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# Extraction and characterization of phosphorus from organic algal biochar

By

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Submitted to the graduate degree program in Civil, Environmental & Architectural Engineering and the Graduate faculty of the University of Kansas in partial fulfillment of the requirements for the degree of Master of Science.

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Date Defended: September 8, 2014

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# Extraction and characterization of phosphorus from organic algal biochar

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Chairperson: Edward Peltier

Date Approved: December 19, 2014

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## Abstract

Algae has become a major research focus in the past few years. Treatments such as pyrolysis, hydrothermal carbonization, hydrothermal liquefaction, hydrothermal gasification are being used to process algae and yield products such as lipids, lipid-rich hydrochar, biocrude, methane and hydrogen. These treatments also produce biochars as a product of thermal decomposition of organic material under limited supply of oxygen at temperatures less than 700$^{o}$C. Biochars have potential for use as a soil amendment, both to improve soil quality and to sequester carbon. Algal biochar used in this study was produced by hydrothermal liquefaction of algae at 350$^{o}$C which was grown at Lawrence Wastewater Treatment Plant using municipal wastewater as the feedstock. This biochar was black in color, very smooth & powdery in texture and tremendously hydrophobic in nature. This study was aimed at extraction and characterization of phosphorus from this organic algal biochar.

Since biochars are often used as a mixture with soils and there are no currently established methods for extraction of phosphorus from biochars, various soil P extraction methods were studied and investigated to be used on this biochar. For estimation of total P, acid digestion of biochar with sulphuric acid was attempted, but failed to achieve good digestion. Microwave acid digestion was tried with different combinations of acids, with reverse aqua regia ($HCl:HNO_3$::1:3) working best to digest this algal biochar. Most elements that were analyzed in the biochar, including Ca and P were present in biochar at a higher concentration than in algae. ICP analysis of the digested samples gave a total P content of 7.4±0.58 % by wt. which was 3.2 times the total

P in algae (2.3±0.03% by. wt.). Similarly, a high amount of total Ca was also found in the biochar (17.10±1.31% by wt.) as compared to 5.70±0.18% by wt. in algae. Approximately 89% of calcium and 95% of phosphorus initially present in the algae were recovered from the biochar after hydrothermal liquefaction.

Because of the high Ca content, it was thought that P in char might be mostly present as calcium phosphates. The Olsen Method was used to estimate bioavailable phosphorus from biochar because it is best suited for calcareous soils. On ICP analysis, a very small fraction (less than 1%) of total P in algal biochar was found to be extractable by the Olsen reagent. However, when shaking time was doubled from 30 minutes to 60 minutes in Olsen method, the amount of soluble P doubled from 287±110 ppm to 581±116 ppm. This meant that the calcium associated P in biochar may slowly release over time. Water extraction was completely ineffective in extracting any P from biochar. These low Olsen and water extraction P results may be because of insufficient contact of biochar with reagents owing to its hydrophobicity. Biochar was difficult to mix well with reagents for estimation of bioavailable P due to its hydrophobic nature. Heating at 105$^{o}$C overnight to get rid of the volatile organic fractions did not improve mixing. This study provides a preliminary idea of the nature of P in this biochar. Based on the extraction results, it appears that P in this biochar is mostly unavailable to plants. Hydrophobic nature of biochar may hinder its ability to mix well with soil and thus it may need to be treated before being used for soil amendment. There is scope for further research on this biochar including testing it with different extraction methods, performing extractions after washing biochar with an organic solvent, engineering the hydrophobicity of this biochar by altering the production conditions, and studying the actual long-term release of P under real or simulated field conditions.

To my parents, loving brother and sister and all dear friends without whose support, this wouldn’t be possible

## Acknowledgements

*(in no particular order)*

Dr. Edward Peltier, my advisor, for introducing me to this topic, being very encouraging and a wonderful support all throughout the duration of my graduate studies and dissertation work.

Dr. Belinda Sturm, faculty and committee member, for being on my committee, providing excellent knowledge and extending great support during my graduate studies.

Dr. Susan Williams, committee member, for making required material (biochar) for the experiments available to me and being on my committee.

Griffin Roberts, for guiding me through the experiments, training me on ICP, extending help whenever needed during the course of this research.

Department of CEAE, KU for awarding me three scholarships that helped me a lot to be financially stable.

My parents, brother Gaurav and sister Shubhra for their abundant encouragement and belief in me that brought me to the US and made it all possible.

All my fellow batchmates, especially Rasha Faraj, Farshid Kiani and Babak Mardan Doost for their invaluable assistance, companionship and support during these two years of study.

Alex Walker, for being my first & best international friend in the United States, making my stay at KU memorable and being a wonderful support.

Arunabha Choudhury, Siddhant Sethi & Ybairy Duin, for always backing me, sharing my problems and being there for me whenever needed during my time at KU.

All my dear friends in India, especially Peeyush Jain, Divir Tiwari, Prahlad Shripathi, Rahul Agarwal who have always believed in me and helped me morally as well as financially to be able to fulfil this dream.

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# 1 - INTRODUCTION

With rapidly increasing human population, environmental concerns have been ever growing. Talks of sustainability, climate-change, emerging renewable resources, and emerging green technologies have been an area of strong concentration over the past few decades. With environmental engineers and scientists working on newer sustainable and renewable technologies all over the world, aquatic biomass, in general, has gained tremendous attention at the energy-environment nexus (Abbasi *et. al.*, 2010). Aquatic plants can be used to perform environmental services such as wastewater treatment (Jernigan *et. al.*, 2013), phytoremediation (Wenzel, 2009) and $CO_2$ removal from flue gas (Mata *et. al.*, 2010). Specifically, algae has become the center of attention of environmental research in past two decades and has become pivotal in production of renewable alternatives of energy (Savage and Hestekin, 2013).

## 1a. Algae as a research focus

The algae are thallophytes (plants lacking roots, stems, and leaves), which have chlorophyllas their primary photosynthetic pigment and which lack a sterile covering of cells around the reproductive cells (Acreman, 1994). There are a hugely vast number of species of algae found with varying properties but these have been categorized broadly into two divisions – macroalgae and microalgae. Macroalgae, as is evident from the name, is bigger in size of the two and is commonly known as "seaweed". These are multicellular and are subdivided on the basis of their color as brown, red and green algae. On the other hand, microalgae are the unicellular really tiny algae that may not be seen with a naked eye inside water bodies. While microalgae is targeted as a source of lipids for algal oil, including the production of biofuels (Christi 2007; Brennan and Owende 2010; Mata *et. al.*, 2010b), macroalgae are lower in lipid content and there is currently

no obvious high-value end product for biomass from these fast growing species (Bird *et. al.*, 2011). Traditionally, algae have been considered for their lipid production potentials. However, high lipid-containing algae grow slowly and require controlled cultivation. Pyrolysis is an attractive option for fuel production from such lipid-deficient algae (DOE, 2010). Figure 1 presents the schematic for conversion of both lipid-rich and lipid-lean algal biomass into useful products (Maddi *et. al.*, 2011)

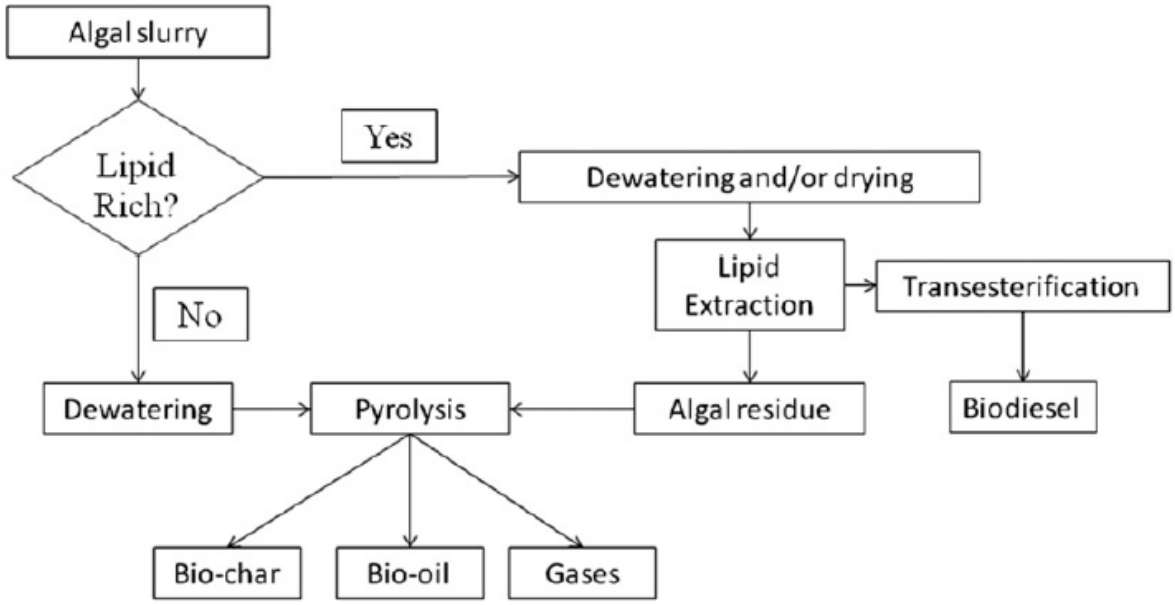


**Figure 1 : Flowchart showing possible algae processing options based on biomass lipid content.**

Hydrothermal treatment is a thermochemical conversion step that is well suited for processing wet algal biomass directly. Hydrothermal treatment simply subjects the biomass slurry to elevated temperatures and pressures that are at or above the vapor pressure of water at the reaction temperature so that the water remains in the liquid phase (when subcritical processing temperatures are used). The combined action of the thermal energy and hydrolytic attack of water molecules on the algae biomolecules lead to chemical reactions that convert the biomass to more useful biofuel intermediates (Savage and Hestekin, 2013).

As Figure 2 shows, hydrothermal treatment can be done at moderate temperatures (200°C) to produce a solid product (hydrochar), at intermediate temperatures (300°C–350°C) to produce a crude bio-oil, or at higher temperatures (400 – 600°C) to produce gaseous products (Savage and Hestekin, 2013).

