TOWARD AN UNDERSTANDING OF THE ROLE OF BIOCHAR AS AN AGRO-ENVIRONMENTAL TOOL: POTENTIAL FOR CONTROL WATER RELEASE, BACTERIAL RETENTION, AND GREENHOUSE GAS EMISSIONS

By

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Dedication

To

My Parents

My Family members

My Teachers

And My Friends
TOWARD AN UNDERSTANDING OF THE ROLE OF BIOCHAR AS AN AGRO-ENVIRONMENTAL TOOL: POTENTIAL FOR CONTROL WATER RELEASE, BACTERIAL RETENTION, AND GREENHOUSE GAS EMISSIONS

Abstract

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This dissertation aims to advance our knowledge on the relationship between biochar physico-chemical properties and its performance as a soil amendment. An emphasis was placed on understanding how the feedstock source and pyrolysis conditions influence biochar bulk and surface properties and what effects these properties have on greenhouse gas emissions, soil water retention, and movement of bacteria in sandy soils.

Three lignocellulosic biomass feedstocks (poplar wood, pine bark, and pine wood) were used to produce biochars at six different pyrolysis temperatures (350, 400, 450, 500, 550, and 600°C). It was found that the content of volatiles, the oxygen to carbon (O/C) ratio, and hydrogen to carbon (H/C) ratio decreased linearly with temperature suggesting a gradual increase in aromatic
structures and thermal recalcitrance. Pine bark-derived biochars had higher ash content than wood-derived biochars, and as the pyrolysis temperature increased, the ash content also increased.

The surface study showed that biochars produced at low temperature (<500°C) retained some surface functionalities characteristics of the feedstock. The XPS and Boehm titration confirmed that most oxygenated surface functional groups (mainly; carbonyl, carboxyl and hydroxyl groups) are gradually removed as pyrolysis temperature increased. Oxidation by air at 250°C was able to introduce several oxygen functional groups onto the biochar surface. Particularly, the formation of carbonyl and carboxyl groups is facilitated in biochars produced at low temperature. The formation of these oxygenated functional groups contributes additional negative charges on the biochar surface.

Upon biochar application to Quincy sandy soil, it was found that oxidized biochar held significantly more water and this is believed to be related to the content of oxygen functional groups and the pores structure. Oxidized biochars facilitated the transport of *Escherichia coli* through soil columns likely due to their negative surface charges that could repel bacteria that often carry an overall negative charge. Our results suggest that unoxidized pine wood-derived biochar was effective in reducing the transport of *E. coli*. Moreover, compared to the soil, biochar amendments did not affect the emissions of N₂O but significantly reduced cumulative CO₂ emissions (at 95% level of confidence).
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CHAPTER 1. INTRODUCTION

1.1 Overview

The crucial challenge of this century is to achieve environmental management and agriculture production goals all while maintaining or elevating the wellbeing of our societies. Multiple challenges such as climate change, water quality, food scarcity, and soil productivity are still causing serious socio-economic and political tensions. Indeed, the potential to overcome these challenges with existing technologies seems considerable but requires inventing or adapting new strategies at both local and global scales. Using biochar in existing agricultural systems is a promising concept with the potential to aid in the mitigation of greenhouse gases (GHGs) and improving soil fertility (Lehmann and Joseph, 2009). However, there are multiple biochar production challenges that need to be overcome before biochar can be used as an effective tool to fight global warming and improve soil fertility. These challenges range from developing production techniques to understanding how biochar behaves in soil. Without a better understanding of the relationship between feedstock characteristics, pyrolysis conditions, biochar physico-chemical properties and its performance as a soil amendment, designing specific biochars to improve soil fertility is very challenging.
1.2 Rational and Significance

Anaerobic thermochemical conversion of biomass (also called pyrolysis) is the main technology used today to produce biochar (Fu et al., 2009). Pyrolysis processes can be refined to produce high yields of a liquid called bio-oil, a process called fast pyrolysis. Biochar can simply be combusted to produce heat or can be post-processed into a higher value product such as activated carbon. The development of engineered biochars for soil amendment is another approach to add value to this material and to develop a tool for environmental services (Rautiainen et al., 2012). The production of biochar from agricultural, forest and urban wastes is also a promising waste management strategy. The identification of the production conditions that result in a biochar capable of improving soil fertility is a subject of great practical and scientific significance. Currently, a knowledge gap exits to guide the selection of feedstock and processing conditions needed to achieve such improvements.

The yields and properties of biochar are strongly dependent on the feedstock composition and pyrolysis temperature (Zhao et al., 2013). For example, feedstocks with high lignin and mineral contents produce higher quantities of biochar (Antal and Grønli, 2003; Collison et al., 2009). Lignocellulosic feedstocks result in carbon-rich biochars compared to other feedstocks such as animal manures which result in nitrogen-rich biochars (Bruun et al., 2011). Low-temperature pyrolysis (<500°C) favors greater recovery of several nutrients (e.g. N, K, and S) and major elements (e.g. O, H, and N) that are removed in the volatile fraction at higher temperatures (Keiluweit et al., 2010). High-temperature pyrolysis (>500°C) chars have higher aromatic carbon content and more developed surface areas but low aliphatic carbons and surface functionality (Bruun et al., 2011; Song and Guo, 2012; Spokas et al., 2012; Mašek et al., 2013). Due to the range
of biomass options and pyrolysis conditions, the variability in biochars that can be produced is high. This variability has significant implications for carbon sequestration and agricultural productivity when biochar is applied to soil (Lehmann and Joseph, 2009).

Application of biochars into agricultural soils can provide long-lasting improvements in carbon sequestration, soil fertility, and water retention especially for sandy soils where agriculture faces large constraints due to low water holding capacity and high leaching of soil nutrients (Uzoma, 2011). This material provides unique opportunities to sequester carbon dioxide (CO₂) from the atmosphere and to enhance plant growth, which then consumes additional CO₂ during photosynthesis. Biochar is also believed to reduce the need for artificial fertilizers due to the retention of nutrients and its potential to improve soil fertility. The retention of nutrients may help to reduce additional emissions of GHGs from the process of fertilizer production and artificially fertilized soils in agronomic production. Interactions between biochar additions, gaseous emissions, and soil microorganisms are known to occur within a short period of time after application to the soil (Lehmann and Joseph, 2009). However, little is known about the extent and implications of these interactions, and this knowledge is needed for an effective evaluation of the use of biochar as a soil amendment and tool for carbon sequestration.

Biochar has also the potential to reduce leaching of manure-borne pathogens through soil. Land application of animal manure is a common agricultural practice used to improve soil fertility and increasing crop productivity. However, improper application of manure can lead to groundwater microbial contamination when pathogenic microorganisms leach downward through the soil profile (Hipel et al., 2003). Massive disease outbreaks have been associated with drinking contaminated groundwater, the most notable being the Walkerton (Ontario, Canada) tragedy when
more than 2300 persons were infected with *Escherichia coli* O157:H7 via the public water supply (Hipel et al., 2003). Investigations for fecal water contamination are typically conducted using indicator organisms such as *E. coli*. The EPA uses this bacterium routinely as predictors of presence of other pathogens in water (Levy et al. 2007). This is because *E. coli* bacteria are detectable at low concentrations, excreted in both human and animal feces, survive longer than other indicators, and act as indictor for highly pathogenic bacteria (Buckley et al., 1998; Levy et al., 2007). A thorough understanding of the transport of *E. coli* within the soil-groundwater system is critical to public health. Although biochar incorporation into soils has a potential for reducing the leaching of manure-borne pathogens, knowledge concerning the impact of biochar addition on the retention and transport of pathogenic bacteria is still nonexistent.

This dissertation contains three separate but supporting studies that address the production conditions, properties and applications for biochars produced from different feedstock sources at different pyrolysis temperatures and oxidation conditions. This research is intended to develop correlations between production conditions (pyrolysis and post-pyrolysis oxidation), and the resulting biochar bulk and surface physico-chemical properties, as well as the impacts on CO$_2$ and N$_2$O emissions, *Escherichia-coli* transport and water retention in biochar-amended sandy soil.
1.3 Research Hypotheses

**Overall hypothesis:** *A better understanding of the relationship between feedstock composition, production conditions and biochar physico-chemical properties with biochar performance as a soil amendment will facilitate the design of biochars engineered to maintain or improve soil fertility, decrease GHGs emissions and attenuate transport of Escherichia coli through sandy soil.*

1.4 Research Objectives

**The overall objective:** *To advance our understanding of how the pyrolysis and post pyrolysis oxidation conditions affect the bulk and surface physico-chemical properties of biochar and the effect these properties have on greenhouse gas emissions, soil water retention, and E. coli transport in soils.*

The following five specific objectives have been identified to achieve this overall goal:

1. To examine how feedstock source and pyrolysis temperature affect the bulk and surface properties of biochar, and to understand the mechanism by which these properties are changed.

2. To study the effect of pyrolysis temperature on the oxidability of biochars and the effect of the oxidation process on the bulk and surface properties of the resultant oxidized chars.

3. To investigate the transport of pathogenic and non-pathogen *E. coli* in biochar-amended sandy soil columns.
4. To understand the role of biochar surface functionality on *E. coli* transport using well-characterized air oxidized biochar simulating subsurface natural conditions.

5. To study the contribution of biochar bulk and surface characteristics on water retention capacity of a sandy soil.

6. To understand the potential role of biochar physico-chemical properties on reducing N$_2$O and CO$_2$ emissions from an agricultural soil.

### 1.5 Dissertation structure

This Ph.D. dissertation has eight chapters, including the present introductory chapter (Chapter 1), which identifies “research gaps” and provides the overall importance of the research including the hypothesis and the objectives. Chapter 2 is composed of a “literature review” that describes the current state of the art biochar research. Chapter 3 investigates the effects of feedstock source and pyrolysis temperature on biochar bulk and surface physico-chemical properties relevant for the use of these materials as soil amendments. As a follow-up study of Chapter 3, Chapter 4 studies the ability of air oxidation to introduce oxygenated functional groups on biochar surfaces. Chapter 5 applies laboratory column experiments and a mathematical model to examine the transport behavior of two *E. coli* strains in a sandy soil amended with oxidized and unoxidized biochars. Chapter 6 explores the potential of oxidized biochars to retain water in a sandy soil. Chapter 7 studies biochar’s ability to reduce emissions of CO$_2$ from microbial respiration and N$_2$O from the Quincy sandy soil. Chapter 8 summarizes the results of all the previous chapters and makes recommendations for future work. A dissertation structure is presented in figure (1).
Figure 1.1. Schematic outline of the dissertation work.
1.6 List of publications and other works related to this dissertation


Works presented in conferences/scientific meetings


Trent, G., Suliman, W., Garcia-Perez, M., and Abu-Lail, N. (2011) Influence of biochar on transport of Escherichia coli through a sandy soil. Poster presentation, showcase for undergraduate research and creative activities (SURCA), April 2011, Pullman, WA.
1.7 References


CHAPTER 2. LITERATURE REVIEW

2.1 Outline

The literature review of this dissertation comprises several key areas, which target the production and applicability of biochar as a soil amendment and environmental tool. A brief history of biochar application to soils is provided. This is followed by a section that definitions biochar as a term followed by a section that deals with the biomass feedstock availabilities and compositions. The biochar production technology (i.e. Pyrolysis) is provided under the sections “Pyrolysis, fast pyrolysis, and slow pyrolysis”. The latter section is focused on the biochar characteristics as affected by feedstock source, pyrolysis temperature, and post-pyrolysis oxidation. A final section includes the conclusions drawn from this literature review.

2.2 Historical perspective

Biochar was used successfully by generations of indigenous farmers to enhance soil fertility in the Amazon (Maia et al., 2011). Accumulation of bulky quantities of biochar, as a result of habitation activities and deliberate soil application in the Amazon Basin, increased soil agronomic quality and formed what is known as Amazonian Dark Earths. These activities were done more than 10,000 years ago (Lehmann and Joseph, 2009; Verheijen et al., 2010; Maia et al., 2011). Some studies classified Amazonian dark earths into two groups based upon their colors, terra preta (black earths) and terra mulata (brown earths) (see Figure 2.1); both are considered to be anthropogenic soils (Barrow, 2012). Amazonian dark soils have a long-lasting fertility that has been related to the biochar stability. In some studies, this biochar has been dated up to 7,000 years ago (Lehmann
and Joseph, 2009; Maia et al., 2011). These observations, combined with the search for carbon sequestration techniques, have created a resurgent interest in biochar as a tool for soil management.

Figure 2.1. Terra preta (F41 and above) and terra mulata (F40). Photo source (Turpin, 2013).

2.3 What is biochar?

The use of the term biochar has generated an important controversy in the scientific community, caused by the fact, that biochar is synonyms to charcoal (Lehmann and Joseph, 2009; Quade, 2010). Charcoal refers to a product of thermal decomposition (in fact it is not a product of the cracking (or pyrolysis reactions) but a product of cross-linking, dehydration, aromatization and polycondensation reactions) of lingo-cellulosic materials at temperatures over 300 °C (most charcoals are produced at around 500 °C). The main criteria used to call a material as “biochar” is
its usage as soil improvement. Charcoal is a term mostly used to define a product of thermochemical processes (slow or fast pyrolysis, gasification) with high C content mostly used in domestic and commercial applications such as heating, cooking, metals refining, chemical production, energy production, purification and other industrial applications (Lehmann et al., 2006; Lehmann and Joseph, 2009).

