laboratory (NREL). The chelating treatments used were recycled and reused, hence reducing the general cost of the treatment.

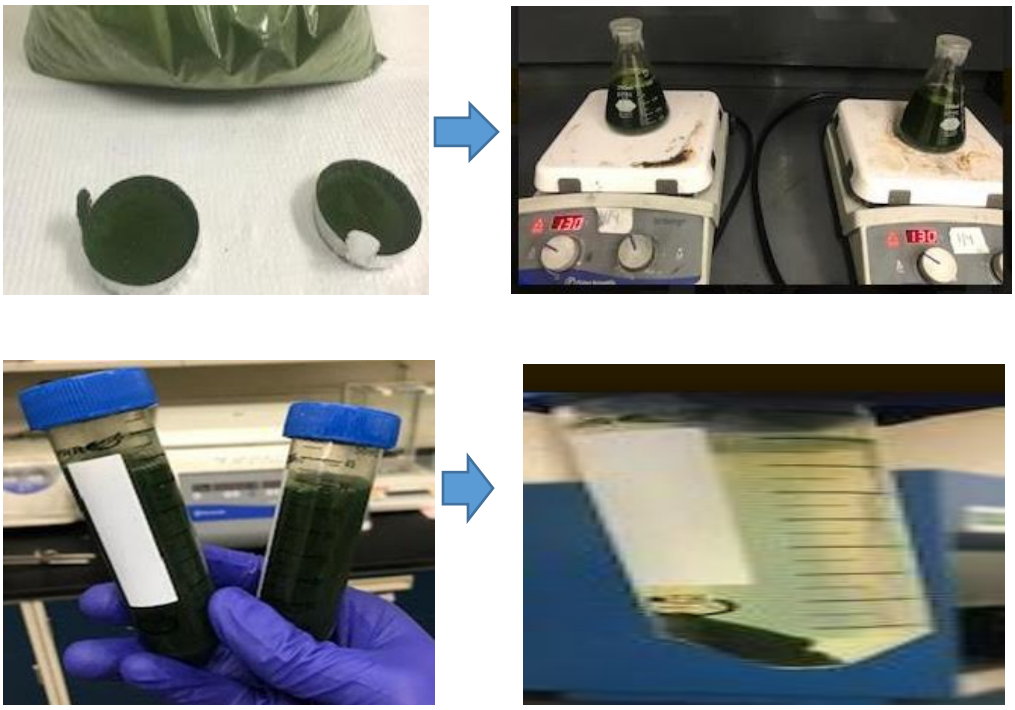

Figure 2

2.3 Successive washing cycles.

Two grams of DI treated algae was added to 100 mL of NTA solution in a 250 mL Erlenmeyer flask and stirred (350 rpm) using a magnetic bar stirrer, for 2 h at 130 ℃. The solution was centrifuged for easy recovery of the NTA solution; the liquid portion was stored in another Erlenmeyer flask so that it could be reused for the removal of trace metals. During the separation of the trace metals from NTA, 0.5M of Na_2S was slowly added and stirred gently, a measured amount of $Ca(OH)_2$ was also added to improve the effective precipitation of the trace metal by Na_2S. The mixture was allowed to sit under a fume hood overnight, the supernatant was gently extracted using a 5 mL syringe and transferred to a 250mL Erlenmeyer flask for it to be reused for

further treatment of the algae sample. This method was repeated twice for effective reduction of ash in algae. The pH of the solution was 10.5 when Na_2S and Ca $(OH)_2$ were added to the recovered NTA solution. [20, 21]

The pH of the recovered NTA used for the initial treatment of the algae was 4.5 prior to the addition of the listed compounds. The pH was adjusted back to 4.5 with the gentle addition of 10% HNO_3 from a pH of 10 after the addition of Na_2S and Ca $(OH)_2$. 0.05M concentration of NTA was used to treat approximately 3 g of algae after three washing cycles, to achieve an effective reduction of ash between 64 -85.1% from the algae. After every successive treatment of the algae using the NTA solution, the treated sample is placed in a 60℃ oven for 24 h. Furthermore, the dry treated algae were then soaked and stirred in a 100 mL DI water for 2 h; this is done to rinse the sample off any trace metal that may be lingering on the outer walls of the algae structure. The ash percentage in the algae for each of the treatment applied was recorded.