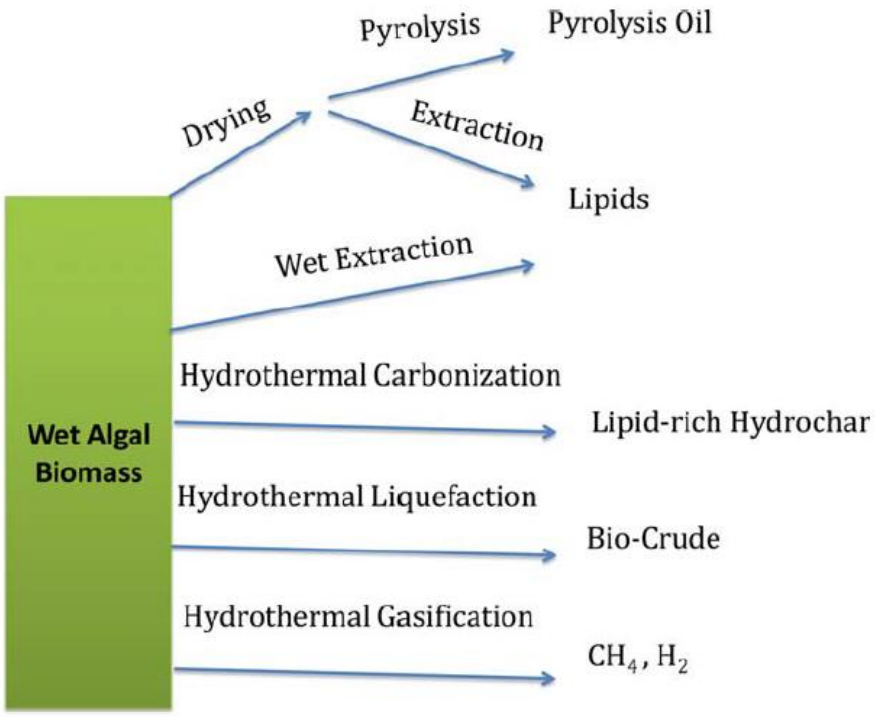


**Figure 2 : Illustrations of different options of converting algal biomass into biofuel intermediates (Savage and Hestekin, 2013).**

These developments in algae related research have encouraged artificial growth of algae in labs and reactors so that it can be used as a raw material to produce more and more renewable fuels and valuable products. Due to fast growing rates of algae and lower space requiremens compared to terrestrial plants, these operations have been quite successful. Research on byproducts from algal processing such as biofuel production and algal biochar have gained momentum recently and has now become one of the most prolific field of research.

## 2- Biochar and Algal Biochar

Biochar is formally defined as "the carbon-rich remnants obtained when biomass including wood, manure and leaves are heated in a confined environment with little to no available oxygen" (Lehmann and Joseph, 2009). It is the product that remains when biomass is heated within a certain temperature range in the absence of oxygen (air). Traditionally, biochar is made by digging an earthen pit that is filled with biomass such as wood and crop residues, setting a fire and then covering the pit with soil to eliminate the oxygen supply. This traditional process is air polluting as it is known to emit gaseous pollutants directly into ambient air (Wimmer, 2011).

More specifically, biochar is the product of thermal decompostion of organic material under limited supply of oxygen at temperatures less than 700 degree celsius (Lehmann and Joseph, 2009). Biochar should not be confused with charcoal, although the process by which charcoal is made is similar (Harris, 1999). The only difference between biochar and charcoal is in its utilitarian intention. Biochar is produced with the intent to be used as a soil amendment, to sequester carbon, or to filter percolating soil water, while charcoal is produced for other reasons (e.g. used as a fuel for heat, as a filter, as a coloring agent, iron making etc.) than biochar. (Wimmer, 2011; Verheijen *et. al.*, 2009). Biochar is a fine-grained, highly porous charcoal that helps soils retain nutrients and water. The carbon in biochar resists degradation and can sequester carbon in soils for hundreds to thousands of years, providing a potentially powerful tool for mitigating anthropogenic climate change (IBI, 2010). Also, biochar should not be confused with ash because these are made through different processes, ash by burning, biochar by pyrolysis, and their chemical composition differs greatly. Ash mainly consists of the minerals calcium and magnesium and inorganic carbonates while biochar is still mostly organic in nature (Lehmann and Joseph, 2009).

## 2.1 Characterization of Biochar

The physical make-up of biochar will depend on the biomass that was used as well as the process of biochar preparation (for example – pyrolysis). Generally, the biochar elemental composition will contain more or less the original composition of the material from which it was made (Laine *et. al.*, 1991; Wildman and Derbyshire, 1991) and this has a significant impact on its physical and structural characteristics. Certain properties such as biochar yield, pH, recalciterance and volatile matter are predominantly controlled by production temperature while properties such as biochar C content, CEC, fixed C, carbon sequestration capacity, mineral concentrations, and ash content are predominantly controlled by the feedstock (Zhao *et. al.*, 2013).

| **Biomass/Property** | **Bagasse*** | **Cocopeat**** | **Paddy** | **Wood Stem** | **Wood Bark** |
|---|---|---|---|---|---|
| **Moisture (wt. %)** | 1.3 | 2.55 | 2.07 | 1.46 | 0.36 |
| **VM (wt.%)[b]** | 9.17 | 14.30 | 6.46 | 12.79 | 18.14 |
| **Ash (wt. %)** | 8.57 | 15.90 | 52.37 | 2.28 | 12.84 |
| **FC (%)[a]** | 80.97 | 67.25 | 39.10 | 83.47 | 68.66 |
| **pH** | 9.3 | 10.3 | 10.5 | 9.5 | 9.6 |

**Table 1 : Some biochar properties produced at 500ºC using different feedstocks (Lee *et. al.*, 2013)**

[a]FC is Fixed Carbon (%, dry basis)
[b]VM is Volatile Matter (%, dry basis)

*Bagasse is the fibrous material remaining after sugarcane stalks are crushed to extract their juice.

**Cocopeat is made from coconut husks which is byproducts of industries using coconuts.

The above table clearly suggests that some biochar properties vary depending upon the feedstock used for the production of biochar. For instance, biochar produced from Bagasse has 80.95% FC on a dry basis while that from Paddy straw has only 39.10% of FC on a dry basis.

| Temperature/ Properties | FC (%)[a] | Yield(%) | VM(%)[b] | Ash(%) | pH | CEC[c] |
|---|---|---|---|---|---|---|
| **200 °C** | 22.5 | 99.3 | 70.2 | 7.21 | 5.43 | 32.1 |
| **350 °C** | 53.2 | 52.5 | 31.3 | 14.7 | 8.69 | 87.2 |
| **500 °C** | 63.7 | 29.8 | 17.6 | 18.0 | 10.2 | 95.5 |
| **650 °C** | 72.1 | 26.8 | 11.1 | 16.2 | 10.2 | 146 |

**Table 2: Some biochar properties produced from Wheat Straw as feedstock at different temperatures (Zhao et. al., 2013)**

[c]CEC is Cation Exchange Capacity (cmol $kg^{-1}$)

The above table suggests that some properties of biochar vary with the temerature at which biochar has been produced. For instance, biochar produced from wheat straw at 200 °C has an acidic pH of 5.43 while biochar produced from the same feedstock is alkaline in nature (pH = 10.2) when produced at temperatures in excess of 500 °C. Research suggests that most biochars are alkaline but can be produced at basically any pH between 4 to 12 (Lehmann, 2007). Carbon content of biochar is broken into three classes - High (> 80%), Medium (60-80%), and Low (< 60%). The carbon composition for biochar produced from wood at 500 degrees Celsius often exceeds 80% (Antal and Gronli, 2003) and some may achieve that percentage below 500 degrees. Biochars having medium carbon content may be derived from feedstocks such as maize stalks, cattle manure with sawdust. Low carbon containing biochars may be produced from feedstocks such as rice hulls and chicken litter (Lehmann and Joseph, 2009).

## 2.2 Why Biochar?

Research on biochar has seen a healthy lift in previous 10 years and this can be judged by the increasing number of publications on "biochar" during these years as illustrated in Fig. 3.

This is due to several factors :

a) It is one of the very few technologies that can actively remove carbon from the atmosphere (Whitman *et. al.*, 2011; Matovic, 2011; Roberts *et. al.*, 2010)

b) It is suitable for a range of environmental applications, in particular amending low quality soils (Cao *et. al.*, 2009; Chen *et. al.*, 2008, Lu *et. al.*,2012)

c) It offers the potential to improve the productivity of soils (while still offering atleast some of the other benefits) (Spokas *et. al.*, 2012; Hossain *et. al.*, 2010)

However the utility of a specific biochar for any particular application depends on its inherent properties. For example, biochar with high specific area may be used as sorbents, whereas those with high recalciterance may function as a carbon fixation mechanism (Zimmerman *et. al.*, 2010). Those rich in available nutrients and minerals and/or showing high water holding capacity could be used as soil amendments to improve fertility (Graber *et. al.*, 2010). Detailed description of biochar application and usefulness is discussed in next section.

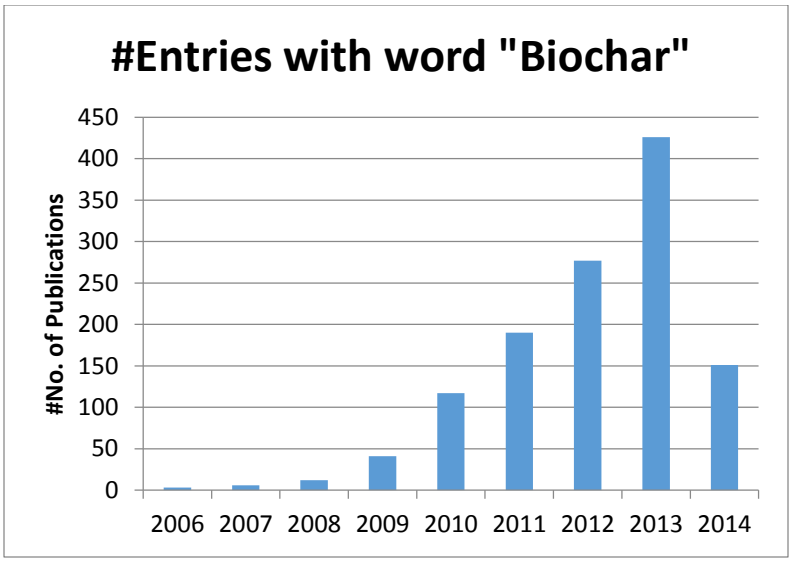


**Figure 3 : Citations by year from a web of science search of "Biochar"**

## 2.3 Major Uses of Biochar

### 2.3a Biochar in Soil Amendment

Biochar has been extensively used in soil amendment and integrated with fertilizers to improve soil productivity and make it more fertile. Many authors have validated biochar application to soil as a successful measure of substantially improving soil quality. However, it is worth noting that the activated carbon that biochar contains alone does not increase soil fertility as additional nutrients are also necessary (Tenanbaum, 2009). It is the combination of the biochar's immense pore space making its nutrient holding capacity high along with additional nutrients that result in increase of soil fertility. Integrating biochar with fertilizers or forms of compost has been shown to cause a substantial productivity increase (Wimmer, 2011). In a study, biochar used in conjunction with chemical fertilizer increased the growth of numerous vegetables, including winter wheat by 25-50% in comparison to just the fertilizer by itself (Guo, 2008). Additional research has shown that addition of biochar can increase nutrient holding capacities, specifically

for phosphorus (Woods *et. al.*, 2009) in low carbon, loamy sand soils of the southeastern United States.