Some literature still considers biochar to be a char or solid fire-derived product which might leave the reader confused especially when the literature does not explain the limitations of using each term. Lehmann and Joseph (2009), for example, defined char in the most general term as the products of pyrolysis and fire, whether they were derived from biomass or other materials. They defined charcoal as a carbonaceous material produced from pyrolysis for use in cooking or heating while biochar is char produced for the purpose of applying it to soil for agronomic enhancements or environmental management. However, in Sohi et al. (2009)’s point of view, char was considered as any solid fire residue of biomass left after a natural fire while biochar as pyrolyzed-carbonaceous matter is produced specifically for application to soil. Consequently, the production process along with intended use determines convention or the meaning of the term biochar (Lehmann & Joseph, 2009).

2.4 Biochar composition

Biochar is a highly aromatic compound that contains random stacks of graphitic layers (Brewer et al., 2009; Spokas et al., 2010). The aromaticity of biochar is a temperature-dependent parameter; as temperature increases, the carbon structure thermally transforms from disordered small polyaromatic ring systems, to ordered graphene sheets (Paris et al., 2005; Harvey et al., 2012), as
illustrated in figure 2.2. Biochar contains mainly C, H, N and O, the proportion of which may change with pyrolysis temperature (Spokas et al., 2011).

![Model biochar structure development with pyrolysis temperature. Source Chia et al., (2015).](image)

**Figure 2.2.** Model biochar structure development with pyrolysis temperature. Source Chia et al., (2015).

A typical surface of biochar possess a diverse range of surface functional groups governing surface functionality (i.e. acidic, basic, hydrophilic, and hydrophobic properties) and adsorption capability of biochar (Amonette, 2009; Lehmann and Joseph, 2009; Farrell et al., 2013). Based on production conditions, different functional groups could be observed on the biochar surface, such as hydroxyl, carbonyl and carboxyl groups. Figure 2.3 shows a typical structure of biochar with different oxygenated functional groups.
**Figure 2.3.** Model of a biochar macromolecule, showing the most important types of surface functional groups, adapted and re-drawn from Bandosz, (2006).

### 2.5 Biomass Precursors for Biochar Production

A wide range of agricultural and forest wastes have been proposed for biochar production including wood chips, wood pellets, bark, field crop residues, and forest residues (dead wood, pole trees, logging residues). Organic wastes, such as animal farm wastes, sewage sludge, urban wastes (yard trimmings, site clearing and wood packaging), and wastes from food, sugar, or juice processing, are other potential sources of biochar (Collison et al., 2009; Verheijen et al., 2010; Laird et al., 2011). The key point is that the suitability of any biomass feedstock, as a potential source for biochar, is absolutely dependent upon chemical, physical and environmental factors, with economic, social and logistical considerations also being important (Collison et al., 2009). In principle any organic waste can be used to produce biochar (Cantrell et al., 2012; Song and Guo,
The idea of producing biochar from organic and agricultural wastes comes certainly as a solution for problems related to the current high-volume waste disposal management issues associated with these industries. For instance, as illustrated in figure 2.4, if agricultural wastes are left to decompose, they can increase greenhouse gas emissions due to releasing of nitrous oxide, methane and CO$_2$ (Lehman & Joseph, 2009).

**Figure 2.4.** Atmospheric CO$_2$ reduction and sequestration pathways in presence and absence of biochar, adapted from Lehmann, (2007).

Lignocellulosic materials (consist by cellulose, hemicellulose and lignin) are the most common form of biomass and the most important source for charcoal making worldwide. The lignocellulusic materials also contains small quantities of other organics extractives (e.g. fats, phytosterols and phenolics) and inorganic compounds known as ash (such as potassium, sulphur, silicon, alkaline
metals, and various trace elements). The structure can vary significantly depending upon plant species, biomass kind, soil type, climate conditions, and the time of harvest (Collision et al., 2009).

Under pyrolysis condition, cellulose and hemicellulose mostly break into compounds of lower molecules weight, which mainly form volatile products. A small fraction of the cellulose and hemicellulose is also subjected to cross-linking, dehydration, aromatization and polycondensation reactions responsible for the formation of biochar. Figure 2.5 shows a suggested mechanism for cellulose pyrolysis (Lin et al., 2009), in which pyrolysis takes place through the initial depolymerization of cellulose occurring at 373-423 K. Cellulose decomposes to the oligosaccharides and continues to complete chain breaks until it reaches the sugar level. The first resulting anhydro-monosaccharide is levoglucosan (LGA), followed by dehydration and isomerization reactions to form levoglucosenone (LGO), 1,4:3,6-dianhydro-Alpha-D-glucopyranose (DGP), and 1,6-anhydro-Beta-D-glucofuranose (AGF). These anhydro-monosaccharides may undergo competitive reactions (i.e. fragmentation/retroaldol condensation, dehydration, decarbonylation, or decarboxylation) resulting in char and gases. In case of lignin, although the thermochemical reactions also result in the production of volatile products (products of cracking reactions), an important part of this material undergoes cross-linking and polycondensation to form a polycondensed aromatic macromolecule (charcoal) (Demirbas, 2004; Kersten and Garcia-Perez, 2013; Wang et al., 2013).
Figure 2.5. A suggested mechanism of cellulose pyrolysis from Lin et al. (2009), (DP indicates ‘degree of polymerization’).
2.6 Biochar Production

Thermochemical conversion of biomass feedstock can be generally divided into three main categories based on temperature and reaction time. These categories are: (1) Torrefaction, (2) Pyrolysis, and (3) Gasification (Laird et al., 2011). In this dissertation, pyrolysis is the thermochemical process under investigation.

Pyrolysis is a thermo-chemical decomposition of carbon-based materials occurring in an oxygen deficient environment (that is, less oxygen is present than required stoichiometrically for complete combustion). Pyrolysis is an exothermic process once the temperature reaches about 300°C mostly due to the acceleration of dehydration, crosslinking, aromatization and polycondensation reactions (Mohan et al., 2006). Pyrolysis has been used for production of a range of compounds including activated carbon, methanol, syngas and some chemicals. The three main constituents of hemicellulose, cellulose, and lignin decompose in ranges of (200-260°C), (240-340°C) and (280-500°C), respectively (Bridgwater and Peacock, 2000). The extent of degradation of each of these components depend on the process parameters of reactor type, temperature, particle size, heating rate and pressure. The final products are water, tars, polymers, gases, and charcoal (Vamvuka, 2011). A very simplified scheme of pseudo reactions is shown in Figure 2.6 to explain the overall pyrolysis process.
Figure 2.6. Mechanisms of pyrolysis, re-drawn from Vamvuka, (2011).
Depending upon the operating conditions, with varied heating rates, residence time, and temperatures, pyrolysis process can be divided into two classes: Slow and Fast processes (Demirbas, 2004). In this introduction, only a brief description on both pyrolysis processes will be presented. More information on these processes can be found elsewhere (Bridgwater and Peacock, 2000; Bridgwater, 2012).

**Slow pyrolysis** is an oldest method that has been operated for thousands of years by using traditional kilns for production of charcoal (Kobya et al., 2005; Lehmann and Joseph, 2009). It is characterized by slower heating rates (5-7°C/min), long residence time, and low temperature (350-550°C). Traditionally, charcoal is produced in slow pyrolysis reactors known as kilns (pits or mounds), and usually used as a fuel for domestic cooking, heating, and metallurgical industry (Sohi et al., 2009; Garcia-Perez et al., 2011). Slow pyrolysis units are often cheap, easy to operate, able to accept a range of feedstock sources, and they do not require finely grounded feedstock (Laird et al., 2011). Probably because of these reasons, the slow pyrolysis technique is widely used in developing countries where charcoal is still sold and used as household fuel; the largest global production is in Africa and South America (21 Mt and 14 Mt respectively) (see a traditional earth-mound kiln in figure 2.7) (Lehmann & Joseph, 2009).
Traditional charcoal production often harms the environment, as it leads to air pollution. With no recovery in traditional processes, Liquid and gas products often escape into the atmosphere causing environmental issues (Antal and Grønli, 2003; Laird et al., 2011). However, current developments allow recirculation of gases to provide internal or external heat and hence slow pyrolyzers have become available for industrial scale processes with no serious air pollution hazards (Vamvuka, 2011). Moreover, while traditional methods convert about 10% of the feedstock into charcoal, controlled industrialized pyrolysis processes achieve about 35% conversion (Sohi et al., 2009).

Fast pyrolysis is described as a rapid high temperature process with heating rates of over 300 °C/min, short residence time, and carefully controlled conditions to generate vapors and aerosols.
with some charcoal (Bridgwater, 2012). According to Bridgwater and Peacocke (2000) the main product of fast pyrolysis is bio-oil which can be obtained in yields up to 80% wt. of dry feed (typical yields are between 60 and 70 wt. %), see figure 2.8. Because of the low thermal conductivity of lignocellulosic material and the mass transfer limitations imposed by the biomass cell structure, small particles are preferred to process in fast pyrolysis. Ablative pyrolysis process is a very special type of fast pyrolysis in which the biochar later formed and the liquid intermediates are continuously removed (Mohan et al., 2006; Niels et al., 2007; Jahirul et al., 2012). Fast pyrolysis area has been extensively reviewed by Garcia-Perez et al., (2011), Bridgwater, (2012) and Bridgwater and Peacock (2000).

Figure 2.8. BioTherm™ flowsheet showing recirculation of gases, (Sandvig et al., 2003)
2.7 Biochar characteristics as a function of feedstock and temperature

Influence of Feedstock Source: The chemical and physical properties of the feedstock biomass are important in influencing the resulting biochar (Basso et al., 2013). Ash and mineral contents of biochar, as an example, vary considerably depending on feedstock ash content and composition (Enders et al., 2012). The ash content of the biochar is about 3-6 times greater than in the original feed, according to the data published by Budai et al., (2014). This is because minerals of original feedstock are mostly concentrated in resultant biochars during thermal degradation process (Antal and Grønli, 2003). Woody feedstocks with low ash content generate biochars with low ash contents while herbaceous and manure feedstocks result in biochars with high ash content (Harris et al., 2007). Biochars derived from different precursors show differences in their mineral composition, porosity, and yield as well. For example, crystalline silica was found in biochar produced from rice straw while calcium carbonate was found in biochar of paper sludge (Verheijen et al., 2010). Some researchers (Lee et al., 2010; Bruun et al., 2011) have hypothesized that inorganic materials of high-ash feedstocks (e.g. manure) may somewhat block access to micropores, thereby reducing the surface area. Biochar yield is another feedstock-dependent parameter which mostly linked to the lignin of the biomass feedstock, regardless pyrolysis type or conditions. Several studies (Antal and Grønli, 2003; Sharma et al., 2004; Collision et al., 2009; Zhou et al., 2014) have shown that high lignin content in the feedstock biomass increases the yield of biochar. Feedstock composition having varying proportions of cellulose, hemicellulose and lignin resulted into significant variation in yield and other properties. A low cellulose/lignin ratio in feedstock decreases volatiles yield and increases char yield (Hodgson et al., 2011).
Influence of Pyrolysis Temperature: The most influential pyrolysis parameter on biochar yield is temperature figure 2.9, followed by heating rate (Amonette, 2009; Cantrell et al., 2012; Song and Guo, 2012). Pyrolysis temperature also leads to alterations in feedstock internal structure, elemental composition and surface characteristics. High pyrolysis temperatures lead to a decrease in surface functional groups and an increase in the aromaticity of the biochar, and thus its recalcitrance to microbial attack. Increasing pyrolysis temperatures enlarges and increases the order of the crystallites as well as increasing surface area (Lua et al., 2004; Copeland et al., 2008).

![Figure 2.9. Effect of pyrolysis temperature on biochar yield, adapted from Jahirul et al., (2012).](image)

Increasing pyrolysis temperature leads to decreases in total N, O, and H, whereas the total carbon, pH, and surface area of biochar increases (Bruun et al., 2011; Song and Guo, 2012). The relative quantity of biochar elements (i.e. O/C and H/C) determines its stability, which in turn determines its suitability for environmental purposes (Cross and Sohi, 2013; Mašek et al., 2013). Biochar with a low H/C ratio, for example, has higher aromaticity and is expected to be more
recalcitrant (Hammes et al., 2006). Studies conducted by Singh et al. (2012) and Farrell et al. (2013) showed that the increase in pyrolysis temperature led to a decrease in H/C and subsequently in the amount of biochar mineralized over an incubation period of 120 days. Such findings are essential for the optimization of pyrolysis conditions for production of biochar with selected properties.