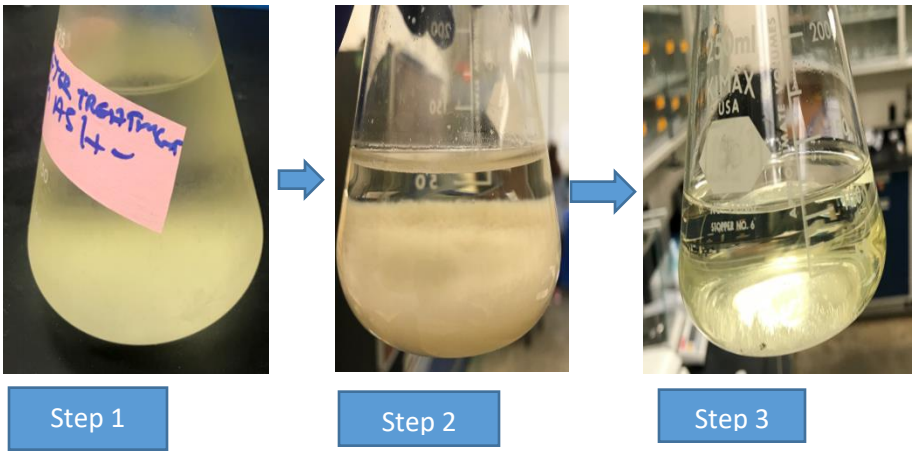

Figure 3: Stages for NTA recycling

2.4 Composition analysis

The total ash content for the untreated algae, DI wash, and chelation-treated algae was carried out using the protocols generated by National Renewable Energy Laboratory (NREL): Determination

of Total Solids and Ash in Algal Biomass. Further chemical analysis for different stages of treatment applied to the algae sample was done by employing various standard protocols developed by NREL which include: Determination of Total Carbohydrates in Algal Biomass; Standard Test Percentage of Moisture, Oven Dry Weight (ODW) and Determination of Protein Content. All the analysis recorded were done in triplicate. [22, 23] [22]

2.5 Ultimate Analysis

The CHN and S analysis was carried out for different stages of the treatment applied, along with the untreated algae using a Flash 2000 (Organic Elemental Analyzer) CHNS-O module. Oxygen was calculated using the difference in the composition of the other element. The sample was put in a 60℃ for 24 h before analysis was done, to ensure a dry basis analysis.

2.6 TOC/TN determination in treatments

Total organic carbon (TOC) and total nitrogen (TN) were analyzed for each of the treatments after the solid samples had been removed from the solution. This was done using a Shimadzu total organic carbon analyzer (TOC-VCSN). Also attached to this is a Shimadzu total nitrogen measuring unit (TNM-1).

2.7 Fourier Transform Infrared Spectroscopy (FTIR)

A Buruker Alpha Platinum- ATR was used in the untreated algae and all the stages of treatment applied to the algae. All the algae samples were analyzed using the infrared spectra (400-4000 cm^{-1}) and grounded up into fine powdered particles, dried at 60℃ for 24 h before analyses was conducted on each of the samples. About 0.05- 0.07g of the dry sample was inserted and pressed up against the zinc crystal using the instrument's diamond surface with its rod. The spectra were acquired using 300 scans for the background and 64 scans for each of the samples analyzed.

Attenuated total reflection (ATR) was the model used for easy and quick molecular information about the samples, the samples remain non-destructive after the analyses were completed.

2.8 Flame Atomic Absorption Spectroscopy (AAS)

Flame AAS was used to analyze the inorganic elemental component of the algae sample for different stages of treatment applied. A 250 µL of 72% (w/w) of H_2SO_4 was added to 0.025 g of dry solid algae sample in a glass tube. The glass tube was placed in a 30℃ water bath for 1 h, while been vortexed every 10 minutes. Additionally, a 7mL of 18.2 Megaohms (MΩ) water was applied to the glass tubes to bring the concentration of the of the sulfuric acid to 4% (w/w). A rubber stopper was place on each of the test tubes to seal it, and the sample was inverted several times to ensure a thorough mix. The tubes were then placed in a rack suitable for autoclaving. The temperature for the autoclave was set at 121℃ using the liquids (slow) settings for 1 h. After the samples were autoclaved, they were allowed to cool for 15 mins before they were removed from the autoclave. Furthermore, the samples were cooled to room temperature for another 30 minutes to 1 h. The recovered samples were then filtered through a 0.2 µm filter using the disposable syringe and compatible filter. The samples were then stored in a 4℃ refrigerator for less than 24 h before AAS analysis was carried on each of the samples digested for inorganic elemental analysis using the FAAS (AA-7000 SHIMADZU)

CHAPTER 3

RESULTS AND DISCUSSION.