Multiple variables contribute to the increase in fertility and thus, crop yield. Variables known to be changed or enhanced by the addition of biochar include: soil pH, cation exchange capacity (CEC), nutrient holding capacity (NHC), bulk density, carbon percentage, microbial environment, and water holding capacity (WHC). The alterations have important effects on the indirect nutrient value of the biochar such as the nutrient holding capacity, which is dependent on its cation exchange capacity (CEC) and anion exchange capacity (AEC) (Liang *et. al.*, 2006; Cheng *et. al.*, 2008). The CEC of biochars has proven to be dependent on the pyrolysis temperature at which it was produced, with higher pyrolysis temperatures making biochar with substantially higher CECs than the very low CECs of low pyrolysis temperature biochar (Lehmann, 2007) as also indicated in Table 2 (Zhao *et. al.*, 2013). Due to their porosity and layered turbostratic structure, most biochars have a much lower density (Oberlin, 2002) around 1.5-1.7 g/cm$^3$ (Janakowski *et. al.*, 1991; Oberlin, 2002). These densities, being lower than that of mineral soils, can change the hydrology of the soil, and the higher porosity allows biochar-enriched soils to hold more moisture (Watts *et. al.*, 2005). The fineness or "dustiness" of biochar also has an impact on production as a finer biochar will result in more surface area and would presumably be more effective at adsorbing and retaining nutrients. Contrary to that common train of thought, biochar with too high a surface area may be less productive as it may reduce the efficacy of soil applied pesticides that are needed for weed and pest control due to its high adsorption capacities(Wimmer, 2011).

A recent review of biochar articles by Spokas *et. al*. (2012) concluded that while application of biochars can lead to positive results in agricultural production, there have been some reports of

no crop yield benefits (Schnell et al., 2012) or even negative yield responses (Lentz and Ippolito,2012). Reported low yields could be because of reduced nutrient release for plant uptake, application of biochar on fertile soils, or a low rate of biochar application. In another study, the yield for biochar-amended soil was found to be reduced by 27,11,36,74 and 2% for rice, wheat, maize, lettuce and tomato respectively, relative to controls (Mukherjee & Lal, 2014). Therefore, even though biochar science is advancing, there may be several areas of uncertainty associated with its large-scale implementation. A systematic road-map for manufacturing classification of different biochars along with cost-benefit analysis, must be developed before implementation of large-scale field application (Mukherjee & Lal, 2014).

## 2.3b Biochar for Carbon Sequestration and reducing overall emissions

The long residence time of biochar in soil makes it an important C sequestration tool (Lehmann et al. 2006). During the conversion of biomass to biochar, about 50% of the original C is retained in the biochar, which offers considerable opportunity for creating a C sink (Lehmann 2007).

Plants convert atmospheric carbon dioxide to organic carbon via photosynthesis. Since plants (biomass) decompose at a quicker rate than they are formed, the carbon dioxide is released back to the atmosphere resulting in no decrease of atmospheric carbon. Application of biochar to soil is popularly known as a means of carbon sequestration (Lehmann *et. al.*, 2006). Converting biomass to biochar slows down the decay process as biochar decomposes very slowly as compared to biomass and consequently the release of carbon back to atmosphere is slowed down (Lehmann, 2007).

Stability of biochar is substantially greater than other OM under the same environmental conditions. It has been predicted that the stable portion of biochar has a mean residence time of

greater than 1000 years (Cheng et al. 2008; Liang et al. 2008). Therefore, the transformation of labile plant organic matter into biochar through pyrolysis not only reduces $CO_2$ emissions from energy production, but biochar additions to the soil constitutes a net withdrawal of carbon dioxide from the atmosphere.

## 2.4 Algal Biochar

Biochar that comes as a result of processing of algal biomass is referred to as 'algal biochar'. Not much research has been carried out on algal biochar to date which is evident from very few publications on the topic as shown in Figure 4. This creates an entirely new research area to look into and presents an opportunity to study and experiment more on this material.

Chaiwong *et. al.* (2012) demonstrated an algal biochar production process by thermal pyrolysis for three types of freshwater algae, namely *Spirulina*, *Spirogyra and Cladophora*. The quality of the biochar obtained was comparable to that of certain established solid fuels such as peat (S-H3), German Braunkohle Lignite, charcoal, and oak char as well as biochar obtained from other forms of biomass. However, the temperature used for production was around 300-330$^{o}$C, lower than those for other forms of biomass. In another continuing study, it was found that the primary components of the bio-char from *Spirulina Sp.* are similar to those of the bio-char from other types of algae. However, *Spirulina Sp.*have a higher carbon content (Chaiwong *et. al.*, 2013). This implies that, even within same kind of biomass, biochar from different species may have different properties.

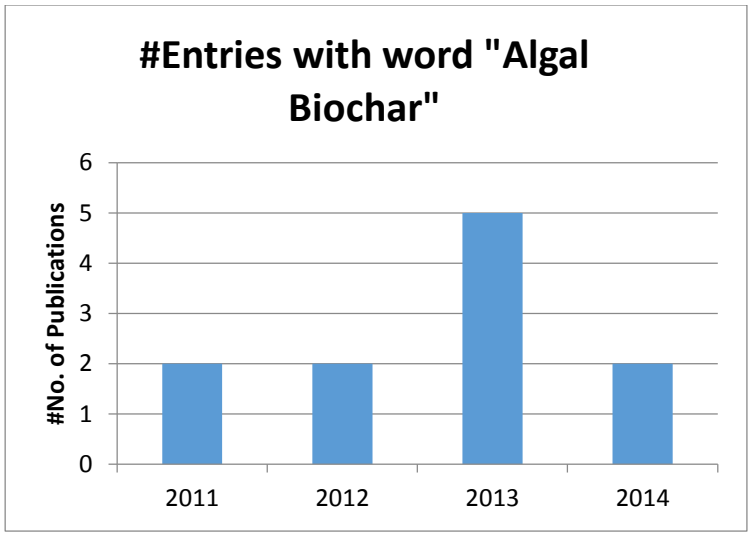


**Figure 4 : Citations by year from a web of science search of "Algal Biochar"**

Bird *et. al.* (2011) produced algal biochar by a pyrolysis process at 450ºC from several species of macroalgae and characterized it for certain elements (C, H, N, Extractable Ca, Na, Mg, K, Total and Extractable P). On characterization, algal biochar in this study were found to be similar to biochars from poultry litter (Chan and Xu, 2009), and dissimilar to biochars from ligno-cellulosic feedstocks. All of the algal biochars in this study were found to be low in carbon content, surface area and cation exchange capacity but high in pH, nitrogen and extractable inorganic nutrients including P, K, Ca and Mg. The total P content of algal biochars in this study was high and ranged from 1712 to 5470 mg $kg^{-1}$. Extractable P was determined using Colwell bicarbonate extraction and was found to be uniformly high in all biochars, ranging from 914 to 2418 mg $kg^{-1}$ and representing between 37% and 83% of the total P in the samples. Tagoe *et. al.* (2008) produced biochar at 500ºC from poultry litter with 12.3% C, 2.6% N, pH of 9.93, and a P content of 18,170 mg $kg^{-1}$. In contrast, ligno-cellulosic biochars tend to have much higher carbon contents, and cation exchange capacities compared to algal biochars (Chan and Xu, 2009; DeLuca *et. al.*, 2009; Ozcimen and Ersoy-Mer-icboyu, 2010).

This study demonstrated that biochar derived from macroalgae has properties likely to make it suitable for use both as a soil amendment and as a tool for long-term carbon sequestration. High extractable P content means that biochar derived from algae can be a potential source of P for plants and be a nutrient resource for soils. Algal biochar, derived from the remediation of wastewater from aquaculture, agriculture, eutrophied natural waterways, or saline wastewater resources could provide a significant revenue stream in the future through energy co-generation, carbon credits from providing long-term soil carbon sequestration, and sale as a soil amendment and fertilizer (Bird *et. al.*, 2012).

## 3 – An Overview of Biochar production and Characterization at KU

Roberts *et. al.* (2013), working at The University of Kansas, performed hydrothermal liquefaction on wastewater derived microalgae for the first time ever. Algal biochar used in this study was produced using microalgae derived from pilot-scale algal cultivation ponds fed with municipal wastewater as the nutrient source. Four 2500 gallon open pond reactors were operated as continuous flow stirred tank reactors at Lawrence Wastewater Treatment Plant (WWTP) in Lawrence, KS from April to October, 2011. The concentrated algae samples (1-1.5% solids) were collected from each sedimentation tank daily and were immediately processed.

The hydrothermal liquefaction reaction was conducted for 1 h at 350ºC with pressures up to 2000 psig and constant stirring at 150 rpm. The municipal wastewater matrix and resultant mixed-culture biomass significantly influenced liquefaction product distribution, yielding a higher proportion of biochar. The product recovery method implemented is outlined in Figure 5 (a). A fraction of biomass was retained in the form of solid biochar because of the high amounts of non-combustible intercellular material. The biochar was 35 wt% organic, containing 20.4% dw

carbon. The measured High Heating Value (HHV) of the biochar was 8-10 MJ $kg^{-1}$, depending upon which fraction was evaluated. They proposed a potential pathway to address some of the main concerns for economic and environmental sustainability of algal biofuels, illustrated in Figure 6. Data collected in the study showed a theoretical production rate of 6-9 barrels $day^{-1}$ biocrude and > 1500 kg $day^{-1}$ biochar from available water and nutrients provided by the Lawrence, KS, municipal wastewater treatment plant. The co-product biochar could greatly enhance sustainability and the value chain for algal biofuels, adding markets in carbon sequestration, soil amendments, adsorbents and fertilizers.