2.8 Post Pyrolysis Oxidation

Surface oxygenated functional groups are by far the most important surface groups that not only influence the surface characteristics such as polarity, acidity, and wettability, but also physico-chemical properties such as catalytic, electrical, and chemical reactivity of carbon materials (Bansal and Goyal, 2005). While increasing pyrolysis temperature (range of 350-750°C) tends to decompose these surface groups, post-pyrolysis oxidation processes have been shown to lead to significant increases in the number of surface oxygenated functional groups (Valdés et al., 2002; Park and Kim, 2005). Wet oxidation method (by acids or bases) are used to chemically modify biochar surface functionality for adsorption of some compounds in aqueous or gas phases, however, use of chemical oxidizers could result in the production of toxic wastes. Dry oxidation (i.e. oxidation by ozone, air, and cold plasma) can be used as an alternative to chemical treatments for surface modification (Strelko and Malik, 2002; R. Wang et al., 2013). Oxidation by hot air, for example, has shown to modify the density and composition of functional groups on surfaces of carbonaceous materials (i.e. biochar and activated carbon) (Strelko et al., 2002; Osswald et al., 2009).
Due to its low energy cost, higher reactivity, less damage and less pollution, oxidation by air has received the most attention by researchers in comparison with chemical treatments. Strelko et al., (2002) indicated that air oxidation of activated carbon increases the concentration of the acidic surface functional groups and allows for significant increase in pore volume and specific surface area. As illustrated in figure 2.10, the oxygenated functional groups could be formed when biochar is treated with oxygen at temperatures below 400°C. In a study conducted by Sakuma et al., (2011), a bamboo activated charcoal was oxidized by air at 350°C for 2 hrs, and results revealed that the surface carboxyl groups increased significantly from 0.02 mmol/L in the unoxidized to 1.19 mmol/L on the oxidized one. In general, their study suggested that air oxidation following low temperature carbonization has the potential to produce a modified bamboo charcoal with a high quantity of acidic surface functional groups as well as greater surface area and pore volume. Similarly, Yamashita and Machida (2010) studied the effect of air oxidation at 280°C for 2 hrs on bamboo char porosity. They found that both total pore volume and specific surface area were significantly increased from 0.14 to 0.24 ml g⁻¹ and from 120 to 240 m² g⁻¹, respectively.
2.9 Effect of biochar addition on soil properties

The use of biochar as a means to ameliorate soil properties has emerged as an important area of research. The main properties associated with the performance of biochar as soil amendments are: cation exchange capacity (CEC), pH, surface area, porosity, and surface functionality (Herath et al., 2013). Recent studies have shown that biochar addition to soil increases pH, total carbon, CEC, water-holding capacity and exchangeable basic cations (Rondon et al., 2006; Novak et al., 2009; Uzoma, 2011; Sika, 2012). Improving soil properties by means of biochar application has been proposed in recent publications. Herath et al. (2013) studied the effect of corn-stover biochar on volumetric water content, bulk density, hydraulic conductivity and aggregate stability of two
soils, and reported positive effects of biochar on these properties. Chan et al., (2007) found that addition of green-wasted based biochar to soil resulted in increased organic carbon, available Na, K, and Ca, and extractable P. Similarly, Major et al. (2010) reported that biochar addition increases available Ca, Mg, and pH in soil. The greater crop yield, they observed, was attributed to nutrient uptake primarily to the 77–320% greater available Ca and Mg in soil where biochar. Generally, the changes in soil after biochar application reflects the properties of the biochar being applied. Since these changes are biochar type-, dose-, and soil-specific, more research is still needed to better assess the potential benefits of biochar for agricultural use.

2.10 Conclusion

A literature review of papers published between 1990 and 2015 was conducted to determine the number of biochar studies in the Web of Knowledge database (May 15th, 2015). Out of 133,700 papers containing the key word pyrolysis, only 300 papers reported the pyrolysis conditions under which the biochar studied was produced. Unexpectedly, about only 30 studies dealt with the use of woody biomass as a biochar feedstock, while 350 papers addressed the possible use of same biomass as a biofuel feedstock. Current knowledge about pine and poplar woods, and pine bark biochars is not sufficient to support recommending its use in agricultural soils; which means further research should be undertaken in this field before wide scale application of these biochars to agricultural soils commences. This dissertation aims to fill the obvious research gap that exists with respect to the adaption of specific biochar production methods, pyrolysis temperature and dry oxidation (post-treatment) to pine and poplar woods, and pine bark biochar properties that produce a high value commercial product with specific chemical, physical and biological properties.
2.11 References


CHAPTER 3. UNDERSTANDING THE EFFECTS OF PYROLYSIS CONDITIONS AND FEEDSTOCK COMPOSITION ON THE BULK AND SURFACE PROPERTIES OF BIOCHARS

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Abstract

Eighteen biochar samples were produced from the pyrolysis of pine wood (PW), pine bark (PB), and hybrid poplar wood (HP) at six temperatures (350, 400, 450, 500, 550 and 600°C) in a lab scale spoon reactor. Changes in the bulk composition of the biochar produced were examined
by elemental and proximate analyses. The content of volatiles, oxygen and the ratios of oxygen to carbon (O/C) and hydrogen to carbon (H/C) decreased linearly with pyrolysis temperature suggesting a gradual increase in aromatic structures and thermal recalcitrance. Surface properties of all the biochars produced (SEM morphology, BET CO$_2$ and N$_2$ adsorption, XPS analysis, Boehm titration, cation exchange capacity (CEC) and ζ-potential) were also studied. The removal of volatiles resulted in the gradual creation of microporosity detectable by CO$_2$ adsorption but which was difficult to detect with N$_2$ adsorption, suggesting that the chars contain micropores mostly less than 1 nm in entrance dimension. For all materials studied the volume of micropores increased linearly with the pyrolysis temperature. At 600°C the volume of microporous obtained by CO$_2$ adsorption was 0.201 cm$^3$/g for PW, 0.170 cm$^3$/g for PB, and 0.167 cm$^3$/g for HP. The XPS and Boehm titration confirmed that most oxygenated surface functional groups (presence of carbonyl, carboxyl and hydroxyl groups) are gradually removed as pyrolysis temperature increased. Consequently, biochar surfaces become more hydrophobic and the negative charge induced by the oxygenated functional groups decreases. The changes in surface charge were studied by ζ-potential measurements and were found to vary directly with the content of oxygenated functional groups. Properties that depend on both, surface area and the surface oxygenated functional groups, such as the cation exchange capacity, showed a more complex behavior. The composition of the ash and associated properties such as pH and electric conductivity (EC) were also measured. The concentration of the mineral matter increased with increased pyrolysis temperature leading to greater concentrations of leachable alkalines, increased pH and EC. Pyrolysis temperature had the greatest influence on biochar properties although the biomass source affected some of the measured properties. This work illustrates the relative
importance of feedstock source and pyrolysis temperature on the bulk and surface properties of biochar products.

1. Introduction

Biochar is a carbon-rich, porous material prepared by the thermochemical decomposition of organic materials in an oxygen-limited environment (Crombie et al., 2013; Ronsse et al., 2013). Biochar is receiving growing attention as a soil amendment due to its potential to enhance soil fertility and sequester carbon (Song and Guo, 2012). The use of biochar as a soil amendment dates back to the Amazonian Dark Earths (known as Terra Preta) in the Amazon basin where charred organic materials appear to have been added purposefully to soil to enhance its fertility. Some of these anthropogenically modified soils date back to 7000 years ago and have long-lasting fertility resulting from the presence and stability of biochar (Maia et al., 2011; Lehman et al., 2009).

A wide range of biomass feedstocks are available for use in the manufacturing of biochar including wood materials, agricultural residues, forest residues and wastes from food, sugar, or juice processing (Mckendry, 2002). Other feedstocks are potentially available for biochar production such as aquatic plants, sewage sludge, and animal farm wastes (Verheijen et al., 2010). Woody biomass is the most important source for charcoal making worldwide. Woody biomass contains varying amounts of hemicellulose, cellulose, lignin and slight quantities of other organics extractives (e.g. fats, phytosterols and phenolics) and inorganic compounds (such as nitrogen, phosphorous, sulfur, silicon, alkali and alkaline earth metals, and various trace minerals). The structure of resulting biochar can vary significantly depending on botanical species, plant part, soil type, climate conditions, and the time of harvest of the feedstock used (McKendry, 2002; Collison
et al., 2009; Maia et al., 2011). In order to obtain a good understanding of the function of biochar in soil, it is important to understand the effect of the feedstock on the biochar properties.

Pyrolysis is the major anaerobic thermochemical conversion method used to convert biomass into liquid fuel, gases, and charcoal (Fu et al., 2009a). Slow pyrolysis is the traditional method for charcoal production, while fast pyrolysis is being actively explored for the production of renewable liquid fuels. Char is a co-product of pyrolysis regardless of the heating rate, although the relative yields of char, bio-oil, and non-condensable gases are strongly dependent on heating rate. The reaction mechanisms of biomass pyrolysis are complex due to the large variation of biomass components and thermochemical reactions (Demirbas et al., 2002; Maia et al., 2011). In general, pyrolysis conditions play a vital role in heterogeneity of chemical and physical properties of biochar (Fu et al., 2009a). Therefore, control of this process is one means of maximizing biochar yield and adapting biomass pyrolysis to site-specific applications.

The feedstock properties along with the pyrolysis temperature are considered the main factors affecting biochar characteristics (Zhao et al., 2013). Higher yield of biochar is obtained from feedstocks with high lignin content and high mineral content (Antal and Grønli, 2003; Collison et al., 2009). Likewise, carbon content of biochars can be varied by the raw material and production conditions; at high pyrolysis temperatures, woody and herbaceous biomass usually provides a more carbon-rich biochar compared to other feedstocks such as sewage sludge and animal manures. Several studies (Bruun et al., 2011; Song and Guo, 2012; Spokas et al., 2012; Mašek et al., 2013) have reported that yield, aliphatic carbons, oxygenated functional groups, and content of nitrogen, oxygen and hydrogen in biochar decrease with increasing reaction temperature. According to a recent study published by Zhao et al. (Zhao et al., 2013), the peak temperature has
a strong effect on surface area, pH, volatile matter and recalcitrance of biochar while CEC, ash content, total carbon, fixed carbon, and mineral concentrations were mainly affected by feedstock properties.

There is clearly a wide range of possible feedstocks and pyrolysis conditions that can be used for thermochemical conversion. The function of biochar in soil is also rather complex. Therefore, there is a need to characterize the bulk and surface properties of biochars produced from any particular feedstock under a given set of pyrolysis conditions in detail in order to understand the properties most relevant for its use as a soil amendment (Wang et al., 2009; Zhao et al., 2013). Thus, the objectives of our research are (i) to examine how feedstock source and pyrolysis temperature affect the bulk and surface properties of biochar, and (ii) to better understand the mechanism by which these properties are changed.

2. Materials and Methods

2.1 Biochar preparation

Three different biomass feedstocks; woods of hybrid poplar (*Populus deltoids*) and pine (*Pinus ponderosa*), and bark of pine; were initially air-dried and milled to a particle size of about 590 µm. Feedstocks were selected that: (1) were appropriate for bioenergy production, (2) could be obtained in large quantities, and (3) were native to the Pacific Northwest. The elemental composition of the biomass feedstocks studied is shown in Table 1.
Table 1. Biomass feedstock composition (wt. % dry basis)

<table>
<thead>
<tr>
<th>Feedstock</th>
<th>C</th>
<th>H</th>
<th>N</th>
<th>O*</th>
<th>Ash</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pine Wood</td>
<td>51.3</td>
<td>8.2</td>
<td>0.4</td>
<td>40.00</td>
<td>0.3</td>
</tr>
<tr>
<td>Pine Bark</td>
<td>53.4</td>
<td>7.6</td>
<td>0.8</td>
<td>35.81</td>
<td>2.4</td>
</tr>
<tr>
<td>Poplar wood</td>
<td>50.4</td>
<td>7.8</td>
<td>1.2</td>
<td>39.37</td>
<td>1.0</td>
</tr>
</tbody>
</table>

*by difference

The milled feedstocks were then pyrolyzed at six different temperatures (350, 400, 450, 500, 550 and 600°C) using a lab-scale spoon reactor (figure 3.1) described elsewhere (Wang et al., 2013). The experiments were conducted under an oxygen free atmosphere by purging the reactor with nitrogen. For each run: heating rate and charring time were fixed at 190°C/min for 30 minutes, respectively. In this paper, the biochars are denoted as HP for the hybrid poplar wood feedstock and HP-350, HP-400, HP-450, HP-500, HP-550, and HP-600 for the resulting biochars created at the six temperatures (350, 400, 450, 500, 550, and 600°C). The same abbreviated procedure was applied to both pine wood (PW) and pine bark (PB). The samples were homogeneously subsampled for analyses after the production process. The biochar yield was calculated as a percentage of the feedstock input and biochar output (Maiti et al., 2006; Song and Guo, 2012).

Figure 3.1. A scheme of the lab-scale spoon pyrolysis reactor used in this study.
2.2 Biochar characterization

2.2.1 Biochar Bulk Properties

*Elemental analysis:* Elemental analysis was performed using a TRUSPEC-CHN® (LECO, US) elemental analyzer. Briefly, 0.05 g oven dried samples were used to determine total carbon, nitrogen and hydrogen. Oxygen content was determined by subtracting the ash, C, N, and H contents from the total mass of the sample. These results were used to calculate atomic H/C, O/C, N/C, and O+N/C ratios which are indicative of the bonding arrangement (Brendova et al., 2012) and polarity (Cantrell et al., 2012).