3.1 Ash Analysis

The initial ash content for the raw untreated algae was 15.2%. The ash content of algae varies for different algae species. This can be as a result of other relative factors such as nutrients used to

culture the algae, method of storage, harvesting period and the preprocessing procedure. All these can be factors that can contribute to how low or high the ash content of the biomass may turn out to be. However, the ash percentage for the algae is high enough to cause damage to the equipment used for the thermochemical conversion of the algae, which will also reduce the efficiency of the organic nutrient that can be extracted from the algae. Different temperatures(90, 100, 110, 120, and 130℃) were investigated for the treatment of ash. The treatment applied to the algae was more effective as the temperature for the treatment increased while, every other parameter for the treatment remained the same (i.e. Time taken, rpm, concentration of NTA solution, and volume of DI).

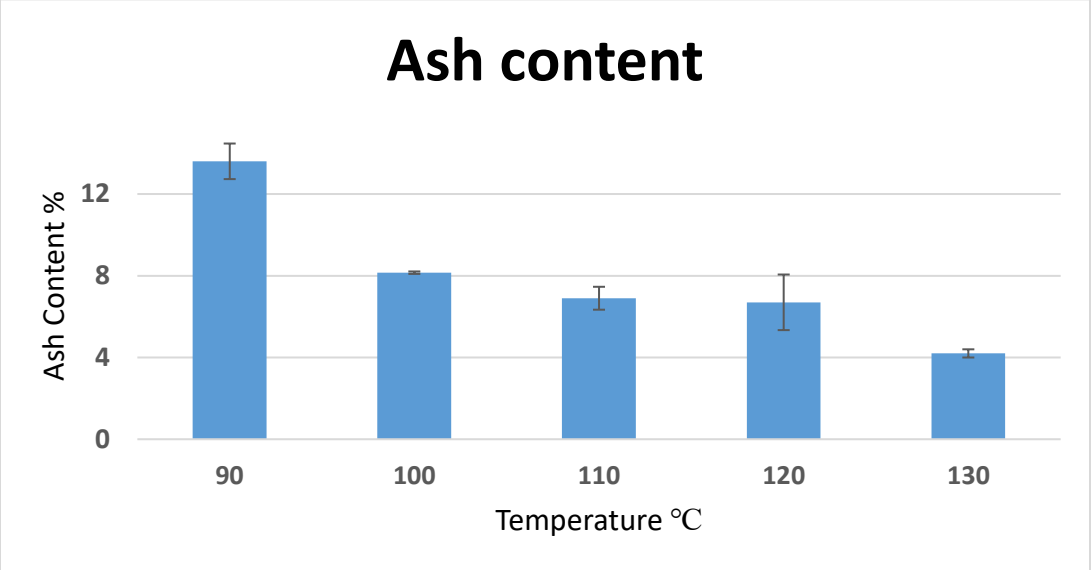

Figure 4: Ash content for Scenedesmus algae for consistent treatments but varying temperature