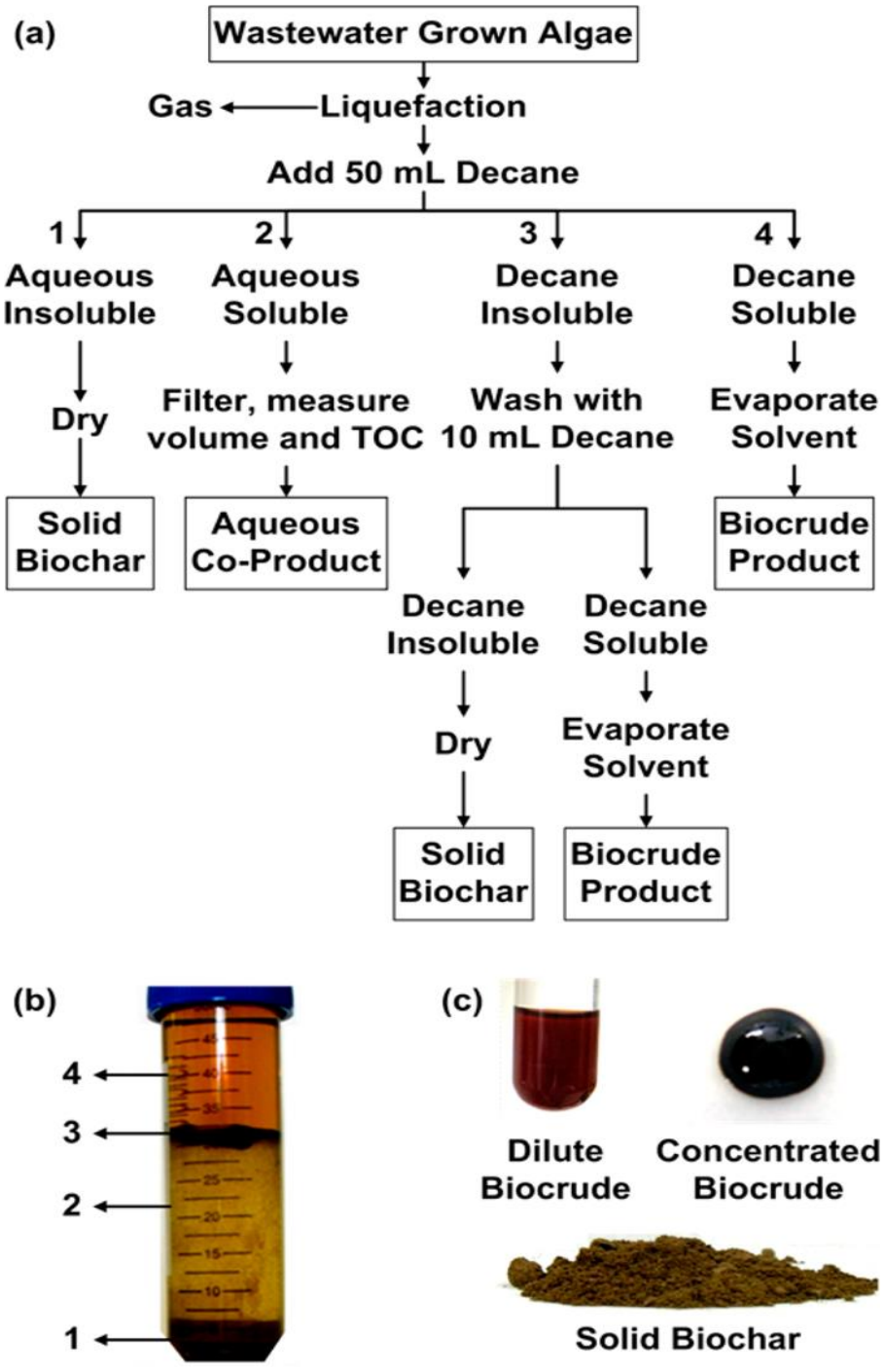


**Figure 5 : (a) Flow Diagram of the solvent extraction and product recovery method used, (b) four layers after centrifugation of HTL products, and (c) photograph of (diluted and concentrated) biocrude and biochar (Roberts et. al., 2013).**

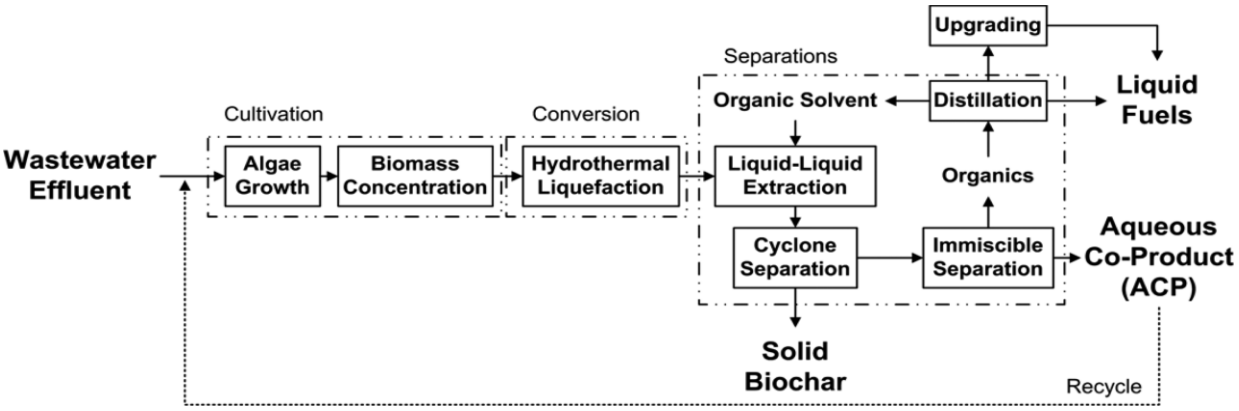


**Figure 6 : Potential block flow diagram of the continuous algal biofuel pathway (Roberts et. al., 2013).**

## 3a. SCOPE OF THIS STUDY

This study characterizes the extractable phosphorus present in algal biochar produced by hydrothermal liquefaction of wastewater derived algae at 350ºC. As properties of biochar are influenced by the feedstock from which it has been derived, process used to make it, and temperature used in the process, this study will investigate the properties of the biochar made out of hydrothermal liquefaction of wastewater derived algae which has been performed only for the first time ever by Roberts *et. al.* (2013). Phosphorus measurements in the form of bioavailable phosphorus, organic phosphates and total phosphorus will provide an idea of suitability of this biochar to soil and crop applications and will ascertain the potential usefulness of this biochar as a source of phosphorus in many feasible settings.

## 4 – Background : Why Phosphorus?

Pure "elemental" phosphorus (P) is rare. In 1934, Alfred C. Redfield, an American oceanographer found that the elemental composition of marine organic matter (dead & living) was remarkably constant globally. The ratios of carbon to nitrogen to phosphorus remained the same from coastal to open ocean regions. When nutrients are not limiting, the molar element ratio C:N:P is 106:16:1 in most phytoplanktons. This ratio is referred to as Redfield Ratio and indicates that Phosphorus is present in really low concentrations in aquatic organisms.

Phosphorus (P) is an essential nutrient for all life forms. In nature, phosphorus usually exists as part of a phosphate molecule ($PO_4^{3-}$). Phosphorus in aquatic systems occurs as organic phosphate and inorganic phosphate. Organic phosphate consists of a phosphate molecule associated with a carbon-based molecule, as in plant or animal tissue. Phosphate that is not associated with organic material is inorganic. Inorganic phosphorus is the form required by plants. Animals can use either organic or inorganic phosphate (EPA, 2014).

Phosphorus typically functions as the "growth-limiting" factor because it is usually present in very low concentrations relative to other nutrients. This means, if all phosphorus is used, plant growth will cease, no matter how much nitrogen is available. The natural scarcity of phosphorus can be explained by its attraction to organic matter and soil particles. Any unattached or "free" phosphorus is quickly removed from the aquatic system by algae and larger aquatic plants. Excessive concentrations of phosphorus can quickly cause extensive growth of aquatic plants and algal blooms. This process is known as eutrophication. Several detrimental consequences may result due to eutrophication. For example - death of aquatic life due to lack of dissolved oxygen in the water body which has been used up by the excessive growth of aquatic plants.

Plants (aquatic or terrestrial) need phosphorus to grow as much as they need water. Many soils

do not have enough to meet the voracious demands for phosphorus of the high-yielding crop varieties (Pearce, 2011).Without phosphorus, plant growth is retarded, resulting in stunted roots, and spindly plants. Deficiency symptoms also include dull greyish-green leaves and red pigment in leaf bases and dying leaves. Phosphorus deficiency is difficult to diagnose, and by the time it is recognized it may be too late to do anything. If plants are starved of phosphorus as seedlings, they may not recover when phosphorus is applied later (Kelly, 2002).

Phosphorus is easily fixed in the soil, i.e. it is present in soils in forms that are more likely to remain bound to soil particles than to get dissolved in water and be available for plants. Thus, plants can take up only a small amount of the phosphorus that is applied. For this reason it is important that phosphorus is applied every year along with regular soil testing to monitor phosphorus levels (Kelly, 2002). Not many people would call phosphate a critical issue or one with serious environmental consequences. But it is an absolutely vital resource for feeding the world. It is also a resource that could start running low within a couple of decades — and one we grossly misuse, pouring it across the planet and recycling virtually none of it. (Pearce, 2011). P is becoming a worldwide limited resource as some estimates indicate that the world's reserves will only last for about 50 to125 years (Gilbert 2009).

Municipal wastewaters contain high amount of nutrients such as nitrogen & phosphorus. Phosphorus levels in wastewater may range between 5-20 mg/L, which is quite high. Integration of phosphorus removal technologies in wastewater treatment processes has gained momentum to prevent discharge of too much phosphorus in water bodies via effluent discharge, preventing eutrophication and other bad impacts of excessive phosphorus in the aquatic sphere. Since the algae which is used as the feedstock to prepare the biochar under investigation by hydrothermal liquefaction was cultivated using municipal wastewater as feedstock, it is expected that the

phosphorus in wastewater must have been taken up by algae to grow. This phosphorus in algae should also be carried forward and concentrated in algal biochar when algae undergoes processing. Thus, it was expected to see concentration of phosphorus in this algal biochar above wastewater levels. Experiments in this study will determine whether & how much phosphorus is present in our algal biochar and how available it will be to plants and organisms.

## 4.1 Phosphorus Speciation and Analysis

In soils P may exist in many different forms. In practical terms, however, P in soils can be thought of existing in 3 "pools": (Busman *et. al*. 2012)

- Solution P
- Active P
- Fixed P

The solution P pool is very small and will usually contain only a fraction of a pound of P per acre. Plants will only take up P in the orthophosphate form. The solution P pool is important because it is the pool from which plants take up P and is the only pool that has any measurable mobility. A growing crop would quickly deplete the P in the soluble P pool if the pool was not being continuously replenished.