*Proximate analysis:* Fixed carbon, volatiles, and ash content were determined by using a high temperature muffle furnace, Isotemp® (Fishe Scientific, US) and a thermo-gravimetric analyzer (TGA), SDTA851e (Mettler Toledo, US) following the methods described elsewhere [2,23]. Briefly, 1.5 g of oven dried samples were weighed into a pre-weighed crucible and heated in air at 575°C for 12 hrs in order to determine ash content of each sample. Moisture, volatile matter and fixed carbon were determined by TGA using Alumina 70 µL crucibles. Five to 8 mg of each biochar sample was heated from room temperature to 105°C at a rate of 10°C/min and held at 105°C for 15 minutes. Next, the samples were heated from 105 °C to 950 °C at 30°C /min and held for 10 minutes. The thermal gravimetric method was performed under N₂ atmosphere (100 mL/min) and the percentage of fixed carbon (FC) was determined by subtracting ash percentage from volatile matter (VM) percentage, after assigning all weight loss up to 150°C to loss of free and non-structural water.
**Thermal recalcitrance analysis:** The thermal recalcitrance of the biochar produced was studied by the thermogravimetric method described by Harvey et al. (Harvey et al., 2012). Mass loss characteristics associated with the thermal oxidation of biochars were determined using a thermo-gravimetric analyzer (TGA), SDTA851e (Mettler Toledo, US). In air atmosphere (air flow rate of 100mL/min), samples between 5 and 8 mg were heated from 25°C to 105°C at 10°C/min and held at this temperature for 15 minutes, and then from 105 °C to 950 °C at 30°C/min and held for 10 minutes. The thermal recalcitrance index (R50) of produced biochar were estimated using equation (2) and equation (3), respectively.

\[
R_{50, \text{biochar}} = \frac{T_{50, \text{biochar}}}{T_{50, \text{graphite}}} \quad \text{..........(1)}
\]

Where \(T_{50, \text{biochar}}\) and \(T_{50, \text{graphite}}\) were the temperature values corresponding to 50% weight loss by oxidation/volatilization of biochar and graphite, respectively. Values for \(T_{50, \text{biochar}}\) and \(T_{50, \text{graphite}}\) were obtained from TG thermo-grams that have been corrected for water and ash content as follows:

\[
W_{i,c} = 100 + \left[ 100 \times \frac{(W_{i,un} - W_{150,un})}{(W_{150,un} - W_{\text{cutoff,un}})} \right] \quad \text{..........(2)}
\]

Where \(W_{i,c}\) and \(W_{i,un}\) were the corrected and uncorrected weights, respectively. \(W_{150,un}\) was the weight at 150°C, and \(W_{\text{cutoff,un}}\) was the weight at the temperature when no further oxidation was apparent.

**2.2.2 Surface Properties**

**Topographic and Microstructural analysis:** The topographic analysis of biochar surface was carried out by scanning electron microscopy (SEM) using a Hitachi S-570 variable pressure instrument (Hitachi, Japan). Biochar samples were mounted on a stub and gold coated prior to
viewing. Magnification ranged between 500x and 1000x. Specimens for the transmission electron microscope (TEM) study were prepared by powdering the biochar samples gently using a Planetary Ball Mill. A DI water-biochar suspensions were then prepared and depositing onto nickel coated grids. Imaging was carried out at 200kV in Philips CM-20 TEM (Eindhoven, Netherlands). Care was taken to minimize exposure to a focused electron beam in order to avoid specimen damage.

**Gas Physisorption Analysis:** Nitrogen (N\(_2\)) and Carbon Dioxide (CO\(_2\)) adsorption isotherms were measured at −196.15°C and 0°C, respectively, on a Micromeritics TriStar II 3030 PLUS Surface Area and Porosity Analyzer (Norcross, GA, USA). Prior to each analysis, samples were degassed at 200°C for 18 hrs under vacuum of about 5-0.1 mbar (the degassing temperatures were chosen on the basis of TGA measurements to avoid sample degradation during preparation). N\(_2\) adsorption isotherms were measured in the partial pressure range P/P\(^0\) = 10\(^{-4}\) – 0.99 using a total of approximately 50 data points, and CO\(_2\) adsorption isotherms were measured in the partial pressure range P/P\(^0\) = 10\(^{-5}\) – 0.03 using approximately 75 data points. The data sampling was programmed to obtain a larger number of data points in the low pressure region to obtain a more accurate description of the microporosity. Where applicable, the apparent BET surface area was determined from the N\(_2\) adsorption data. The micropore volumes were estimated from both N\(_2\) and CO\(_2\) adsorption using the the Dubinin–Radushkevich (DR) equation. Density functional theory (DFT) calculations were carried out from CO\(_2\) adsorption isotherms using commercial software (MicroActive\(^{\text{TM}}\) v. 1.01, Micromeritics) to calculate the micropore size distribution assuming a slit pore shape. DFT also provided an independent assessment of the volume of micropores with entrance dimension < 1 nm.
**X-ray photoelectron spectroscopy (XPS) analysis:** The XPS measurements were obtained with an Axis-165 (Kratos Analytical Inc. Manchester, UK) using an Achromatic X-ray radiation of 1253.6 eV as the XPS excitation source for acquiring all photoelectron spectra. The curve fitting of the C1s and O1s data was performed with XPSPEAK software version 4.1 (programed by R. Kwok, Chinese University of Hong Kong) with Shirley background subtraction for all components of the same peak. The deconvolution of XPS spectra was set to the same Gaussian-Lorentzian line shape (GL≈30%) and full width half maximum (FWHM≈2 eV). The C1s bending energy for C-C, C=C, and C-H was assigned at 285 eV whereas C-O, C=O, and COO− were at 286.5 eV, 287.8 eV and 289.5 eV, respectively (Cheng et al., 2006, 2008; Park and Kim, 2010).

**Boehm Titration:** The acidic groups covering the biochar surface were quantified by using the general Boehm’s method as described by Goertzen et al. (Goertzen et al., 2010). Briefly, 150 mg of oven dried biochar samples were placed in 50 mL of each of three 0.05M reaction bases: NaOH, Na2CO3, and NaHCO3. The mixtures, along with a control solution, were agitated on an orbital shaker for 24 hours and then filtered through Whatman filter paper (No. 42). After filtration, 5 mL of aliquots from NaOH, Na2CO3 and NaHCO3 filtrates were acidified by addition of 10 mL, 15mL and 10mL, respectively, of 0.05 M HCl to ensure complete neutralization of bases. The solutions were then back titrated with 0.05 NaOH to a pH of 4 in order to avoid any buffering issues related to the atmospheric CO2. The numbers of acidic groups were calculated under the assumption that NaOH neutralizes carboxylic, lactonic, and phenolic groups; Na2CO3 neutralizes carboxylic and lactonic groups; and NaHCO3 neutralizes only carboxylic groups. The difference between molar NaHCO3 and Na2CO3 was considered as the lactonic group content while the difference between molar NaOH and Na2CO3 was assumed to be the phenolic functional group content (Bandosz, 2002; Mukherjee et al., 2011).
**Cation Exchange Capacity (CEC):** The CEC of the samples were determined according to the method of passive barium exchanged with forced magnesium exchange (Lee et al., 2010). Firstly, 0.4 g of biochars were washed with 20 ml of 0.1M HCl for 1 hour, and then washed with e-pure water three times. 30 ml of 0.1M of BaCl$_2$ was added and agitated for half an hour. The samples were then filtrated and mixed with 0.025 M of BaCl$_2$; pH of the mixtures was adjusted at 5.3 using Ba(OH)$_2$ or HCl. The mixtures were agitated for 24 hours. After that, the BaCl$_2$ was washed out of the samples with e-pure water and then dried at 105°C. In triplicates, 100 mg of dried samples were re-suspended in 10 ml of 0.01 M MgSO$_4$ solution. After one hour of agitation, samples were filtered and the filtrates were analyzed by Atomic Adsorption, Spectra-AA220 (Varian Analytical Instruments Inc., US).

**Zeta potential (ζ):** The zeta potential of biochar was determined using the method described by Julien et al.(Julien et al., 1998). Briefly, biochar particles were firstly ball milled in a PQ-N2 planetary ball mill (Across International LLC, NJ, USA) for 24 h to pass through a 200 mesh (opening: 74 mm). 5 mg of each biochar sample was added to 50 ml deionized water and agitated on an orbital shaker for 24 h at 25°C. The suspension pH was adjusted by addition of 0.1 mol l$^{-1}$ HCl and 0.1 mol l$^{-1}$ NaOH. The ζ of biochar suspensions was measured using a Nano-Zetasizer 3000 (Malvern Instruments Ltd., Malvern, UK). Measurements were done by maintaining constant tension (100V), recommended for suspensions having EC lower than 1 mS cm$^{-1}$. The point of zero charge (pH$_{pzc}$) was determined by multiple measurements of ζ as a function of equilibrium pH, adjusted by 0.1 mol l$^{-1}$ HCl and 0.1 mol l$^{-1}$ NaOH.
2.2.3 Properties related with the mineral matter

*Mineral and metal analyzes:* Inductively coupled plasma atomic emission spectroscopy (ICP-AES), 4500 series (Agilent Technologies, USA) was used to determine the content of Na, K, Mg, Ca, Fe, Al, Mn, Cu, Ba, Pb, and Zn in biochar samples. Briefly, 30-50 mg of the material was weighed into 50 ml glass tubes and dissolved in a wet acid digestion (conc. HNO$_3$ + 30% H$_2$O$_2$) using Microwave digestion via a Discover SP-D microwave (CEM, NC, US). The acid solution recovered after filtration was analyzed by ICP-AES.

*pH and electrical conductivity (EC):* Biochar pH and electrical conductivity were determined by methods described elsewhere (Ahmedna et al., 1997; Ekpete et al., 2011; Wang et al., 2012). Briefly, 1% (w/v) suspensions of biochar were prepared with deionized water, and agitated for 24 hrs. The biochar suspensions were then measured for both pH and EC using Hanna HI 9813-6 pH/EC/TDS meters (HANNA instruments Inc., US). The pH reading was considered stable when it did not change more than 0.1 units per 30 second. The analyses of pH and EC were performed in duplicate.

2.3 Data analysis

The degree of linear association between two measured variables was examined using the Pearson product-moment correlation coefficient (PMCC), stated here as correlation coefficients (r). Labeling systems exist to approximately categorize r-values as absolute values by considering $r \leq 0.35$ to represent weak correlation, 0.36–0.67 moderate correlation, and 0.68–1.00 strong correlations with $\geq 0.9$ to be a very high correlation (Taylor, 1990). Moreover, two categories, $RV_F$
(feedstock-depended relative variability) and $RV_T$ (temperature-depended relative variability) were introduced to quantify the biochar-feedstock-pyrolytic temperature relationships based on statistical analysis of Relative Variability (RV) (known as Relative Standard Deviation-RSD); the higher RV values the more influence on biochar properties. Relative variability (%) equals the standard deviation divided by the mean, and multiplied by 100. We also analyzed the statistical differences between mean values using the General Linear Model. This analysis was performed using Minitab version 17 and differences were considered significant at $P < 0.05$.

3. Results and Discussion

3.1 Biochar yield

The yields of biochar obtained as a function of pyrolysis temperature for the three feedstocks studied (pine wood, pine bark, and poplar wood) are shown in Figure 3.2. The biochar yield sharply decreased as pyrolysis temperature increased from $350^\circ$C to $500^\circ$C, corresponding to the temperature range at which most of the thermal decomposition of lignocellulosic materials occur (Paris et al., 2005). Between $500^\circ$C and $600^\circ$C, the biochar yield did not change much indicating that most of the volatile fraction had been removed at lower temperatures (Novak et al., 2009). The yields of biochars were also dependent on the original feedstock source. Pine bark (PB) resulted in the largest biochar yield. This may be the result of this feedstocks high ash and lignin content, which is known to contribute to char formation (Song and Guo, 2012). The differences in biochar yields between pine (PW) and poplar (HP) woods were relatively small and most likely due to the differences in their lignin content (Paris et al., 2005). Biochar yields were generally
more sensitive to feedstock source as indicated by its higher $RV_F$ (1.6) compared to $RV_T$ (1.25). Our findings are generally consistent with other reports in the literature for other feedstocks (Kim et al., 2012; Al-Wabel et al., 2013; Crombie et al., 2013).

**Figure 3.2.** Biochar yields as a function of temperature for Pine wood (PW), Pine bark (PB), and Hybrid poplar wood (HP).

### 3.2 Biochar Bulk Properties

*Elemental analysis:* The elemental composition of the biochars produced at different pyrolysis temperatures is shown in Figure 3.3. By increasing pyrolysis temperature from 350 to 600°C, the C content was increased from 70.5 wt. % in pine wood chars (PW) to 87.8 wt. %, from
69.9 wt. % in poplar wood chars (HP) to 83.1 wt. %, and from 66.1 wt. % in pine bark chars (PB) to 78.1 wt. % (Figure 3.3a). In contrast, the O and H contents decreased as pyrolysis temperature increased (Figures 3.3b and c). The losses of O and H are attributed to the cleavage and cracking of weak oxygenated bonds within the structure of the solid pyrolysed (Demirbas, 2004). These results are similar to those reported elsewhere (Brendova et al., 2012; Enders et al., 2012; Ronsse et al., 2013). Among the feedstocks, pine wood biochars (PW) contained the highest concentrations of C (fig. 2a), especially at elevated temperatures. There were convergences of H (fig. 2b) for all biomass types, likely due to the similarity in rate of dehydration reactions typically at low temperature (250-350 °C) and release of hydrogen-rich gases like CH$_4$ and H$_2$, especially at above 400°C (Wang et al., 2009). Total N contents varied based upon original biomass; the highest conservation of N was observed in biochars made from pine bark while the lowest in pine wood biochars, PB>HP>PW. The content of N can be attributed to the formation of heterocyclic N such as pyridines and pyrrols (Enders et al., 2012). The feedstock sources seemed to have a slight influence on elemental composition, with small and inconsistent differences between samples produced under the present conditions of pyrolysis. The C, H, and O concentrations of biochars were mainly influenced by temperature (i.e., RV$_T$ of C, H, and O are 0.84, 1.27, and 1.01, respectively) and to a lesser extent by feedstock source (i.e., RV$_F$ of C, H, and O are 0.69, 1.11, and 0.86, respectively).
**Figure 3.3.** Total C, O, H, and N of PW, PB, and HP biochars generated through pyrolysis at six different temperatures (350, 400, 450, 500, 550, 600°C).