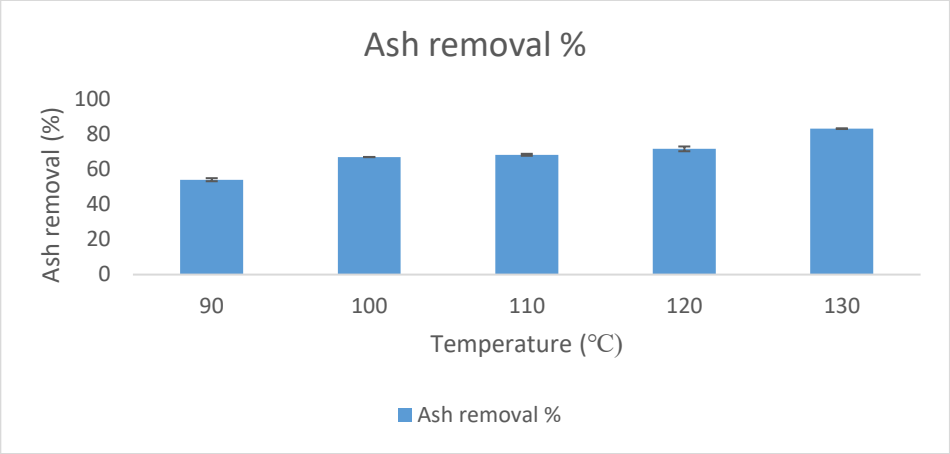

Figure 5: Ash removal in Scenedesmus algae for consistent extraction treatment but different temperature

The ash removed from the treated sample was at the highest when subjected to a temperature of 130℃ for 2 h, at this temperature the ash content for the algae was at 3.83%. The least effective treatment was recorded at 90℃ for both the ash content as well as the ash removed. The ash content of various treated algae at different temperatures is presented in Fig 4. The ash removal from the extraction varies from 54 to 85.1%. At 130℃ the initial DI wash pretreatment showed the least removal rate of 24 % and the ash content was 12.86 %, while the removal rate after NTA was applied to the algae at 56%. This also resulted in ash content reducing to 7.2 %, which was the highest removal rate of all the treatments applied to the algae. The final treatment applied to the algae was a DI wash, this was essential due to the NTA applied treatment of algae, which may react strongly with some of the metals in the algae such as Al, Ca and Mg, which may be detrimental to the organic structure of the algae during its thermochemical conversion. The ash removal after another DI was applied at 130 ℃ and the ash content of the algae was 54% and 3.8% respectively.

Table 2: Ash content and the ash removed by each stage of the treatment applied to the algae.

Pretreatment	Ash content %	Ash Removed %
After DI	12.86 (±1.2)	24.2
After NTA	7.22 (±0.4)	54
After NTA + DI	3.83 (±0.8)	56

Table 3: Ash analysis after reusing NTA

SUCCESSIVE RECYCLING	**ASH CONTENT (%)**
1	**7.52 (±0.5)**
2	**8.10 (±0.05)**
3	**23.72 (±1.8)**

The maximum temperature applied for the treatment of algae was 130℃, this temperature was not exceeded because a higher temperature will cause the degradation of the organic components of the algae such as hemicellulose, cellulose, and protein. [17].

NTA indicates the highest reduction of structural ash in the treatment applied on the algae. A concentration of 0.05 M of NTA was applied after DI wash was applied, DI wash was applied prior to NTA so that the non-physiological ash can be washed, and structural inorganics can be more vulnerable for removal after been exposed to NTA. A total ash removal of 85% was recorded at 130℃.

3.2 Successive recycling.

The extraction performance of metals from algal biomass with reclaimed NTA in a couple of successive washings proved to be effective, but the ash reduction decreased with each successive washing cycle. In the first two trials the metals were fairly precipitated with the addition of Na_2S and Ca $(OH)_2$ treatment which made the reclaimed NTA free of inorganic metals, any lingering metal surrounding the algal biomass after it's treated with the reclaimed NTA has been washed with DI water after it's dried in a 60℃ oven . Although some NTA is lost through the adsorption of the chelating agents by the algal biomass, 0.05M of NTA was used to treat 6 g of algal biomass. The NTA solution was less effective at the third trial, the ash content of the algae after DI treatment was higher than the original ash content of the algae (15.2%).

3.3 Ultimate Analysis.

The results of the ultimate analysis for the raw sample and for the different stages of treatment applied to the algae is shown in Table 3**.** The results indicate similar hydrogen and nitrogen content when compared to the raw algae. An increase in oxygen was observed when compared to that of the initial raw sample. However, carbon showed a slight decrease after the first two treatments were performed, but an increase can be observed after the final treatment was carried out on the algae. The ash content is lower in the treated samples than the untreated sample, a significant

decrease is shown after the addition of NTA followed by DI wash. This indicates the importance for the sample to be washed in the DI after it as been subjected to NTA. The ash content reflects the reduction of the inorganic compound, while the elemental components are preserved after the treatment was carried out. The treated algae analyzed below is treated at a 130℃, 300 rpm, and for a duration of 2 h, all the factors listed remained constant for all the stages of treatment applied to the algae.