The active P pool is P in the solid phase that is relatively easily released to the soil solution, the water surrounding soil particles. Because the solution P pool is very small, the active P pool is the main source of available P for crops. The ability of the active P pool to replenish the soil solution P pool in a soil is what makes a soil fertile with respect to phosphate. The fixed P pool contains phosphate that may remain in soils for years without being made available to plants and may have very little impact on the fertility of a soil (Busman *et. al.*, 2002).

The ability of soil to provide P to biota depends on what forms of P are present and their relative amounts. The most commonly used differentiation of soil P is between inorganic and organic forms. At the molecular level, nearly all inorganic P in soil is orthophosphate, and its chemistry is determined by the strength of ionic bonds to surrounding atoms. Transformation of inorganic forms of P are controlled by the processes of precipitation, dissolution and sorption (Bunemann & Oherson). Organic forms of soil P are distinguished from inorganic forms by the presence of at least one covalent bond to a carbon atom generally via an ester linkage (i.e. through an oxygen atom). Most transformations of organic P, and in particular their conversion to inorganic P, require the breaking of this covalent bond. Precipitation, dissolution and sorption also affect other forms of P (Berg and Joern, 2006). The other reason that the differences between inorganic and organic forms is so fundamental to P speciation is that this distinction has been easy to analyze with long-established techniques. Inorganic P is traditionally detected spectrophotometrically as a blue-colored phosphomolybednum complex formed when free phosphate reacts with an acidified molybdate reagent. Organic P does not form a colored complex with this reagent, so can be determined as the difference between Total P and Inorganic P. However, there are drawbacks to this method (Turner *et. al.*, 2003, 2006). Organic P is overestimated when inorganic polyphosphates are present because they also do not react with the molybdate reagent, and therefore are included in the organic P fraction. Total P determination is usually done using sodium carbonate fusion or acid digestion. Both of these methods are explained in detail in section 5.1.

The bioavailability and fate and behavior of an element in the soil is primarily a function of speciation, both in the solid and solution phases. P speciation can be influenced by soil parent material (McDowell *et. al.*, 2005; McDowell & Stewart, 2006). Soil P concentrations range from

35 to 5300 mg $kg^{-1}$, with a median of 800 mg $kg^{-1}$ (Bowen,1979; Sparks, 2003). The majority (>99%) occurs in inorganic and organic solid phases and in microbial biomass (Hesterberg, 2010).Although inorganic orthophosphate ($PO_4$) combines with over 30 elements into approximately 350 minerals (Nriagu, 1984), $PO_4$ in soils is thought to be largely bound with Ca, Fe, and Al (Hesterberg, 2010). Soil organic P includes that bonded into organic moieties of soil organic matter (OM) and in biologically derived molecules like DNA, phytic acid, and phospholipids (Frausto da Silva and Williams, 2001).

There are several spectroscopic techniques that may be used to do P Speciation, including X-Ray Absorption Near-Edge Surface Spectroscopy, Nuclear Magnetic Resonance Spectroscopy, Fourier-transform Infrared Spectroscopy, Raman Spectroscopy (Kizewski *et. al.,* 2011). Because of the complexity of environmental matrices, no single spectroscopic technique can comprehensively characterize the variety of coexisting inorganic and organic P species in these systems. System complexity limits the level of specificity that can be achieved in P speciation analysis and increases uncertainty in the analysis.

Solution NMR spectroscopy has detected orthophosphates ($PO_4^{3-}$) in essentially all soil and sediment samples, and pyrophosphates ($P_2O_7^-$) are the second most frequently detected general type of inorganic phosphate species (Kizewski *et. al.,* 2011). Pyrophosphates are water soluble and their condensed nature results in higher P analyses. In spite of their water solubility, pyrophosphates are not considered to be as efficient as orthophosphates in terms of plant nutrition (Ahmad & Kelso, 2001). It is well known that pyrophosphate is a relatively ineffective source of P for plants prior to hydrolysis to the orthophosphate form (Sutton and Larsen, 1964). Generally the effectiveness of pyrophosphates as a source of phosphorus for plants depends on its reactions with the soil constituents and the distribution of phosphorus between ortho- and

pyro-phosphates at different times during the growing season (Hughes and Hashimoto,1971). Phosphate diesters are present in small proportions (<3%) in a few soils. Polyphosphate, a more condensed form of inorganic P, is found in most manured soils (<2%) but absent in untreated soils (Dou *et. al.*, 2009).

Although organic forms of Phosphorus (P) may play an important role in non-biological availability of soil P, the concentrations, forms, and dynamics of organic P in soil are poorly understood. (Hansen *et. al.,* 2004). Normally, there is more organic phosphorus in the soil solution than there is inorganic phosphorus. In light textured soils (sands) as much as 90 percent of the total phosphorus in solution may be organic. This has considerable implications for the role of organic phosphates in the movement of phosphates in soils and to plant nutrition. The major forms of organic P in soil are meso-inositol hexaphosphate, phospholipids, and nucleic acid P (Tisdale *et. al.,* 1985).The relatively simple organic phosphates in soil extracts are probably in high molecular weight complexes in the soil. In addition, inositol phosphates are strongly adsorbed on charged surfaces and decrease phosphorus availability to plants by forming sparingly soluble Fe and Al complexes. However, there is evidence that plant roots and rhizosphere organisms which are found around plant roots excrete phosphatase enzymes that are capable of hydrolyzing some organic phosphate compounds. These hydrolyzed organic phosphate compounds are released as inorganic phosphates for absorption by the plants (Cornforth, NZIC).

# 5– Development of Phosphorus Extraction Method for Biochar

## 5.1 Existing Extraction Tests

Chemical extraction techniques are used to separate a particular constituent from a matrix by selectively causing the reagents to react with the targeted element or compound and extracting it. Phosphorus is present in different amounts and forms in different soils depending upon the type of soil. To estimate the amount and availability of phosphorus in a sample of soil, several soil phosphorus extraction methods have been researched and published. However, no extraction method is effective for evaluating phosphorus in all kinds of soils. Each extraction method gives the best result for a specific nature of soil. This is because of the different reagents used in the various extraction methods react with specific forms of phosphorus present in the soil sample to facilitate extraction. Use of any chemical extractant over a different soil than what it was designed for can result to the buffering of the extractant and dissolution of non-labile P, or under-extraction of labile fractions (Myers *et. al.*, 2005).

When extracting solution is added to soil, there are four basic reactions by which P is removed from the solid phase: 1) dissolving action of acids, 2) anion replacement to enhance desorption, 3) complexing of cations binding P, and 4) hydrolysis of cations binding P. (Elrashidi, 2001) Therefore, the selection of a P soil test depends on the chemical forms of P in the soil. Many authors (Bray and Kurtz, 1945; Olsen, 1954; Mehlich, 1984) have designed P-testing methods using chemical extractants to determine soil available P. These conventional P extractants may not give a clue on the level of available P for plant absorption as the chemicals used for the extraction may solubilize non-labile P. (Abdu, 2006). Some prevalent methods that were considered to be used for phosphorus extraction from algal biochar are explained in this section.

## 5.1a Water Extraction

Water probably was the first extractant researchers used to measure P in soils. A water extract removes dissolved forms of P but very little of the absorbed and mineral forms. It is suitable for both acid and calcareous soils. The amount of P extracted is small for most soils, and may not reflect all forms of labile P. (Elrashidi, 2001)

## 5.1b Bray and Kurtz Test

The Bray and Kurtz P-1 soil test phosphorus (P) method was developed by Roger H. Bray and Touby Kurtz of the Illinois Agricultural Experiment Station in 1945 (Bray and Kurtz, 1945). Phosphorus extracted by the Bray and Kurtz P-1 method has been shown to be well-correlated with crop yield response on most acid and neutral soils in Midwestern and North Central United States (Frank *et. al.*,1998). The reagent used in extraction in this test (Bray's Reagent) is 0.025 M HCl + 0.03 M $NH_4F$. (Bray and Kurtz, 1945). For acid soils, the fluoride in the Bray and Kurtz extractant enhances P release from aluminum phosphates by decreasing Al activity in solution through the formation of various Al-F complexes. (Sims, 2000). The Bray soil test is not suitable for silt clay loam or finer-textured soils that are calcareous or have a high pH value (pH > 6.8), soils with a calcium carbonate equivalent > 7% of the base saturation, or soils with large amounts of lime (> 2% $CaCO_3$) (Sims, 2000).

## 5.1c Mehlich – I

The Mehlich I soil test for Phosphorus (P) was developed in the early 1950s by Mehlich and his co-workers (Mehlich, 1953). The Mehlich 1 extracting solution is (0.125 M $H_2SO_4$ + 0.05 M HCl) and is also referred to as a dilute double acid or the North Carolina extractant. The Mehlich 1 extracts P from aluminum, iron, and calcium phosphates and is best suited to acid soils (pH <

6.5) with low cation exchange capacities (< 10 cmol/kg) and organic matter contents (< 5%) (Sims, 2000). The Mehlich 1 soil test is unreliable for calcareous or alkaline soils because it extracts large amounts of non-labile P in soils with pH > 6.5 (Kuo, 1996). In soils such as these the acidity of Mehlich 1 solution is neutralized, reducing the capability of the dilute acid to extract P. (Sims, 2000)

### 5.1d Mehlich – III

The Mehlich 3 soil test was developed by Mehlich in 1984 as an improved multi-element extractant for P, K, Ca, Mg, Cu, Fe, Mn, and Zn (Mehlich, 1984). The Mehlich 3 Extracting Solution is (0.2 M $CH_3COOH$, 0.25 M $NH_4NO_3$, 0.015 M $NH_4F$, 0.013 M $HNO_3$, 0.001 M EDTA). The Mehlich 3 is similar in principle to the Bray and Kurtz P-1 test because it is an acidic solution that contains ammonium fluoride. Acetic acid in the extractant also contributes to the release of available P in most soils (Sims, 2000). It is more effective than the Mehlich 1 soil test at predicting crop response to P on neutral and alkaline soils because the acidity of the extractant is neutralized less by soil carbonates (Tran and Simard, 1993). Several studies showed that the Mehlich 3 soil test is highly correlated with P extracted from soils by the Bray and Kurtz P-1, Mehlich 1, and Olsen P methods (Sims, 1989; Tran *et. al.*, 1990; Wolf and Baker, 1985). However, Mehlich 3 may not be a reliable test for calcareous soils as it is found to correlate well with Olsen P results for some calcareous soils but not so well for others (Carrow*et. al.*, 2004)

### 5.1e Olsen

The “Olsen P” or sodium bicarbonate soil test phosphorus (P) method was developed by Sterling R. Olsen and co-workers in 1954 (Olsen *et. al.*, 1954). The Olsen P extracting solution is 0.5 M

$NaHCO_3$ at pH 8.5. The Olsen P method is best suited for calcareous soils, particularly those with > 2% calcium carbonate, but has been shown in some research to be reasonably effective for acidic soils (Fixen and Grove, 1990). The method is based on the use of carbonate and hydroxide ions to decrease the solution concentrations of soluble $Ca^{2+}$ (by precipitation as $CaCO_{3)}$ and soluble $Al^{3+}$and $Fe^{+3}$ (by formation of Al and Fe oxyhydroxides), thus increasing P solubility. (Sims, 2000). Contact time between the reagent and sample matters is an important variable in this method, as the reaction occurs under non-equilibrium conditions.