*Aromaticity and Maturation of biochar:* Figure 3.4 shows the change in the O/C and H/C ratio as a function of pyrolysis temperature. The atomic ratios of H/C and O/C are typically correlated with the degree of aromaticity, polarity, and maturation of biochar [5, 21]. As can be seen in Figure 3.4, the O/C ratio of all biochars significantly decreased as the temperature increased demonstrating the loss of oxygenated compounds through the carbon-concentrated process as previously observed by Wang et al. (Wang et al., 2009). Slight reductions in H/C were observed.
in this range indicating that most of H loss occurred at temperatures below 350°C. In general, the high temperature biochars had low H/C and O/C ratios compared to biochars produced at low temperatures; this is an indication of a gradual increase in aromaticity (Wiedner et al., 2013), low polarity (Wang et al., 2009), more graphite-like structure (Enders et al., 2012), and low aliphatic content (Kim et al., 2012). The H/C and O/C ratios are in all cases lower than those recommended by the International Biochar Initiative (IBI) and European Biochar Certificate (EBC) for biochar materials (H/C and O/C must be <0.6 and 0.4, respectively).

**Figure 3.4.** Total C, O, H, and atomic ratios of PW, PB, and HP biochars generated through pyrolysis at six different temperature (350, 400, 450, 500, 550 and 600°C).
In figure 3.5, the H/C and O/C ratios are plotted against each other in a Van Krevelen diagram. This diagram confirms the importance of dehydration (loss of O and H as H$_2$O) and carbonization reactions during pyrolysis. The rates of decarboxylation (loss of CO$_2$ and/or CO, indicated by a decreasing O/C ratio) and de-methylation (loss of CH$_3$, indicated by a decreasing H/C ratio) are dependent upon both the feedstock source and the pyrolysis temperature. The de-methylation seems to be more important for pine bark than for pine and poplar woods. The influence of both pyrolysis temperature and feedstock type on decarboxylation and de-methylation followed the order HP>PW>PB and PB>PW>HP, respectively. The diagram also shows that increases in both processes (de-methylation and decarboxylation) were attributable to thermal transformations of feedstock materials to form increasingly aromatized structures.

![Figure 3.5. Van Krevelen diagram showing the relationship between the O:C & H:C atomic ratios.](image)

*Each arrow represents the direction of loss of a particular gas molecule in the course of evolution.*
**Proximate analysis:** Figure 3.6 presents the results of our proximate analyses (ash content, volatile matter (VM) and fixed carbon (FC) as well as the FC/VM ratio as a function of pyrolysis temperature. In all cases, the ash content, fixed carbon and FC/VM ratio increased with increasing temperature, while the volatile matter decreased considerably with temperature. Ash content was more sensitive to feedstock ($RV_T = 1.21$) than to temperature ($RV_T = 1.03$) while FC and VM were moderately influenced by feedstock as indicated by $RV_F$ (0.89 and 0.69, respectively) compared to $RV_T$ (0.86 and 0.67, respectively).

**Figure 3.6.** Influence of pyrolysis temperature and feedstock source of prepared biochars on fixed carbon, volatile matter, ash content, and FC/VM ratio.
The ash content (Figure 3.7a) varied most among feedstock materials (2.9-7.7 wt. %) and less due to differences in pyrolysis temperatures. Irrespective of production temperature, the lowest proportions of ash were found in the pine wood biochars (0.60 wt. %) and the greatest proportions in pine bark and poplar wood chars (8.85 and 7.17 wt. %, respectively). The ash content increases with the increase in pyrolysis temperature. This is due to the accumulation of mineral elements during the decomposition of organic constituents (Enders et al., 2012). The contents of volatile matter, fixed carbon and ash are dependent upon the feedstock used and the pyrolysis temperature.

The volatiles content per unit of ash free mass (fig. 5b) decreased from 49.82 to 15.72 wt. %, 42.15 to 17.81 wt. %, and 43.78 to 17.25 wt. %, for PW, HP, and PB, respectively, as temperature increased from 350 to 600°C. For the full set of biochars, low variations in volatile matter were observed at high temperature (e.g., 15.72-17.25 wt. % at 600°C) and were a result of complete decomposition of cellulosic components due to the relatively longer period of de-volatilization associated with elevated temperatures (Antal and Grønli, 2003). Pine wood biochar (PW) was the material with the highest quantity of volatile matter (49.82 wt. %) when produced at 350°C and the lowest volatiles content when pyrolyzed at 600°C. The amount of fixed carbon (i.e., degree of carbonization) was relatively sensitive to both pyrolysis temperature and feedstock type; it increased by 33.57 for PW, 20.67 for HP, and 22.39% for PB with increasing the temperature from 350 to 600°C (Figure 3.7a). Among 18-biochar samples, pine wood biochar (PW) produced at 600°C contained the highest fixed C contents (83.15 wt. %). The content of fixed C depends on the severity of pyrolysis and partially by the composition of feedstock. Enders et al.(Enders et al., 2012), for example, considered the ash content as a potential reason for the notable increase in fixed C content; they established that the quantity of fixed C increases consistently with temperature due to the interactions between organic and inorganic feedstock constituents during
pyrolysis. Our findings corresponded fairly well with this observation due to the low contents of ash (<9 wt.%) comparable to those studied by Enders and his colleagues. However, our results are in good accordance with the results of Ronsse et al., (Ronsse et al., 2013) who reported that the increase in the fixed C content is due to the reduction in the overall biochar mass rather than additional ‘carbon-fixing’ reactions.

Figure 3.7 shows a linear correlation between atomic ratios (O:C and H:C) and content of volatile matter and fixed carbon. It indicates that volatile matter released during pyrolysis is mainly composed of rich oxygen and hydrogen-compounds than the remaining solid matter. It also shows that fixed carbon, as a material, has very low oxygen and hydrogen contents (Ronsse et al., 2013).

![Figure 3.7. Correlations between proximate and elemental analysis data; (a) atomic ratios [O:C & H:C] vs. volatile matter, and (b) atomic ratios [O:C & H:C] vs. fixed carbon. (Arrows show the direction of temperature increase).](image)
**Thermal recalcitrance of biochars:** An understanding of the influence of feedstock and pyrolysis temperatures affects on the ability of biochar to resist abiotic and biotic degradation (herein referred to as recalcitrance) is vital to understanding the potential use of these materials to sequester carbon in soil (Crombie et al., 2013; Zhao et al., 2013). Harvey et al. (Harvey et al., 2012) have developed a new recalcitrance index (referred as the $R_{50}$) to evaluate the recalcitrance of biochars based on the energy required to oxidize a unit mass of biochar-C to CO$_2$. As can be seen in figure 3.8, biochars exposed to higher pyrolysis temperature contain a higher fraction of recalcitrant C (as determined by $R_{50}$) comparable to those produced at low temperatures. This phenomenon occurs because of the formation of polyaromatic structures as the production temperature increases. As the temperature increases the H and O is released from the biochar matrix, making it more aromatic, and consequently more recalcitrant.

Our results revealed that the recalcitrance of biochar is not only a temperature-governed property ($RV_T=0.9$) but also depends on the composition of the feedstock biomass ($RV_F=1.1$). Differences in thermal recalcitrance were clearly observed in biochars produced from different feedstocks at the same temperature. Biochars made from pine wood showed higher $R_{50}$ values compared to biochars produced from poplar wood and pine bark ($R_{50} = 0.73, 0.61$, and $0.66$, respectively). This is due to the higher quantity of fixed carbon combined with low ash content in pine wood (Enders et al., 2012). It is important to mention that additional factors such as particle size, surface area, volatiles content, H/C ratio, O/C ratio, and atomic arrangement could also influence $R_{50}$ values, a parameter of thermal oxidation (Harvey et al., 2012).
Figure 3.8. Thermal recalcitrance of resultant biochar as a function of temperature and feedstock sources.

3.3 Surface Properties

Surface morphology (SEM analysis): Figure 3.9 shows SEM micrographs (200-1600X) of PW and PB biochar particles. Sponge- and honeycomb-like porous structures were observed in all biochar particles. These structures consists mainly of heterogeneous veins, lateral pits, and helical fibrils originated from the tissue structure in the precursor plants (KRZESINSKA et al., 2006; Ma et al., 2013). The observed pores in these carbonaceous structures are generally large macro-pores, which serve as a passage for the adsorbates to the micro-porous system located on the cell walls (Lin et al., 2014).
Figure 3.9. SEM images of PW (a), PB (b) and HP (c) biochars (magnification 800X, 200X and 300X, respectively).
Micro-nanostructure: The nano-structure of cell walls of PW chars produced at 350 and 600 °C was visualized by TEM technology (see Figure 3.10). Although the 350°C biochar resembles an amorphous carbon phase, as other organic materials (Müller et al., 2007), the carbon atoms were arranged irregularly in worm-like structures when pine wood feedstock was pyrolyzed at 600°C. In this TEM structure, we can clearly see the disordered small graphene structures on the biochar cell walls. The TEM image of 600°C PW biochar (Fig. 9b) was characterized by roughly irregular fringes from 0.2 to 1.4nm in length. These fringes occurred in packets of 2-4 layers; many of them are curved. The shapes and length of the observed fringes makes such an interpretation uncertain (Kovalevski et al., 2001). Regardless the production temperature, both biochars exhibit smooth-like surfaces with irregularities.

Figure 3.10. TEM images of (a) 350°C PW biochar (mag. 310kx), (b) 600°C PW biochar (mag. 310kx). Image (b) shows the ‘worm-type’ architecture.
**Gas physisorption analysis:** Figure 3.11 shows the CO₂ adsorption isotherms for the biochars produced at different temperatures. As the pyrolysis temperature increased, the amount of CO₂ adsorbed at \( P/P^0 = 0.03 \) also increased, indicating an increase in the volume of narrow micropores. Most of the char samples were difficult to characterize with N₂ adsorption. It was observed that equilibration took a relatively long time so that properly equilibrated N₂ adsorption isotherms could not be collected before the liquid N₂ used to control the analysis temperature would evaporate from the analysis Dewar flask (> ~35 hours would be required for N₂ adsorption isotherm acquisition). This finding indicates that most of the micropores present in char samples were smaller than approximately 1 nm in entrance dimension or that there were constrictions present at the entrances to the microporosity. Only chars from pine wood and bark prepared at 550°C and 600°C could be characterized with N₂ adsorption using our adsorption system. These N₂ isotherms were type I isotherms (according to IUPAC isotherm classification), indicating a completely microporous material with little mesoporosity. The differences between N₂ and CO₂ adsorption behavior between the different feedstocks carbonized at higher temperatures demonstrate that PW contains a greater fraction of larger micropores compared to PB, and HP chars contained little or no microporosity with entrance dimension greater than 1 nm in the range of pyrolysis temperatures studied.
Figure 3.11. CO$_2$ adsorption isotherms (1-3) and pore size distributions (2-6) at 0°C for PW (1&4), HP (2&5), and PB (3&6) biochars produced at different pyrolysis temperatures.
The surface areas and pore volumes of biochars produced at various pyrolysis temperatures obtained with CO$_2$ and N$_2$ adsorption are shown in Figure 3.12. As the pyrolysis temperature increases, a gradual increase in the surface area-CO$_2$ (SA$_{CO2}$) was observed due to the formation of very small micropores on the biochar surface. SA$_{CO2}$ increased from 145.87 to 500.4 m$^2$/g in PW, 208.22 to 416.91 m$^2$/g in HP, and from 171.47 to 423.65 m$^2$/g in PB as temperature was elevated from 350 to 600°C. In the same way, micropore volume increased from 0.06, 0.08, and 0.07 cm$^3$/g at 350°C to 0.2, 0.17, and 0.17 cm$^3$/g at 600°C, for PW, HP, and PB, respectively.

We couldn’t obtain useful data for N$_2$ adsorption for samples produced below 550°C most likely due to that the pore sizes of the biochars were too small for the N$_2$ to penetrate and get adsorbed. Biochar produced from pine wood and pine bark at 600°C showed adsorption of N$_2$ (522.17, and 222.36 m$^2$/g, respectively), which is a clear indication that the pore size of these materials has increased as the pyrolysis temperature increased. This evolution of BET surface area is somehow similar to that reported in the literature (Fu et al., 2009b; Lee et al., 2010; Kloss et al., 2012).
**Figure 3.12.** Surface area and pore size volume determined by CO\(_2\) (1&3) and N\(_2\) (2&4) adsorptions using Dubinin-Radushkevich method.