Table 4: Ultimate analysis of untreated and treated algal biomass

SAMPLE DESCRIPTION	RAW ALGAE (SCENEDESMUS)	AFTER DI WASH	AFTER NTA	AFTER NTA +DI
ASH (%)	15.2	12.9	7.2	3.8
H (%)	6.4	6.2	6.1	6.8
C (%)	46.2	45.6	45.6	50.6
N (%)	8.4	7.7	7.2	7.6
O (%)	23.4	27.6	33.9	31.2

3.4 Protein Determination (Protein_N)

The protein value for the algae before and after treatment were measured using a CHNS elemental analyzer (ThermoFIsher Scientific, Waltham, MA) with 2,5-Bis(5-terte-butylbenzoxazol-2-yl) thiophnene (BBOT) standard. The values recorded form the analyzer and converted the protein content using a literature conversion factor of 4.78, that was recommended by NREL.[23, 24].

% protein = % N × Nfactor.

Table 5: Protein content of raw and treated algae

SAMPLES	PROTEIN %
RAW	40.15
AFTER DI	36.81
AFTER NTA	34.42
AFTER NTA + DI	36.32

The protein percentage is highest in the raw algal biomass than in any of the treated sample. The difference in the protein percentage is not significant, protein content in the algae was lowest after NTA was applied to reduce the inorganics in the algal biomass.

3.5 FTIR analysis of raw and treated algae.

The FTIR indicates bond energies of characteristic groups in algae, it identifies the changes in the molecular formation resulting from different stages of treatment applied to the algae sample. [25]. By identifying the peaks of FTIR spectra, it signifies and identifies the chemical structure of the sample being analyzed, which is a result of vibrations of various functional groups. Table 5 below shows the functional groups of each wavelength. The initial shape of the *Scenedesmus* showed peak intensities at particular wavelengths.

Table 6: IR absorption corresponding to various functional groups[26-28]

WAVE NUMBER ($\mathbf{cm^{-1}}$)	Functional groups	Possible compounds
1733	C= O stretching	Ketone and hemicellulose
1638	C = C	Lignin
1603-1618	C = C stretching	Cellulose, lignin
1485	O $-CH_3$	Lignin

1426	OH bending	Acid
1367	CH bending	Acid
1244	C −O−C stretching	Cellulose
1213	C−O stretching	Lignin
1,186, 1,163	C−O−C stretching vibration	Cellulose, lignin
1,140, 1,103	OH association	Alcohol, hemicellulose
1036	C−O stretching C−O deformation	Alcohol
896	C−H	Cellulose, hemicellulose