### 5.1f Total Phosphorus

Two of the most commonly used methods of total phosphorus determination are Sodium carbonate ($Na_2CO_3$) fusion and acid digestion. The Fusion method involves fusing 0.5-1 g of soil with sodium carbonate, extracting the melt with 4.5 M sulphuric acid and digesting the melt for one hour on a boiling water bath. The acid digestion method involves digesting 0.5-1 g of soil with a concentrated strong acid or mixture of strong acids. Of these methods, $Na_2CO_3$ fusion is thought to give more reliable results (Syers*et. al.*, 1967; Syers*et. al.*, 1968; Sherrell and Saunders, 1966; Sommers and Nelson, 1972). Underestimation of total P by acid digestion is thought to be due to inability of these methods to extract P from apatite inclusions (Syers*et. al.*, 1967). The ability of an acid digestion to extract P from apatite inclusions depends upon the acid or combination of acids used. Syers*et. al.* (1967) showed that the effectiveness of extraction generally followed the order: Fusion > HF digestion > $HClO_4$ digestion > $H_2SO_4$ > ignition. In the TP determination, the $PO_4$ reacts with ammonium molybdate in the presence of $H_2SO_4$ to form a phosphomolybdenum complex. Potassium antimonyl tartrate and ascorbic acid are used to reduce the complex, forming a blue color which is proportional to the TP concentration. (ESS,

1992). Bird *et. al.* (2011) determined total P from biochar using Total P Kjeldahl digestion (Taylor, 2008)

## 5.2 Physical Properties of Algal Biochar used in this experiment

The algal biochar used to determine total phosphorus by microwave acid digestion was derived from macroalgae harvested from surface waters in Kansas. For Olsen and water extraction experiments, algal biochar derived from microalgae harvested from Lawrence wastewater treatment plant was used. Both the biochars had similar physical characteristics such as similar color, texture and high hydrophobicity.

### 5.2a Color

The algal biochar used in this research was a black colored, powder-like material.

### 5.2b Texture

It was very light and had a smooth texture much like very fine soils.

### 5.2c Hydrophobicity

This biochar was very hydrophobic in nature and did not mix well with water. This property made it difficult to perform water extraction and other P extraction methods with this material because all extraction reagents contain a large percentage of water. Several authors have reported that biochar is hydrophobic in nature. The hydrophobicity of biochar has been found to be influenced by the initial material (biomass type and particle size) and pyrolysis temperature (Aston *et. al.*, 2014). Biochar hydrophobicity can be explained by the presence of alkyl groups on the surface of the particles (Kinney *et. al.*, 2012). For each biomass type, hydrophobicity was reduced with increasing pyrolysis temperature. This can be attributed both to the destruction of

alkyl functionalities and shrinkage of particles during pyrolysis, smoothing their surfaces. (Aston *et. al.*, 2014)

The algal biochar in our study was heated at 105$^{o}$C overnight to get rid of the organic fractions that might be the cause of its hydrophobicity. The heated biochar was cooled and mixed with water to test its hydrophobicity. This biochar was still found to be hydrophobic with not much improvement in its ability to mix with water.

## 5.3 Materials & Methods

Three methods were used for determination of phosphorus in algal biochar samples – Total Microwave digestion method, Olsen phosphorus method, Water extraction method.

### 5.3a Initial Approach

Initially, Mehlich 3 extraction method was tested on our algal biochar as it is a well known method for estimation of phosphorus. It works well on most acidic, neutral and alkaline soils. To start the extraction procedure, char sample and Mehlich reagent were combined in a 1:20 ratio (w/v). However, even after being allowed to settle for a while, almost all of the char material remained over the surface of the Mehlich extractant which indicated there would be no proper contact between the material and solution on shaking. Thus, there were 2 reasons that led to rejection of Mehlich 3 extraction method for our algal biochar – i) Insolubility of biochar with Mehlich 3 extractant, & ii) inability of Mehlich 3 extractant to reliably estimate P in calcareous soils (Carrow *et. al.*, 2004). Since it was later estimated that our biochar contained substantial calcium carbonate, the Olsen Method was substituted for P extraction as it is the best and reliable extraction method for Phosphorus from calcareous soils.

For Total Phosphorus estimation, Webb & Adeloju (2013) used four different combination of acids for digestion of Australian soils – i) Nitric acid, ii) Sulphuric acid, iii) Nitric & sulphuric Acid, iv) Nitric Acid & Potassium persulphate ($K_2S_2O_8$) and found that the phosphate concentrations revealed by these digestions followed the order ii) > iii) > iv) >i). Thus, we leveraged the best method ((ii) sulphuric acid digestion) and performed it on our algal biochar. However, after exactly heating for the same time at the same temperature, no major progress with digestion was observed. Thus, this method was discarded and microwave acid digestion was performed with different combination of acids. Microwave acid digestion of our algal biochar with reverse aqua-regia yielded the best digestion.

### 5.3b Total Microwave Digestion Method

0.3 grams of material (char or algae) was carefully weighed into liners such that the maximum amount of material was at the bottom of the liner. 8 ml of reverse aqua regia mix (6 ml Nitric acid + 2 ml Hydrochloric acid) was added to the liners (following the biochar addition). Liners were sealed tightly with liner caps and were placed into the rotary device to be placed inside the microwave. The rotary device with all the sample containing liners was placed inside the microwave. Microwave settings were set according to the method mentioned in the manual for sample material “algae”. The entire digestion cycle was 70 minutes long. Power was ramped up from 0 W to 240 W for 30 minutes, held at 240 W for 20 minutes and cooled down at 0 W for 20 minutes. Pressure was set to 0.3 bar/s. After completion of this 70 minute microwave cycle, the rotating container was taken out and the lids of the liners half rotated inside a fume hood to let the gases escape the liner. The escape of gases can be heard in form of a hiss sound. The liner was opened and contents were filtered through a 0.45 micron syringe PTFE filter after cooling.

The filtrate was diluted to 100 ml in a volumetric flask with deionized water and this sample was tested for phosphorus by ICP.

## 5.3c Olsen Phosphorus Method

All the glassware was acid-washed prior to use. 0.5 g of material (char or algae) was weighed in a small plastic bottle. It was made sure that there was minimum material sticking to inside walls of the bottle and all of the material was at the bottom (to ensure full contact with reagent). 10 ml of 0.5 M $NaHCO_3$ solution was then added (1:20::Char:Reagent). Different samples were shaken


**Figure 7: Microwave used for Total Phosphorus Microwave Digestion**

for different length of time (30 minutes, 60 minutes and 120 minutes).Shaken samples were filtered using vacuum filtration and 2.5 µm Whatman filter paper. Filtrates were tested by ICP.

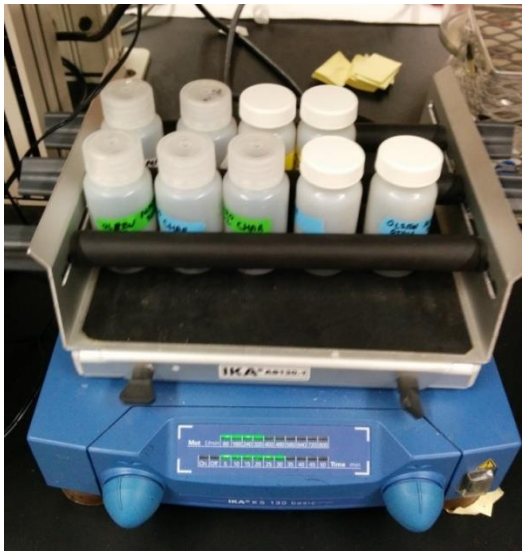

**Figure 8 : Samples shaken in a reciprocating shaker for Olsen Phosphorus Method**

## 5.3d Water Extraction Method –

0.5 g of material (char or algae) was weighed in a centrifuge tube. 10 ml of water was added to the tube (to maintain a 1:20 ratio of Char:Water).This was shaken for 1 hour in a reciprocating shaker and then centrifuged for 15 minutes at 6000 rpm. Centrifuged samples were filtered using vacuum filtration and 2.5 µm Whatman filter paper. Filtrates were tested in ICP.

## 5.3e ICP v Calorimetry

Colorimetric quantification of the phosphorus based on the phosphoromolybdenum blue complex has always been the most widespread analytical method. Recently, many soil test laboratories are moving from traditional colorimetric methods to inductively coupled plasma optical emission spectrometry (ICP OES) to quantify phosphorus in soil test extracts, due to the advantage of allowing the determination of many elements in one analytical process (Ivanov *et. al.*, 2010). Also, It has been established that ICP-OES consistently yields significantly higher P concentrations than the colorimetric method for extraction tests, with the relative differences

being greatest for lower P concentrations (Ivanov *et. al.*, 2012). However, calorimetric methods have one advantage over ICP. Calorimetric methods have a lower detection limit (~10 ppb) as compared to ICP (~100 ppb) which means a very low amount of an element that might not be detected in ICP, may be detected when estimated using calorimetric methods.

Considering these factors, Phosphorus determination from all the extraction samples was performed by ICP-Analysis.

# 6 – Results and discussion

Phosphorus content in algae and char samples was determined by running samples through ICP. Many other elements were also analyzed while the samples were being run through ICP. These elements were Ca, K, Mg, Fe, Mn, Zn, Sr, Na, Ba, S. ICP was calibrated using phosphorus standards of concentration 1 ppm, 2 ppm, 5 ppm and 10 ppm.