**XPS analysis:** The XPS analysis indicates that the surface composition of biochar changed substantially as a result of charring temperature and feedstock source (Figure 3.13). As in the bulk of the biochar, the content of oxygen on the surface of the biochar also decreases as the pyrolysis temperature increases. Figure (3.13d) shows the high-resolution XPS spectra of C1s excitation that
represented the content of graphite carbon (area of peak A-assigned on 285), and oxygenate carbon (sum of peaks B+C) on the biochar surface. In figures 3.13 a, b, and c, we can clearly observe a linear increase in the content of C atoms without O from 56.00 to 71.50%, 51.85 to 89.41%, and 63.03 to 81.25% for PW, PB, and HP, respectively. The content of oxygenated carbon decreases from 43.94 to 26.50%, 48.15 to 10.59%, and 36.97 to 18.75% for PW, PB, and HP, respectively, as the carbonization temperature increased from 350 to 600°C. These results are due to the removal of oxygenated functional groups (lactonic, phenolic and carboxylic) from the surface of the biochar produced. Our data also showed that higher C content was observed on PB biochar surfaces (89.41%) compared to PW and HP biochar surfaces (71.50 and 81.25%, respectively) at 600°C, similarly for oxygenate C content at 350°C. It is worth mentioning that the sum of all oxygen-containing functional groups of PW biochar produced at 600°C was two times higher than that of PB and HP produced at same temperature demonstrating less loss of oxygenated compounds in PW through the carbon-concentrated process.
**Figure 3.13.** Influence of Temperature and feedstock on the relative amount of carbon atoms (area of peak A -assigned on 284.5) and oxygenate carbon atoms of peaks (sum of B, C etc.) of PW (a), HP (b), and PB (c) biochars.
**Boehm Titration:** Figure 3.14 shows the content of oxygenated functional groups (phenolic, lactone and carboxylic) on the surface of the biochars produced as a function of pyrolysis temperature as determined by Boehm titration. In all the cases, we observe a linear decrease in the content of these oxygenated functional groups as a function of pyrolysis temperature. This result is similar to those reported in the literature [34, 57] and is in agreement with our XPS results (Figure 3.13). The reverse trend was found for the total basic surface functional groups of biochars (Figure 3.14 d), the high-temperature biochars had higher total basic groups than their low-temperature counterparts. The basic functional groups are mainly associated with the ash fraction (figure 3.6). The low-ash biochars made from pine wood contained fewer basic functional groups that increased with a concomitant increase in ash content. The highest content of basic functional groups was found in the hybrid poplar wood biochars.
Figure 3.14. Variation in surface acidic and total basic functional groups as a function of pyrolysis temperature.

*Surface Charge:* Figure 3.15 shows the measured zeta potential as a function of pH for three biochars produced from pine wood, pine bark, and hybrid poplar wood at three different pyrolysis temperatures (350, 450, 600°C). As expected, all the ζ-potentials monotonically decrease as the pH of the environment increases. The ζ-potential for almost all the biochars produced increased with increasing pyrolysis temperature. This result is mainly due to the removal of oxygenated functional groups from the surface of the biochar which are responsible for the formation of
negatively charged surfaces. The pine wood feedstock source produced the biochar with the highest ζ-potentials. This is due to abundant acid surface functional groups combined with high surface area which provides more surface charges (Mukherjee et al., 2011). Our ζ-potential results agree with those obtained by Boehm titration and XPS analysis.

Figure 3.15. Variation in Zeta potential (ζ) as a function of pH for PW (a), HP(b), and PB(c) biochars generated at 350, 450, and 600°C. Values are averages over the replicates of the treatments (n = 3), and straight lines are drawn to facilitate interpretation of the graph.
The study of the influence of pyrolysis temperature on the zero charge point (pH\text{pzc}), at which the biochar becomes uncharged, shows that as the temperature increases the zero potential can be achieved at higher pHs, figure 3.16. In reference to PW-350 (pine wood char produced at 350°C) with pH\text{pzc} of 3, pine wood biochar produced at 600°C showed higher pH\text{pzc} by about 2.8 pH units. However, pine bark biochar produced at 350°C had lower pH\text{pzc} than that of PW-350 by about 1.8 pH units. The Variation of the zero potential based on feedstock source and/or pyrolysis temperature is a function of acidity, in particular the carboxyl acidity. These results are generally in agreement with the results reported by Julien et al. (Julien et al., 1998) and Qiu et al. (Qiu and Ling, 2006) and with our XPS and Boehm titration results.

**Figure 3.16.** pH at point of zero charge as a function of pyrolysis temperature and feedstock source of PW, PB, and HP biochars generated at 350, 450, and 600°C.

*Cation Exchange Capacity (CEC):* Figure 3.17 shows the changes in the cation exchange capacity of the resultant biochars. The cation exchange capacity is the total capacity of a biochar to adsorb and exchange positively charged species (Carrier et al., 2012). The CEC is a function of
the presence of oxygenated functional groups (Carboxylic, phenolic, and lactonic) in the biochar and the surface area of the material. Our findings indicated that the CEC did not change much with the temperature for the biochars derived from pine wood (PW) but decreased slightly for the biochars derived from pine bark (PB) and hybrid poplar (HP). This slight decrease could be in part due to the reduction of the content of oxygenated functional groups on the biochar surface (Figure 3.13). The general decrease in CEC of pine bark and hybrid poplar biochars produced at 600°C compared to 350°C is consistent with the data published by Mukherjee et al. (2011)(Mukherjee et al., 2011). In terms of feedstock source, the CEC of PW biochars was significantly higher than the CECs of PB and HP biochars. The relatively high CEC of PW biochars, compared to PB and HP biochars, could be attributed to the combination of carboxylic functional groups, which contribute most of the CEC among the acidic functional groups, and the BET surface area (Singh, 2010; Carrier et al., 2012).

![Figure 3.17. Influence of pyrolysis temperature on CEC values of biochars produced from Pine wood (PW), Pine bark (PB), and Hybrid poplar wood (HP).]
3.4 Properties Related with the Mineral Composition

The composition of mineral matter in three biomass feedstocks was investigated as a function of temperature using an inductively coupled plasma atomic emission spectroscopy (ICP-AES). Figure 3.18 shows the content of alkali (K, Na) and alkaline earth metals (Mg, Ca) for all the biochars produced. The content of these metals correlates very well with the content of ash and the basic functional groups on the biochar surface. The mineral content was the lowest in the pine wood biochar, which had the lowest content of ash. While the K was much higher in the hybrid poplar wood biochar (HP) than pine bark and wood biochars, the Ca was the highest in the pine bark biochar. This could be linked to the variation in nutrient content of soils where these feedstocks were grown. Na was the lowest detectable cation in all biochars produced. These results are consistent with the observation made by Singh (2010) that exchangeable (and soluble) Na is low in woody biomass.
Figure 3.18 Mineral content of PW, HP, and PB biochars made at six pyrolysis temperatures.
**pH and EC:** Figure 3.19 shows the changes in the pH and electric conductivity (EC) of the water in contact with the biochars produced. The pH of all biochars in water was alkaline (ranging from 7.9 to 10.4), increased with the pyrolysis temperature, and varied significantly among feedstocks. Pine wood biochar had lower pH values (from 8.3 to 8.8) comparable to pine bark and hybrid poplar wood biochars. This result could be explained by the low surface alkalinity and low ash content of pine wood biochars. The electrical conductivity (EC), total water-soluble ions in the biochars, increased linearly with the pyrolysis temperature and varied very significantly among feedstocks. The most obvious interpretation is that EC increases were due to a progressive loss of acidic functional groups and the gradual increase in ash as the pyrolysis temperature increases. Among feedstocks, the highest conductivities were obtained from the hybrid poplar wood biochar which is the material with the highest content of leachable alkalines and lowest surface acidity. Our results are in agreement with those reported elsewhere (Mukherjee et al., 2011; Carrier et al., 2012; Enders et al., 2012; Kloss et al., 2012).

![Figure 3.19. pH (a) and EC (b) values of resultant biochars.](image)
4. Conclusion

In this work, we investigated how the physico-chemical properties of biochars are affected by the feedstocks (pine wood, pine bark, and poplar wood) and pyrolysis temperatures in the range between 350 to 600°C. The results clearly showed that certain properties, e.g. BET surface area, carbon stability, ζ-potential, and the content of oxygen-containing functional groups, were mainly controlled by the pyrolysis temperature. Unambiguously, the surface area and carbon stability were enhanced at high pyrolysis temperature (<500°C). The ζ - potential is well-correlated with the presence of O functional groups on the biochar surface, so any applications of biochar requiring ζ-potential and/or O functional groups would call for greater attention to the pyrolysis temperature. Furthermore, feedstock source had a notable impact on other properties, e.g., ash content, CEC, and mineral concentrations. The CEC, for example, is affected by both the surface O content, and the surface area. Our results reveals that CEC is mostly affected by the choice of feedstock, possibly due to ash content, pH, micropore structure, and/or mineral composition, in addition to surface oxygen content. Overall, our study indicates that by appropriate combination of pyrolysis temperature and feedstock source it is possible to repeatedly produce a biochar with a wide range of physiochemical properties.

5. Acknowledgement

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6. References


CHAPTER 4. MODIFICATION OF BIOCHAR SURFACE

BY AIR OXIDATION: ROLE OF PYROLYSIS TEMPERATURE

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Abstract

This paper reports results on the effect of pyrolysis temperature in the range between 350 and 600 °C on the oxidability of biochars derived from hybrid Poplar, Pine Wood and Pine Bark. The oxidation step for all the biochar produced was conducted at 250°C in the presence of air in a spoon reactor. The elemental and proximate analyses of all the oxidized and un-oxidized chars suggest that the carbonaceous materials produced at low temperature are more susceptible to oxidation than those obtained at high temperature. A number of surface properties of resultant biochars were
examined to better understand how pyrolysis temperatures and feedstock sources relate to the development of surface characteristics. The removal of volatiles during the pyrolysis step resulted in the gradual creation of microporosity detectable by CO\textsubscript{2} adsorption but which was difficult to detect with N\textsubscript{2} adsorption, suggesting that the chars contain micropores mostly less than 1 nm in entrance dimension. In some cases the surface area decreased after being oxidized likely due to the blockage of micropores by oxygen-containing functional groups. The surface composition determined by XPS and Boehm titration confirms that the formation of carbonyl and carboxyl groups is easier for biochars produced at low temperature. The formation of these oxygenated functional groups contributes to add negative charges on the surface and consequently the pH at the point of zero charge is always higher for un-oxidized biochars.

1. Introduction

The global warming caused by the release of greenhouse gases is a source of serious political concern all over the world. On this context, biomass conversion technologies are being studied for their potential to mitigate global warming. Biomass is our only renewable source for carbon-based fuels and chemicals. Although the growth of terrestrial vegetation removes 120 billion tons y\textsuperscript{-1} of carbon from the atmosphere (far more than the 8.5 b tons released by the combustion of fossil fuels), most of this carbon is released back by the action of microorganisms through processes of decomposition of soil organic matter. Given its high resistance to microbial attack (Granatstein et al., 2009); biochar offers the potential to stabilize some of the carbon fixed by terrestrial vegetation. Biochar production is being considered as one of the most promising strategies for large scale, low cost carbon sequestration (Lehmann et al., 2006; Lee et al., 2010).
Without additional treatment, the price obtained from crude biochar is likely to be determined by its calorific value (approximately 66 $ per ton), which is still higher than even the most aggressive carbon tax plans recently proposed (Bauman, 2010). *If the U.S. develops a mandatory carbon cap and associated markets, if biochar is approved for carbon storage payments, and if biochars are engineered to improve the fertility of our soils, then the economics of this technology could dramatically improve.* The cost of mono-ammonium phosphate fertilizers to farms is currently over $ 900 per ton. By using biochars to adsorb nitrogen and phosphorous from waste water streams, and by increasing its cation/anion exchange capacity it is possible to add extra value to biochar and thus obtain higher selling prices. Because biochars are significantly more stable than the fast and slow-cycling fractions of soil organic matter, the effects of biochar additions to soil could have significant large long-term benefits on soil C sequestration and fertility. Any value added chemical processing treatment performed on the char to improve its nutrient adsorption capacity is going to increase production costs. However, the goal of further processing treatment is to increase the economic value by creating a greater margin of return on the cost of production.

While biochar has significant potential as a soil amendment, studies with freshly produced biochars have not been able to reproduce the effectiveness of the centuries old Terra Preta soils of the Amazonian Basin. One possible reason is that when the char is left in the soil for long periods of time the surface is slowly oxidized (Liang et al., 2006). The surface oxygenated complexes (hydroxyl, carbonyl and carboxyl groups), typically at the edges of aromatic ring systems in the form of sheets are responsible for many of the physico-chemical properties of these materials.

The carboxylic acid groups are essential for improving the biochars nutrient holding capacity, as well as polarizing the surface, which should also increase water retention of the material. A high
proportion of carboxylic acids as well as other oxygen groups should also provide biochar many of the desirable properties of humic acid which is an important degradation product of soil organic matter that contains a high proportion of carboxylic acid functional groups, on the order of 2-5 milliequivalents per gram (meq/g) with total acidic groups as high as 9 meq/g (Mbagwu and Piccolo, 1997). The relatively high concentration of acidic groups can allow the formation of chelates with metal ions which will help to bind positively charged ions to the surface of the carbon. When the surface density of carboxylic acid groups is very high, chelates with metal ions can be also be used to almost completely immobilize potentially toxic metal compounds (Fuchs et al., 2012).

The results from numerous studies indicate that the acidic groups can be formed on the surface of activated carbons in quantities of a similar order of magnitude as found in various humic materials. The results obtained by Valdes et al. (Valdés et al., 2002) indicate that total acidic groups on activated carbons (AC) can reach at least 2 meq/g with half the acidic groups being due to carboxyl groups.