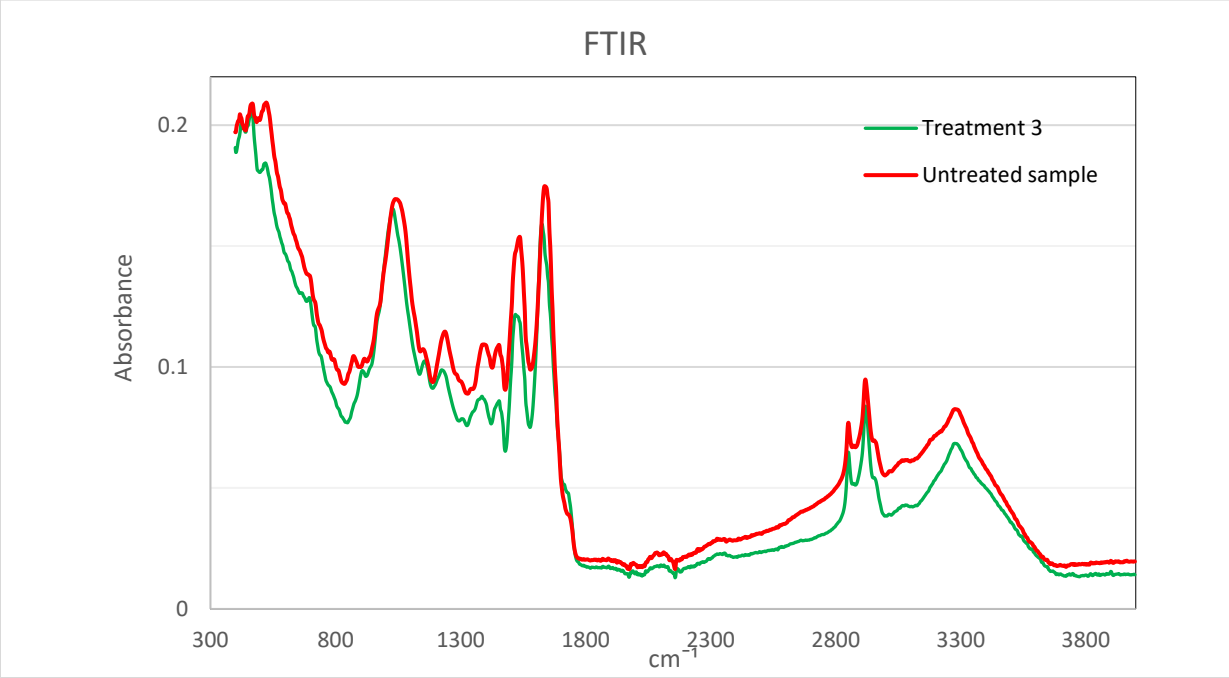

Figure 6: FTIR spectra of raw algae and after NTA and DI was applied to the algae

The FTIR shows the spectra for the raw algae sample and for the treated sample after all the treatments were completed. The spectra shows no indication of any significant changes have been experienced for any of the functional groups present in the raw sample of the algae, especially that of the aromatic carbon-hydrogen bond of hemicellulose (C−H). All the initial peaks are still sharp, above FTIR spectra demonstrates that all the treatments applied to the algae do not alter the chemical structure of hemicellulose, lignin, or cellulose.

3.6 Sugar concentration in raw and treated algae.

The concentration of biomass monomeric sugars (glucose, xylose, galactose arabinose, and mannose) were analyzed quantitatively using the high-performance liquid chromatography (HPLC) using the protocol developed for determination of total carbohydrate in algal biomass by NREL. [22]. All the monomeric sugars had a baseline resolution. Figure 7 shows the sugar concentration of the raw sample and the sugar concentration of all the treatment applied to the algae.

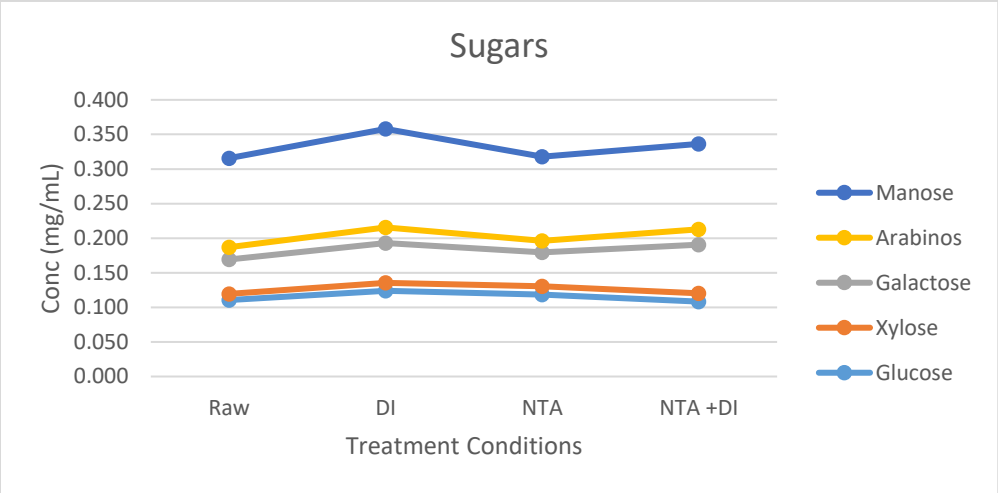

Figure 7: Sugars present in raw and treated algae

The concentration of sugar in the treated sample shows sharper peaks when compared to the raw sample. This simply implies an increased concentration of these components after the treatments were applied.

3.7 TOC/TN analysis of all treatments.

TOC/TN was used as a technique in this study to monitor the total amount of organic and total nitrogen content of all the treatments used for ash reduction in the algal biomass.