## 6.1 - Microwave acid digestion

2 samples of algae and 6 samples of algal biochar were subjected to microwave acid digestion. Table-1(a) and 1(b) lists the percentage weight of the elements in the acid digested samples. This percentage represents the mean total amount of the element present in the sample.

| **Elements** | **Ca (%)** | **P (%)** | **Mg (%)** | **K (%)** | **Fe (%)** | **S (%)** |
|---|---|---|---|---|---|---|
| **Algae** | 5.70±0.18 | 2.31±0.03 | 0.42±0.002 | 0.37±0.01 | 0.13±0.001 | 0.82±0.03 |
| **Char** | 17.10±1.31 | 7.40±0.58 | 1.41±0.07 | 0.29±0.084 | 0.37±0.02 | 0.54±0.14 |

**Table 1(a): Percentage of elements present in algae and biochar samples**

| | Mn (%) | Zn (%) | Sr (%) | Na (%) | Ba (%) |
|---|---|---|---|---|---|
| **Algae** | 0.014±0.0003 | 0.0154±0.0006 | 0.022±0.0002 | 0.06±0.005 | 0.011±0.0004 |
| **Char** | 0.05±0.0034 | 0.03±0.0074 | 0.06±0.004 | 0.09±0.013 | 0.07±0.003 |

**Table 1(b): Percentage of elements present in algae and biochar samples in trace amounts**

From the above data, it is observed that calcium and phosphorus are present in significant amounts in both algae and biochar samples while all other elements are present in lesser amounts. Also, for every element with the exceptions of potassium and sulphur, elements have been found to be more concentrated in biochar as opposed to algae samples. For example, the weight percentage of calcium increased from 5.70% in algae to 17.10% in biochar. Similarly, the percentage of phosphorus increased from 2.31% in algae to 7.40% in biochar. However, the ratio of calcium to phosphorus was found to be approximately equal in both algae and char, shown in Table-2.

| | Total Ca (mg/g) | Total P (mg/g) | Ca : P |
|---|---|---|---|
| **Algae** | 57±1.80 | 23.1±0.31 | 2.46 |
| **Char** | 171±13.2 | 74±5.8 | 2.31 |

**Table 2 : Mean amount of Ca and P in mg/g and their ratio in algae and char**

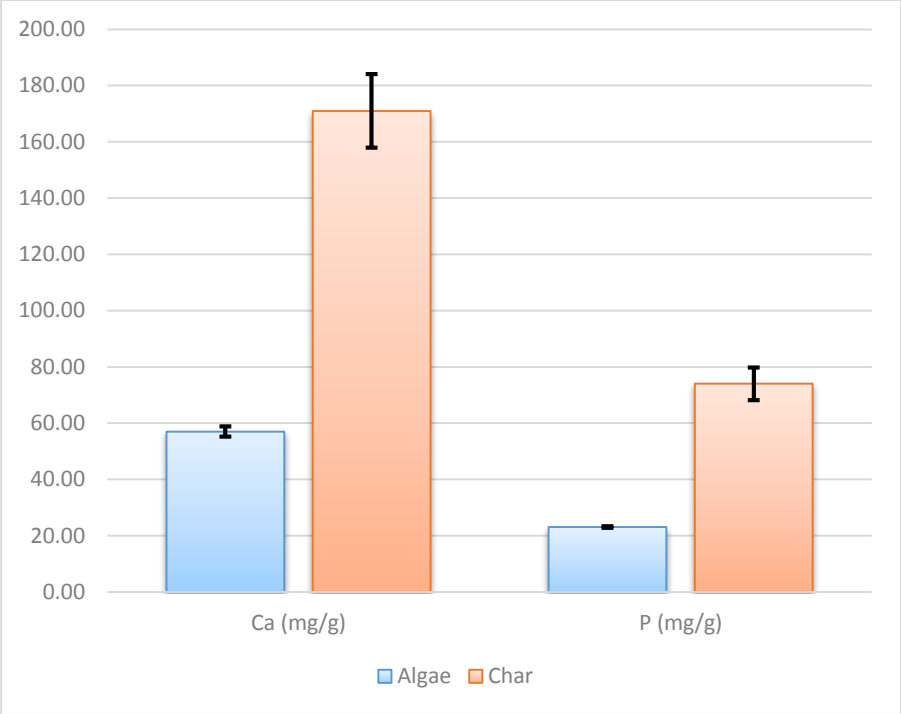


**Figure 9 : Increase in concentration of Ca and P in char as compared to algae**

Although the concentration of elements in char was found to be greatly increased as compared to algae, the source of these elements in the char was the algae which was tranformed to biochar by hydrothermal liquefaction. In the process of algae production and transformation into products by Roberts et al at the University of Kansas, biochar yield was found to be 29.5% which means 29.5 g of biochar was produced per 100 g of algae processed. Production rate of algae from Lawrence WWTP was 10,070 kg/day. Therefore, production rate of biochar, calculated as (Production rate of algae* Biochar yield) was 2,970 kg/day.

Table 3 presents the concentration of elements present in algae and biochar in mg/g, percentage of each element that was recovered from algae into biochar during processing and amount of each element that can be recovered in char per day.

| Element | (A) % in algae | (B) % in char | (C)* %recovery | (D)** Conc. factor | (E)** Production rate (kg/day) |
|---|---|---|---|---|---|
| **Ca** | 5.7 | 17.1 | 88.48 | 3 | 508 |
| **P** | 2.31 | 7.4 | 94.48 | 3.2 | 220 |
| **Mg** | 0.42 | 1.41 | 99.01 | 3.42 | 42 |
| **K** | 0.37 | 0.29 | 23.12 | 0.78 | 9 |
| **Fe** | 0.13 | 0.37 | 83.94 | 2.84 | 11 |
| **S** | 0.82 | 0.54 | 19.42 | 0.66 | 16 |
| **Na** | 0.014 | 0.05 | 105.33 | 3.57 | 1.5 |
| **Ba** | 0.015 | 0.03 | 58.99 | 2 | 0.9 |
| **Sr** | 0.022 | 0.06 | 80.44 | 2.72 | 1.8 |
| **Mn** | 0.06 | 0.09 | 44.24 | 1.5 | 2.7 |
| **Zn** | 0.011 | 0.07 | 187.69 | 6.36 | 2.1 |

**Table 3: Concentration and recovery rates of each element into biochar after algal processing**

*(C) % recovery is calculated as $\frac{(percentage\ of\ element\ in\ char)*(biochar\ yield)}{(percentage\ of\ element\ in\ algae)}$

**(D) Concentration factor is calculated as $\frac{(percentage\ of\ element\ in\ char)}{(percentage\ of\ element\ in\ algae)}$, i.e. a concentration factor of 3 indicates that the % of element in biochar is 3x higher than in algae.

*** (E) Production rate of an element is calculated as:

$$\frac{(percentage\ of\ element\ in\ char)*(biochar\ production\ rate)}{100}$$

As observed from the table above, Ca, P and Mg had some of the highest recovery rates in the biochar and thus the highest concentration factors. It is found that approximately 89% of calcium and 95% of phosphorus were recovered from algae into char during processing. The microwave acid digestion results indicate that the algal biochar contains a substantial amount of total phosphorus (7.40% by weight). Since, it was also found to contain a significant amount of calcium (17.10% by weight), it appeared safe to assume that the majority of this phosphorus may

be existing as calcium phosphates in the char. Assuming the phosphate in char exists as Calcium associated P (say, as hydroxyapatite in which the ratio of Ca:P is ~ 1.67), there is enough Ca in char (2.3x times P) to bind all the phosphorus in phosphates. This was one of the determining factors in selection of 'Olsen phosphorus extraction' method to estimate bioavailable phosphorus as 'Olsen method' is best suited to be used for calcareous soils.

Zn had a very high concentration factor of 6.36 but the percentages of Zn in algae and char were very small (0.011% & 0.07%, respectively).

## 6.2 - Olsen method

The extraction samples developed using Olsen method were shaken for different times – 30 minutes, 60 minutes & 120 minutes. Duplicate samples were prepared for each shaking time. One sample each for 120-minute shaking time of algae & char both gave back clearly erroneous results (P values were nearly negligible). Thus, those were ignored and the results for one sample only were used for both. On running the samples through ICP, following values for amount of phosphorus (P) were obtained. Table 4 shows the extracted P estimated after ICP analysis of the solution. While the concentration of extractable P in algae was found to be 0.70, 0.87 and 1.23 mg/g for contact times of 30, 60 and 120 minutes respectively, extractable P from the char was very low around 0.28, 0.58 and 0.33 mg/g for 30, 60 and 120 minutes respectively.

| **Contact Time (minutes)** | **30** | **60** | **120** |
|---|---|---|---|
| **Algae (mg/g)** | 0.70±0.16 | 0.87±0.039 | 1.23 |
| **Char (mg/g)** | 0.28±0.11 | 0.582±0.11 | 0.33 |

**Table 4: Concentration of extractable P by Olsen extraction method for algae and char in mg/g**

Table 5 shows the % of phosphorus that was extractable out of total phosphorus in both algae and char when shaken for different contact times.

| Contact Time (minutes) | 30 | 60 | 120 |
|---|---|---|---|
| **Algae** | 3.03±0.7% | 3.76±0.16% | 5.32% |
| **Char** | 0.38±0.14% | 0.79±0.15% | 0.45% |

**Table 5: % of extractable P out of total P for both algae and char**

The above table indicates that P extractable by Olsen method in both algae and char is much less (>3% in algae and < 1% in char). Note that the char contained higher total P, but lower absolute concentrations in the Olsen extract, so P in the char was much less mobilized.

Varying contact times were used for identical samples while shaking with Olsen reagent to determine whether the contact time has an impact on the amount of phosphorus extracted. Phosphorus extracted was found to be directly related to the contact time for algae but this behavior wasn't consistent for char. For algae, the amount of Olsen P against contact times followed the order 120 minutes > 60 minutes > 30 minutes. For char, the Phosphorus content increased on shaking the samples for 60 minutes rather than 30 minutes but was found to be decreased for a shaking time of 120 minutes as compared to that for 60 minutes. Figure 4 shows the variation of amount of extractable Phosphorus with different reagent contact times in both algae and biochar.