The acidic nature of the oxidized biochars means that they are particularly well suited for the retention of basic ions such as ammonia or other cation compounds (Kastner et al., 2009). Investigation of the effectiveness of oxidized biochars for the removal of ammonia from gas streams and ammonium from liquid streams has received considerable attention. Chiang et al. (Chiang, Chiang, et al., 2002) has shown a strong correlation between the quantity of ammonia adsorbed by the carbon and the concentration of acid groups on the surface.

Oxygenated functional groups can be formed when carbonaceous surfaces are attacked by oxidizing agents. The oxidation of carbonaceous materials (mainly coal) with nitric acid (HNO₃),
hydrogen peroxide (H$_2$O$_2$), (NH$_4$)$_2$S$_2$O$_8$ and ozone (O$_3$) has been extensively studied (Pradhan and Sandle, 1999; Valdés et al., 2002). Treatment by ozone at room temperature has been shown to be an effective way to rapidly oxidize the surface of carbonaceous materials in a controlled environment. The oxidation process has been shown to lead to significant increases in the number of acidic surface oxygenated groups including carboxylic acid groups (Chiang, Huang, et al., 2002; Valdés et al., 2002; Park and Jin, 2004). Most of the biochar oxidation tests reported in the literature were conducting using aggressive reagents (ozone, hydrogen peroxide, strong acids). Although the oxidation of coal and activated materials in air in the range of temperature between 120 and 250 °C has extensively studied (Nordon et al., 1979; Oda et al., 1981; Koch, 1998), it was not possible to find any study on the effect of pyrolysis conditions determining the structure and the oxidability of the resulting carbonaceous materials. Air oxidation is of great interests for the biomass pyrolysis community because it can be easily and cheaply integrated during the biochar cooling step. Thus, the main objective of this paper is to study the effect of pyrolysis temperature on the oxidability of biochars and the effect of the oxidation process on the bulk and surface properties of the resulting oxidized chars.

2. Materials and Methods

All Biochar were prepared and characterized as described in chapter 3. For the oxidation, dry air was used as oxidizing agent to modify the surface of produced biochars. Oxidation of about 50 mg of each biochar sample was carried out in the same spoon reactor operated at 250°C for 30 min with air stream. In this chapter, the oxidized samples have same abbreviations, as in Chapter 3, followed by capital A, which refers to air oxidation.
3. Results and discussion

3.1. Oxidized biochar Yield

The yields of biochar obtained after pyrolysis and oxidation as a function of pyrolysis temperature are shown in Figure 1. Oxidized biochar yields changed nonlinearly in response to pyrolysis temperature for each biomass feedstock. This type of nonlinearity is a common biomass response to pyrolysis and post pyrolysis conditions and is mainly associated to the existence of temperature regions where different thermal degradation temperatures of the cellulose, hemicellulose and lignin. Within the pyrolysis temperature range utilized to produce our char samples, the yield decreased dramatically due to the removal of carbon, oxygen, and hydrogen atoms as volatiles by thermal decomposition. Losses in biochar mass during oxidation by air at 250°C ranged from 0.61 to 6.08 wt.% overall based upon production temperature for each biomass type. These weight losses can be due to the removal of some of the oxygenated groups (burn off) from some of the edges of biochar aromatic ring systems.
3.2. Bulk properties

3.2.1. Proximate analysis

The proximate analysis [fixed carbon (FC), volatile matter (VM), and ash content] of unoxidized and oxidized samples is shown in Fig. 2. The fixed carbon and ash content increased with increasing pyrolysis temperature (350-600°C), while the volatile matter decreased. When the
chars were oxidized by air at 250°C, the fixed carbon decreased by 4.25 ± 2.22 wt.% (overall average) while volatile matter increased by 4.91 ± 2.18 wt.% (overall average) comparable to unoxidized samples, indicating that some of the fixed carbon was converted to structures with less thermal stability during oxidation. Feedstock source did not show any significant alteration on FC, VM, nor ash content after samples were oxidized, except the ash content for poplar wood biochars (HP) which decreased by 1.1 ± 0.4 wt.% compared to unoxidized ones.
Figure 4.2 Contents of Volatile matter (VM) and Fixed Carbon (FC) of oxidized and un-oxidized biochars (Dry biochar basis).
3.2.2. Elemental composition

The results for elemental analysis of three different biochars produced at six pyrolytic temperatures and their oxidized derivatives are illustrated in Table 4.1 and Figure 4.3. Compared to the un-oxidized biochars, all oxidized biochars showed relatively very small decreases in amount of carbon, hydrogen, and nitrogen. These changes were more pronounced for low-temperature (<500°C) biochars than for high temperature (>500°C) biochars, regardless feedstock sources. Consequently, lower temperature biochars are more susceptible to oxidation due to the removal of labile C and volatile organic compounds present comparable with higher temperature ones.

Table 4.1  Elemental composition and ash content of oxidized HP, PW and PB biochars

<table>
<thead>
<tr>
<th>Biochar ID</th>
<th>C\textsuperscript{a} (wt.%)</th>
<th>O\textsuperscript{b} (wt.%)</th>
<th>N\textsuperscript{c} (wt.%)</th>
<th>H\textsuperscript{d} (wt.%)</th>
<th>Ash (wt.%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HP-350-A</td>
<td>65.43 (±0.70)</td>
<td>24.94 (±0.69)</td>
<td>0.458 (±0.01)</td>
<td>4.48 (±0.01)</td>
<td>4.70 (±0.64)</td>
</tr>
<tr>
<td>HP-400-A</td>
<td>69.90 (±0.29)</td>
<td>22.05 (±0.29)</td>
<td>0.356 (±0.04)</td>
<td>3.89 (±0.04)</td>
<td>3.80 (±1.04)</td>
</tr>
<tr>
<td>HP-450-A</td>
<td>71.61 (±0.81)</td>
<td>18.85 (±0.84)</td>
<td>0.426 (±0.03)</td>
<td>3.72 (±0.01)</td>
<td>5.40 (±0.39)</td>
</tr>
<tr>
<td>HP-500-A</td>
<td>74.71 (±0.09)</td>
<td>16.37 (±0.11)</td>
<td>0.426 (±0.03)</td>
<td>3.64 (±0.02)</td>
<td>4.85 (±0.61)</td>
</tr>
<tr>
<td>HP-550-A</td>
<td>78.75 (±1.12)</td>
<td>11.27 (±1.29)</td>
<td>0.438 (±0.03)</td>
<td>3.69 (±0.21)</td>
<td>5.85 (±0.18)</td>
</tr>
<tr>
<td>HP-600-A</td>
<td>81.41 (±0.83)</td>
<td>8.78 (±0.80)</td>
<td>0.410 (±0.02)</td>
<td>3.44 (±0.02)</td>
<td>5.96 (±0.28)</td>
</tr>
<tr>
<td>PW-350-A</td>
<td>65.37 (±0.19)</td>
<td>29.17 (±0.23)</td>
<td>0.260 (±0.01)</td>
<td>4.79 (±0.04)</td>
<td>0.42 (±0.93)</td>
</tr>
<tr>
<td>PW-400-A</td>
<td>70.07 (±0.12)</td>
<td>25.00 (±0.06)</td>
<td>0.303 (±0.01)</td>
<td>4.08 (±0.07)</td>
<td>0.55 (±0.12)</td>
</tr>
<tr>
<td>PW-450-A</td>
<td>72.65 (±0.21)</td>
<td>22.58 (±0.33)</td>
<td>0.347 (±0.01)</td>
<td>3.69 (±0.12)</td>
<td>0.73 (±0.04)</td>
</tr>
<tr>
<td>PW-500-A</td>
<td>76.30 (±0.32)</td>
<td>18.86 (±0.31)</td>
<td>0.332 (±0.03)</td>
<td>3.57 (±0.03)</td>
<td>0.93 (±0.08)</td>
</tr>
<tr>
<td>PW-550-A</td>
<td>81.69 (±0.42)</td>
<td>13.61 (±0.44)</td>
<td>0.341 (±0.05)</td>
<td>3.57 (±0.03)</td>
<td>0.82 (±0.05)</td>
</tr>
<tr>
<td>PW-600-A</td>
<td>85.08 (±0.35)</td>
<td>10.27 (±0.31)</td>
<td>0.319 (±0.05)</td>
<td>3.44 (±0.04)</td>
<td>0.89 (±0.12)</td>
</tr>
<tr>
<td>PB-350-A</td>
<td>63.85 (±0.32)</td>
<td>27.11 (±0.29)</td>
<td>0.600 (±0.03)</td>
<td>4.49 (±0.01)</td>
<td>3.95 (±1.05)</td>
</tr>
<tr>
<td>PB-400-A</td>
<td>66.10 (±0.39)</td>
<td>23.56 (±0.36)</td>
<td>0.613 (±0.01)</td>
<td>4.15 (±0.03)</td>
<td>5.58 (±0.74)</td>
</tr>
<tr>
<td>PB-450-A</td>
<td>68.92 (±0.56)</td>
<td>20.03 (±0.56)</td>
<td>0.658 (±0.06)</td>
<td>3.77 (±0.06)</td>
<td>6.62 (±0.53)</td>
</tr>
<tr>
<td>PB-500-A</td>
<td>69.84 (±0.35)</td>
<td>18.59 (±0.43)</td>
<td>0.684 (±0.04)</td>
<td>3.51 (±0.11)</td>
<td>7.38 (±0.27)</td>
</tr>
<tr>
<td>PB-550-A</td>
<td>75.23 (±0.48)</td>
<td>12.90 (±0.41)</td>
<td>0.668 (±0.04)</td>
<td>3.46 (±0.03)</td>
<td>7.75 (±0.39)</td>
</tr>
<tr>
<td>PB-600-A</td>
<td>78.07 (±0.32)</td>
<td>10.21 (±0.32)</td>
<td>0.731 (±0.01)</td>
<td>3.29 (±0.02)</td>
<td>7.70 (±0.53)</td>
</tr>
</tbody>
</table>

C\textsuperscript{a} = Total Carbon content (wt.%), O\textsuperscript{b} = Total Oxygen content (by difference), N\textsuperscript{c} = Total Nitrogen content (wt.%), H\textsuperscript{d} = Total Hydrogen content (wt.%). Standard deviation are shown in parentheses for analytical replicates (n=3).
The behavior of oxygen content is also shown in Figure 4.3. The bulk oxygen content increased by 29%, 28.39% and 20.75% for the pine wood, poplar wood and pine bark biochars produced at 350 °C, respectively, indicating formation of oxygenated functional groups. The results show that the air oxidation of biochar considerably enhanced the oxygen incorporation into the carbon structures especially when the char was produced at low temperature. This could be directly related to the increase in acidic oxygen-containing functional groups during thermal oxidation by air (Harry et al., 2006). The biochars produced at low temperature (which higher content of volatile, less stabilized aromatic carbon) were observed to have a higher susceptibility to air oxidation than the biochars produced at high temperature.
Figure 4.3 Total oxygen contents oxidized and unoxidized biochars.
The atomic ratios (O/C, H/C, N/C and O+N/C) are shown in Table 2. This table encapsulates how the production parameters influencing carbonization affect the oxidation degree of the biochar produced (see Table, 2). Air oxidation increased atomic O/C ratios mostly due to the formation of oxygenated functional groups into the carbon structures.

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>O/C</th>
<th>H/C</th>
<th>N/C</th>
<th>O+N/C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UO</td>
<td>AO</td>
<td>UO</td>
<td>AO</td>
</tr>
<tr>
<td>HP-350</td>
<td>0.298</td>
<td>0.381</td>
<td>0.076</td>
<td>0.068</td>
</tr>
<tr>
<td>HP-400</td>
<td>0.183</td>
<td>0.315</td>
<td>0.059</td>
<td>0.056</td>
</tr>
<tr>
<td>HP-450</td>
<td>0.147</td>
<td>0.263</td>
<td>0.056</td>
<td>0.052</td>
</tr>
<tr>
<td>HP-500</td>
<td>0.145</td>
<td>0.219</td>
<td>0.052</td>
<td>0.049</td>
</tr>
<tr>
<td>HP-550</td>
<td>0.121</td>
<td>0.143</td>
<td>0.048</td>
<td>0.047</td>
</tr>
<tr>
<td>HP-600</td>
<td>0.082</td>
<td>0.108</td>
<td>0.041</td>
<td>0.042</td>
</tr>
<tr>
<td>PW-350</td>
<td>0.328</td>
<td>0.446</td>
<td>0.078</td>
<td>0.073</td>
</tr>
<tr>
<td>PW-400</td>
<td>0.255</td>
<td>0.357</td>
<td>0.068</td>
<td>0.058</td>
</tr>
<tr>
<td>PW-450</td>
<td>0.216</td>
<td>0.311</td>
<td>0.057</td>
<td>0.051</td>
</tr>
<tr>
<td>PW-500</td>
<td>0.187</td>
<td>0.247</td>
<td>0.054</td>
<td>0.047</td>
</tr>
<tr>
<td>PW-550</td>
<td>0.112</td>
<td>0.167</td>
<td>0.046</td>
<td>0.043</td>
</tr>
<tr>
<td>PW-600</td>
<td>0.080</td>
<td>0.121</td>
<td>0.041</td>
<td>0.040</td>
</tr>
<tr>
<td>PB-350</td>
<td>0.358</td>
<td>0.425</td>
<td>0.074</td>
<td>0.070</td>
</tr>
<tr>
<td>PB-400</td>
<td>0.269</td>
<td>0.356</td>
<td>0.065</td>
<td>0.063</td>
</tr>
<tr>
<td>PB-450</td>
<td>0.213</td>
<td>0.291</td>
<td>0.057</td>
<td>0.055</td>
</tr>
<tr>
<td>PB-500</td>
<td>0.171</td>
<td>0.266</td>
<td>0.050</td>
<td>0.050</td>
</tr>
<tr>
<td>PB-550</td>
<td>0.156</td>
<td>0.171</td>
<td>0.046</td>
<td>0.046</td>
</tr>
<tr>
<td>PB-600</td>
<td>0.115</td>
<td>0.131</td>
<td>0.042</td>
<td>0.042</td>
</tr>
</tbody>
</table>
3.3 Surface properties

3.3.1. CO₂ physisorption analysis

Biochar samples were analyzed by CO₂ adsorption to determine evaluate the amount and dimensions of narrow microporosity. Figure 4 shows the CO₂ adsorption isotherms PW, PB and HP biochars oxidized by air at 250°C. The isotherm shape and the quantity of CO₂ adsorbed by the oxidized biochars were dependent mainly on production temperature and slightly on feedstock source. In general, adsorbed quantities of CO₂ increased consistently as pyrolysis temperature increased from 350-600°C, indicating an increase in micropore volume with increasing pyrolysis temperature. The highest adsorption were achieved with biochars produced at 600°C (63.69, 61.73, and 61.62 cm³/g STP for HP, PB, and PW, respectively, at 0.03 P/P₀), which due to the pronounced porosity. The pine wood biochars (PW) showed the best adsorptive behavior.