Table 7:TOC/TN analysis of all treated sample

	Total Carbon (g/mL)			**Total Nitrogen (g/mL)**		
	After DI(g)	After NTA	NTA&DI	After DI	NTA	NTA &DI
	0.09	0.10	0.07	0.06	0.03	0.02
	0.09	0.13	0.06	0.07	0.03	0.02
Avg	**0.09**	**0.12**	**0.07**	**0.07**	**0.03**	**0.02**

The NTA solution after treating about 2.5g of algal biomass shows the highest organic removal of all the three treatment stages applied to the algal. Next to the NTA was the DI water, and this is mostly due to the 200 mL volume of DI wash that the algal was subjected to initially for the removal of the non-structural ash present in the algae. The DI was after the NTA recorded the least TOC/TN in its solution. Though the treatments resulted in the removal of organic components, it was not significant as that of the inorganic trace metals removed. The conditions for the three stages of the treatment remained the same except for the volume of the first DI wash.

3.8 Inorganic analysis of raw/untreated and treated algal biomass.

The inorganic content of the raw algal biomass is presented in Table 8. Calcium cation was the dominant inorganic element of all the inorganic elements analyzed. Flame absorption spectrometry (FAAS) was used to analyze the metals in the algal biomass. All the raw/untreated and treated algal were weighed out in concurrence with the laboratory analytical procedure (LAP) developed by NREL. The results of the analysis showed reduction of metal concentration in the treated algae, which explains the reduction seen in the ash content of the treated algal biomass.

Table 8: Inorganic elemental analysis of algal biomass

	Ca (ppm)	K (ppm)	Cu (ppm)	Zn (ppm)	Pb (ppm)
Raw	6.72	4.43	0.55	3.60	0.27
After DI	6.52	2.77	0.33	3.30	0.14
After NTA	BDL	2.33	0.28	1.99	0.12
After NTA+DI	BDL	BDL	0.20	0.57	0.03

The three treatments applied for the reduction of the overall ash content in the algal biomass showed to be effective, with slight or complete removal of the inorganic metals. There was a significant decrease in the concentration of calcium, the concentration of calcium was below detection limit. A Similar result was detected for potassium after experiencing DI wash shortly after NTA was applied. The concentration of copper, zinc, and lead also decreases with successive treatments applied. This explains the reduction in the overall ash of the algal biomass.

CHAPTER 4

Conclusion.

This study investigated the effective treatment of algal biomass for total ash reduction using DI wash followed by subjecting the algal to a chelating agent (NTA) and lastly rinsing it off with DI at 130℃. NTA and the metals were separated in multiple trials so that NTA can be reused for further treatment of algal biomass.

The three stages of treatment applied for the removal of both physiological (structural) and non-physiological ash proved to be effective in the reduction of the overall ash in the algal biomass. DI wash afforded significant reduction, however, NTA proved to be the most effective removal of ash

from the algal biomass. Furthermore, NTA removes the overall ash without having any significant degradation to the structural components of the treated algae. This project indicates the benefits of a chelator-mediated extraction treatment of inorganic metals reduction in algae. Lastly, due to the high cost of NTA, future research towards more successive recycling of NTA will be investigated. Future study will aim towards recycling NTA efficiently for a total of seven successive usages, so that NTA can become a more economically viable treatment for removal of ash from algae. Further analysis on the inorganic metals present in the algae such as Si, Cl, Fe, and Ti would give a better understanding of the reduction and removal of ash from the treated sample.