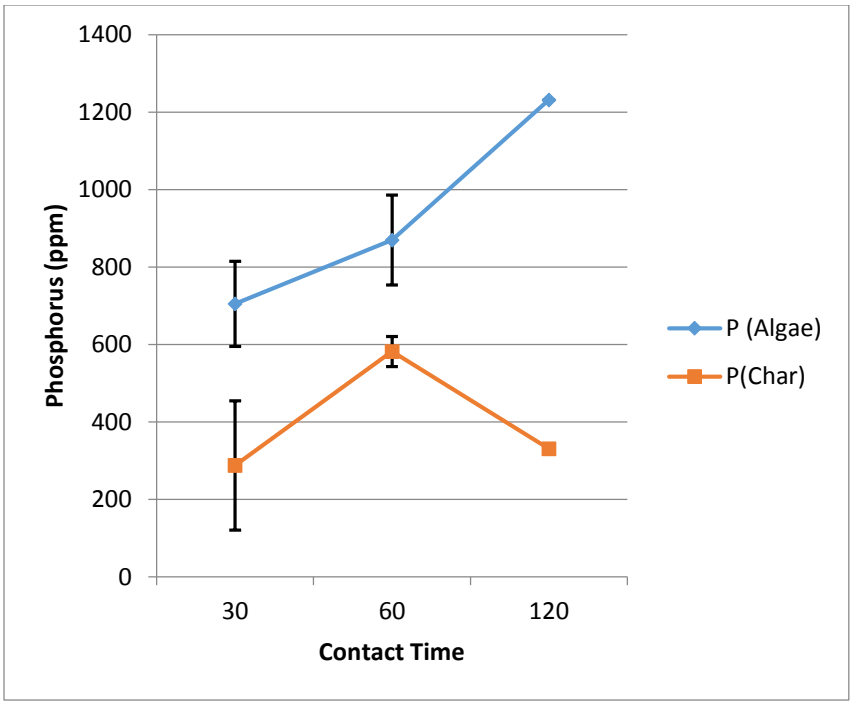


**Figure 10: Phosphorus extracted as a function of Contact time**

A comparison of Olsen extractability of phosphorus with the total phosphorus reveals that the phosphorus present in char is not readily bioavailable and is locked inside the material. In fact, bioavailability appears to decrease in the char relative to the starting algal feedstock.

Calcium was not detected in the samples in Olsen extraction solution by ICP. 0.3 g of char contained 51.3 mg of calcium (17.1% of Ca in char),

Amount of Ca in char = 0.171*0.3/40 = 1.28 millimoles of Ca

At pH 8.5, most of the sodium biocarbonate in solution exists as the bicarbonate ion. But since bicarbonate can deprotonate to replace carbonate as it precipitates, it can all be considered to be available for reaction.

Available Carbonate in solution = 0.5 mol/l * 10 ml = 5 millimoles of carbonate

Since the expected Ca is less than the available carbonate from the reagent, all of the calcium could potentially be extracted from the char. So all calcium-associated phosphate in the char is theoretically extractable using this approach.

As the ratio of Ca and P in char is in excess of 2, it is possible that most of the phosphorus in char is calcium associated and exists as hydroxyapatite. In contact with the Olsen solution, it is expected that some dissolution of hydroxyapatite ($HA_p$) will occur since the phosphate concentration in the starting solution is effectively zero. The Ca in $HA_p$ is expected to dissolve in solution as $CaCO_3$. $K_{sp}$ of $CaCO_3 \approx 3.7*10^{-9}$ at 25°C.

$$CaCO_3 \rightleftharpoons Ca^{2+} + CO_3^{2-}$$

If we assume all of the carbonate reacts with all of the Ca,

$$\text{Final } [CO_3] = 50.5\text{-}0.13 = 0.37 \text{ mol/l.}$$

$$\text{So, } Ca = 3.7*10^{-9}/0.37 \sim= 10^{-8} \text{ M or} \sim 0.1 \text{ ppb.}$$

So we have no real expectation of detecting Ca in the Olsen solution. Thus, no Ca detection by ICP in Olsen extract is justified as the maximum possible concentration of dissolved Ca is well below the detection limit of ICP.

## 6.3 - Water extraction

All samples extracted using the water extraction method yielded no results detectable P, i.e. water extraction proved to be ineffective to extract phosphorus out of both algae and char for all the samples.

ICP-OES used for estimation of P in samples has a detection limit of 0.1 ppm, i.e. the instrument cannot detect the presence of an element if the element is present in concentration lower than the detection limit.

0.1 ppm of P = 0.1 µg/g of P

In 0.3 g of material, there could be 0.03 µg of water extractable P that wouldn't be detected by ICP. That means, as much as 10 µg of water soluble P could be present in 100 g biochar that cannot be detected.

The results followed the expected trend of phosphorus content of algae and biochar based on the method used to estimate the amount –

Total phosphorus > Olsen phosphorus >Water extractable phosphorus

These findings suggest that there is substantial amount of total phosphorus present in our algal biochar. However, this phosphorus is not very extractable or bio-available. Olsen extraction method was only able to extract a very small percentage of total phosphorus out of the algal biochar while water extraction method proved to be ineffective in extraction of phosphorus within the instrument detection limit.

## 6.4 Discussion

A low amount of Olsen P certainly suggests that phosphorus in algal biochar under investigation is not readily available to plants. However, there are uncertainties present in these results due to limitations of the experimental method. As discussed earlier, the algal biochar was very hydrophobic in nature and did not maintain a good contact with water or Olsen reagent. Due to lack of proper contact and relatively short contact time, it is possible that the P extracted is far

lower than it could actually be. Heating biochar to 105$^{o}$C overnight in an attempt to drive off the insoluble organic fractions was not successful in reducing its hydrophobicity. No detection of P in water extract of algal biochar could be partially or completely due to this hydrophobicity because the miscibility of biochar with pure water was even worse than it was with Olsen Reagent. However, it also suggests that P in this biochar is not soluble. Secondly, Olsen P estimates plant available inorganic P levels; it makes no assessment of the organic component of P in the soil. Organic P component mineralizes over time by decomposition of organic matter and becomes available. If this algal biochar contains Organic P as a sizeable fraction of total phosphorus, it might have been ignored by Olsen test and the plant available phosphorus may have been underestimated. An increase in extracted P on increase in contact time from 30 minutes to 60 minutes indicates that the calcium associated P in biochar may slowly release over time. The Colwell bicarbonate method which uses the same reagent as Olsen P method but recommends a contact time of 16 h instead of 30 minutes might be a better judge of slow release ability of calcium associated P from algal biochar. Thus, where this study provides a preliminary idea of nature of phosphorus in this biochar and suggests that it contains phosphorus which is mostly unavailable to plants, it doesn't prove it. Even though, the phosphorus in this biochar isn't readily bioavailable, it may still provide other benefits such as carbon sequestration, reducing run off due to high permeability, conditioning soils, retaining pesticides etc. and therefore, may be used as a soil amendment. However, when used as a soil amendment, the char may not mix with soil owing to its hydrophobicity. Highly hydrophobic nature of biochar indicates that some oil still remains on the surface of biochar which may hinder its ability to mix with soil. Therefore, for end use, the char may need to be treated before being applied to soil which would increase costs.

Analysis of this algal biochar is a subject of potential research considering the uncertainties involved and concrete conclusions about the value and appropriate uses of this biochar can only be reached thereafter. Slowly releasable P and ignored organic component of P by Olsen test are indicators that this biochar may indeed be a useful resource in soil amendment and be used to add to nutrient value of soil. Further research may include analyzing P in this biochar using different extraction methods such as Colwell bicarbonate method, Kjeldahl method, Mehlich III method and comparing the results. Water extraction and Olsen extraction of this biochar can also be done after washing this biochar with an organic solvent such as Isopropyl Alcohol (IPA) to consolidate the powdery texture of biochar and make it be in better contact with water and Olsen reagent. Hydrophobicity of this biochar may also be attempted to be reduced by altering its production temperature. Existing research on different biochars has shown that hydrophobicity of biochar can be minimized by altering the temperature used to produce biochar (Kinney et. al., 2012). It is possible that an engineered less hydrophobic biochar produced using the same algae as feedstock produces better results for soluble and available P.

## 7 – CONCLUSION

The objective of this study was to analyze phosphorus in algal biochar produced from algae as a feedstock which was produced at Lawrence WWTP. This study was an attempt to estimate the amount of phosphorus present in this organic algal biochar and test what fraction of it was soluble and plant available.

The biochar derived from wastewater algae from Lawrence WWTP as a feedstock was found to contain a substantial amount of total phosphorus when digested using reverse aqua-regia in a

microwave and subjected to ICP analysis. The biochar had a mean concentration of 74±5.8 mg/g of total phosphorus and a high concentration of calcium (171±13.1 mg/g) as well. Many other elements were analyzed in ICP simultaneously and were detected in small amounts. On an average, the char contained 1.41% dw of magnesium, 0.37% dw of iron, 0.29% dw of potassium and 0.82% dw of sulphur as significant inorganic constituents. Approximately 89% of calcium and 95% of phosphorus were recovered from algae into char after algal processing. The ratio of calcium to phosphorus content in both algae and char was approximately the same around 2.4.

Due to presence of a high amount of calcium, biochar was expected to be calcareous in nature and thought to contain phosphorus as calcium phosphate. Since the Olsen method is considered the best test for phosphorus extraction from calcareous soils, the algal biochar was subjected to Olsen extraction. A very small fraction (less than 1%) of total phosphorus in the algal biochar was found to be extractable by Olsen reagent on ICP analysis. Different amounts of phosphorus were estimated for different shaking times. An Olsen extractable phosphorus concentration of 0.28±0.11 mg/g for 30 minute shaking time, 0.58±0.11 mg/g for 60 minute shaking time and 0.33 mg/g for 120 minute shaking time was found. These observations suggest that the phosphorus content almost doubled when shaking time was increased from 30 minutes to 60 minutes. However, the same was not found consistent when shaking time was increased from 60 minutes to 120 minutes. This inconsistency might have been due to improper contact between biochar and Olsen reagent for the particular sample.

Water extraction was completely ineffective in extracting any phosphorus from algal biochar. No phosphorus was detected when water extraction solution from biochar was analyzed in ICP. The amount of water extractable phosphorus might have been insignificant and less than the detection limit of the instrument. These results followed the expected decreasing trend of

phosphorus content in algal biochar from using different extraction methods – total phosphorus being the greatest, followed by Olsen and no results for water extraction.

These findings suggest that there is very less plant available P present in this biochar which may be available over time. Increase of Olsen extractable P at longer shaking times encourages the idea that this biochar may indeed be a good resource for soil amendment and as a fertilizer because it may release P over time for plant consumption. However, ability of biochar to mix with soil may be hindered by its hydrophobic nature. Concrete conclusions can only be made after further research on this biochar which includes trying more soil P extraction methods on this biochar and comparing the results, attempting to reduce its hydrophobicity to ensure better contact with reagents, extracting biochar with reagents after consolidating its texture by washing with organic solvents such as IPA.

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