REFERENCES

1. Ullah, K., et al., *Assessing the potential of algal biomass opportunities for bioenergy industry: a review.* Fuel, 2015. **143**: p. 414-423.
2. Klass, D.L., *Biomass for renewable energy, fuels, and chemicals*. 1998: Elsevier.
3. Singh, A., P.S. Nigam, and J.D. Murphy, *Renewable fuels from algae: an answer to debatable land based fuels.* Bioresource technology, 2011. **102**(1): p. 10-16.
4. Brennan, L. and P. Owende, *Biofuels from microalgae—a review of technologies for production, processing, and extractions of biofuels and co-products.* Renewable and sustainable energy reviews, 2010. **14**(2): p. 557-577.
5. Bixler, H.J. and H. Porse, *A decade of change in the seaweed hydrocolloids industry.* Journal of applied Phycology, 2011. **23**(3): p. 321-335.
6. Demirbas, A., *Use of algae as biofuel sources.* Energy conversion and management, 2010. **51**(12): p. 2738-2749.
7. Gust, D., et al., *Engineered and artificial photosynthesis: human ingenuity enters the game.* MRS bulletin, 2008. **33**(4): p. 383-387.
8. Huber, G.W., S. Iborra, and A. Corma, *Synthesis of transportation fuels from biomass: chemistry, catalysts, and engineering.* Chemical reviews, 2006. **106**(9): p. 4044-4098.
9. Vassilev, S.V. and C.G. Vassileva, *Composition, properties and challenges of algae biomass for biofuel application: an overview.* Fuel, 2016. **181**: p. 1-33.
10. Froment, K., et al., *Inorganic species behaviour in thermochemical processes for energy biomass valorisation.* Oil & Gas Science and Technology–Revue d'IFP Energies nouvelles, 2013. **68**(4): p. 725-739.
11. Weiss, N.D., J.D. Farmer, and D.J. Schell, *Impact of corn stover composition on hemicellulose conversion during dilute acid pretreatment and enzymatic cellulose digestibility of the pretreated solids.* Bioresource technology, 2010. **101**(2): p. 674-678.
12. Jenkins, B., R. Bakker, and J. Wei, *On the properties of washed straw.* Biomass and bioenergy, 1996. **10**(4): p. 177-200.
13. Das, P., A. Ganesh, and P. Wangikar, *Influence of pretreatment for deashing of sugarcane bagasse on pyrolysis products.* Biomass and Bioenergy, 2004. **27**(5): p. 445-457.
14. Turn, S.Q., C.M. Kinoshita, and D.M. Ishimura, *Removal of inorganic constituents of biomass feedstocks by mechanical dewatering and leaching.* Biomass and Bioenergy, 1997. **12**(4): p. 241-252.
15. Stefanidis, S.D., et al., *Optimization of bio-oil yields by demineralization of low quality biomass.* Biomass and Bioenergy, 2015. **83**: p. 105-115.
16. Liu, X. and X.T. Bi, *Removal of inorganic constituents from pine barks and switchgrass.* Fuel processing technology, 2011. **92**(7): p. 1273-1279.
17. Edmunds, C.W., et al., *Using a chelating agent to generate low ash bioenergy feedstock.* Biomass and bioenergy, 2017. **96**: p. 12-18.
18. Anderson, R.L., et al., *A review of the environmental and mammalian toxicology of nitrilotriacetic acid.* CRC critical reviews in toxicology, 1985. **15**(1): p. 1-102.
19. Dickey, R.W. and W.W. Dickhoff, *Dispersants and Seafood Safety Assessment of the potential impact of COREXIT® oil dispersants on seafood safety.* 2011.
20. Zeng, Q., et al., *Recycling EDTA solutions used to remediate metal-polluted soils.* Environmental Pollution, 2005. **133**(2): p. 225-231.
21. Lindsay, W., *L. 1979. Chemical equilibria in soils.* New York.
22. Van Wychen, S. and L.M. Laurens, *Determination of total carbohydrates in algal biomass: laboratory analytical procedure (LAP)*. 2016, National Renewable Energy Lab.(NREL), Golden, CO (United States).

23. Laurens, L.M., *Summative Mass Analysis of Algal Biomass-Integration of Analytical Procedures: Laboratory Analytical Procedure (LAP)*. 2016, National Renewable Energy Lab.(NREL), Golden, CO (United States).
24. Lourenço, S.O., et al., *Distribution of intracellular nitrogen in marine microalgae: basis for the calculation of specific nitrogen-to-protein conversion factors.* Journal of Phycology, 1998. **34**(5): p. 798-811.
25. Reza, M.T., *Upgrading biomass by hydrothermal and chemical conditioning*. 2013.
26. Reza, M.T., et al., *Ash reduction of corn stover by mild hydrothermal preprocessing.* Biomass Conversion and Biorefinery, 2015. **5**(1): p. 21-31.
27. Viera, R.G., et al., *Synthesis and characterization of methylcellulose from sugar cane bagasse cellulose.* Carbohydrate Polymers, 2007. **67**(2): p. 182-189.
28. Saddawi, A., et al., *Commodity fuels from biomass through pretreatment and torrefaction: effects of mineral content on torrefied fuel characteristics and quality.* Energy & Fuels, 2012. **26**(11): p. 6466-6474.