The values of the characteristic adsorption energy ($E₀$) of Dubinin-Radushkevich equation were plotted against pyrolysis temperature in Figure 4 (d, e and f). It is known in the literature that decreases in $E₀$ values correlate well with increases in micropore sizes (Parra et al., 1995). Our results showed that $E₀$ increase with oxidation by air at 250°C, particularly in biochars produced below 500°C, corresponding to narrower micropores. Conversely, $E₀$ values decreased in oxidized biochars produced at pyrolysis temperature equals or above 550°C, which, in turn, indicates that the micropores are being wider. In general, the oxidizer biochars exhibit narrower microporous structure (as indicated by $E₀$ trends) than the unoxidized samples. This can be attributed to the formation of new carbon-oxygen surface functional groups blocking some of the pores hindering CO₂ adsorption (Parra et al., 1995).
Figure 4.4 CO₂ adsorption isotherms for oxidized PW (a), PB(b) and HP(c) biochars, and characteristic energy for PW (d), PB(e) and HP(f) obtained by DR method.
The micropore volume of the biochar samples are shown in Table 4.3. A side-by-side comparison of unoxidized and oxidized samples shows that microporous structure, in general, did not alter significantly till pyrolysis temperature reached its highest degrees. As seen, for example, micropore surface area increased from 500 to 570, 424 to 550, and 417 to 557 m²/g in PW, PB, and HP biochars, respectively, when produced at 600°C. On the other hand, we observe a slight increase or a slight decrease in microporous characteristics. The increase in area can be explained by the oxidation and removal of some of the walls of the carbonaceous material, the decrease in micro-porous volume can be attributed to the partial blockage of micropores by oxygen-containing functional groups, which limit CO₂ diffusion and adsorption (Parra et al., 1995; Harry et al., 2006).
Table 4.3 Characteristics of microporous structure of unoxidized (UO) and oxidized (AO) PW, PB, and HP biochars as a function of pyrolysis temperature.

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>PV$_{\text{mic}}$ (cm$^3$ g$^{-1}$)</th>
<th>PV$_{\text{tot}}$ (cm$^3$ g$^{-1}$)</th>
<th>P$_{\text{cap}}$ (cm$^3$ g$^{-1}$)</th>
<th>SA$_{\text{CO}_2}$ (m$^2$ g$^{-1}$)</th>
<th>Pore size distribution of oxidized biochar (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UO</td>
<td>AO</td>
<td>UO</td>
<td>AO</td>
<td>UO</td>
</tr>
<tr>
<td>PW-350</td>
<td>0.06</td>
<td>0.08</td>
<td>0.14</td>
<td>0.23</td>
<td>32</td>
</tr>
<tr>
<td>PW-400</td>
<td>0.10</td>
<td>0.11</td>
<td>0.28</td>
<td>0.43</td>
<td>57</td>
</tr>
<tr>
<td>PW-450</td>
<td>0.13</td>
<td>0.13</td>
<td>0.44</td>
<td>0.48</td>
<td>73</td>
</tr>
<tr>
<td>PW-500</td>
<td>0.16</td>
<td>0.15</td>
<td>0.43</td>
<td>0.54</td>
<td>88</td>
</tr>
<tr>
<td>PW-550</td>
<td>0.18</td>
<td>0.20</td>
<td>0.44</td>
<td>0.59</td>
<td>98</td>
</tr>
<tr>
<td>PW-600</td>
<td>0.20</td>
<td>0.23</td>
<td>0.57</td>
<td>0.75</td>
<td>110</td>
</tr>
<tr>
<td>PB-350</td>
<td>0.07</td>
<td>0.07</td>
<td>0.20</td>
<td>0.24</td>
<td>38</td>
</tr>
<tr>
<td>PB-400</td>
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<td>0.09</td>
<td>0.20</td>
<td>0.29</td>
<td>46</td>
</tr>
<tr>
<td>PB-450</td>
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<td>0.11</td>
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<td>0.38</td>
<td>64</td>
</tr>
<tr>
<td>PB-500</td>
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<td>0.14</td>
<td>0.36</td>
<td>0.47</td>
<td>70</td>
</tr>
<tr>
<td>PB-550</td>
<td>0.16</td>
<td>0.15</td>
<td>0.54</td>
<td>0.45</td>
<td>87</td>
</tr>
<tr>
<td>PB-600</td>
<td>0.17</td>
<td>0.22</td>
<td>0.43</td>
<td>0.81</td>
<td>93</td>
</tr>
<tr>
<td>HP-350</td>
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<td>0.07</td>
<td>0.27</td>
<td>0.20</td>
<td>46</td>
</tr>
<tr>
<td>HP-400</td>
<td>0.10</td>
<td>0.10</td>
<td>0.31</td>
<td>0.35</td>
<td>57</td>
</tr>
<tr>
<td>HP-450</td>
<td>0.11</td>
<td>0.10</td>
<td>0.38</td>
<td>0.48</td>
<td>61</td>
</tr>
<tr>
<td>HP-500</td>
<td>0.14</td>
<td>0.13</td>
<td>0.50</td>
<td>0.91</td>
<td>79</td>
</tr>
<tr>
<td>HP-550</td>
<td>0.15</td>
<td>0.15</td>
<td>0.57</td>
<td>0.43</td>
<td>84</td>
</tr>
<tr>
<td>HP-600</td>
<td>0.17</td>
<td>0.22</td>
<td>0.65</td>
<td>0.87</td>
<td>91</td>
</tr>
</tbody>
</table>

a = Micropore volume, b = total pore volume, c = pore capacity, d = surface area calculated from fitting of the DR equation.

A correlation between burn-off during pyrolysis and oxidation and micropore volume is shown in figure 6. As can be seen, the volume of the micropores increases with increase in the degree of burn-off. This means that the increasing burn-off increases the micropore distribution, and consequently, increases the number of larger micropore. It is interesting to note that the large micro-porosity was improved to a large value when the burn off is increased to 80% in PW and
HP biochars, but 73% in case of PB biochar. The behavior of PW and HP was very similar. In the case of PB higher pore volumes were observed for lower levels of burn off.

![Evolution of micropore volume as a function of burn-off (pyrolysis + oxidation) for PW, HP and PB biochars.](image)

**Fig. 4.5** Evolution of micropore volume as a function of burn-off (pyrolysis + oxidation) for PW, HP and PB biochars.

### 3.3.2. Surface functionality

The content of oxygenated surface functional groups obtained by Boehm titration method are shown in Table 4.4. The concentrations of carboxylic and total acidic functional groups of unoxidized biochars and oxidized samples are shown in Fig. 4.6. The results clearly show that the oxidation with air increases the acidic functional groups, especially for the formation of carboxyl on biochar surfaces. Air oxidation of pine wood biochars introduces a large number of carboxylic functional groups; the pine wood biochar functionalities rapidly improved due likely to their surface area. These findings are consistent with Salame and Bandosz (Salame and Bandosz, 2001).
Slight increase of carboxylic groups were observed in pine bark and poplar wood biochars in comparison with pine wood biochars, regardless pyrolysis temperature.

**Table 4.4 Surface functional group contents (Boehm titration results)**

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Carboxylic groups (mmol/g)</th>
<th>Lactonic groups (mmol/g)</th>
<th>Phenolic groups (mmol/g)</th>
<th>Total basic groups (mmol/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UO(^a)</td>
<td>AO(^b)</td>
<td>UO</td>
<td>AO</td>
</tr>
<tr>
<td>PW-350</td>
<td>0.08</td>
<td>0.28</td>
<td>0.02</td>
<td>0.22</td>
</tr>
<tr>
<td>PW-400</td>
<td>0.07</td>
<td>0.28</td>
<td>0.01</td>
<td>0.16</td>
</tr>
<tr>
<td>PW-450</td>
<td>0.06</td>
<td>0.28</td>
<td>0.01</td>
<td>0.17</td>
</tr>
<tr>
<td>PW-500</td>
<td>0.05</td>
<td>0.22</td>
<td>0.01</td>
<td>0.12</td>
</tr>
<tr>
<td>PW-550</td>
<td>0.02</td>
<td>0.07</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>PW-600</td>
<td>0.01</td>
<td>0.03</td>
<td>0.01</td>
<td>0.03</td>
</tr>
<tr>
<td>PB-350</td>
<td>0.05</td>
<td>0.06</td>
<td>0.07</td>
<td>0.22</td>
</tr>
<tr>
<td>PB-400</td>
<td>0.02</td>
<td>0.05</td>
<td>0.06</td>
<td>0.23</td>
</tr>
<tr>
<td>PB-450</td>
<td>0.01</td>
<td>0.05</td>
<td>0.05</td>
<td>0.17</td>
</tr>
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<td>PB-500</td>
<td>0.02</td>
<td>0.03</td>
<td>0.02</td>
<td>0.16</td>
</tr>
<tr>
<td>PB-550</td>
<td>0.01</td>
<td>0.03</td>
<td>0.01</td>
<td>0.06</td>
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<tr>
<td>PB-600</td>
<td>0.01</td>
<td>0.02</td>
<td>n.a.</td>
<td>0.03</td>
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<tr>
<td>HP-350</td>
<td>0.06</td>
<td>0.09</td>
<td>0.03</td>
<td>0.19</td>
</tr>
<tr>
<td>HP-400</td>
<td>0.03</td>
<td>0.06</td>
<td>0.02</td>
<td>0.19</td>
</tr>
<tr>
<td>HP-450</td>
<td>0.03</td>
<td>0.07</td>
<td>0.03</td>
<td>0.12</td>
</tr>
<tr>
<td>HP-500</td>
<td>0.02</td>
<td>0.03</td>
<td>0.01</td>
<td>0.11</td>
</tr>
<tr>
<td>HP-550</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.06</td>
</tr>
<tr>
<td>HP-600</td>
<td>n.a.</td>
<td>0.01</td>
<td>n.a.</td>
<td>0.03</td>
</tr>
</tbody>
</table>

\(^a\) UO = unoxidized biochar, \(^b\) AO = air-oxidized biochar. n.a. = not applicable.

The data obtained from Boehm analysis clearly show that the content of total acidic functional groups is very dependent on the pyrolysis temperature. The amount of oxygen surface groups forming decreases as the structure of the biochar formed gains in stability with the increase in production temperature. It is clear that low temperature biochars appeared to be more suitable for oxidation by air than their derivative, which produced at high temperatures. The results show in
Figure 4.6 suggest that the creation of acid functional groups is easier for biochars that have been produced at very temperature. It seems that the oxidability of biochars decreases as the pyrolysis temperature increases and more stable poly-aromatic structures are formed.

Figure 4.6 Carboxylic and total acidic functional groups of oxidized and unoxidized PW, HP and PB biochars.
3.3.3. XPS analysis

XPS is an alternative technique for surface functional groups determination since chemical titration is usually controlled by wettability factor between solution and biochar surface (Oda et al., 2006). Figure 8 shows the influence of pyrolysis and post-pyrolysis conditions on total oxygen content on C1s peak of studied biochars. The XPS analysis indicates that the surface composition of biochar changed substantially as a result of charring temperature and feedstock source. The concentration of surface oxygen on C1s peak decreased from 48 to 19 atom %, from 57 to 10 atom %, and from 42 to 10 atom % for PW, PB and HP, respectively, as the pyrolysis temperature increased from 350 to 600°C. This is due to the gradual removal of oxygenated functional groups (e.g. lactonic, phenolic and carboxylic) during pyrolysis, as indicated previously by Boehm titration. These findings are in accordance with the results found by Dong et al. (Dong et al., 2013) who reported that low temperature biochars contained more oxygen functional groups than high temperature biochars. Our XPS data also showed that the air oxidation increases the final oxygen content on biochar surfaces which likely due to introducing oxygen-containing groups on biochar surfaces. Shifts of C1s spectra to higher binding energy for oxidized samples indicated that the increase of oxygen content were due to the formation of oxygen-containing functional groups not merely an adsorption of oxygen (Cheng et al., 2008). These findings were similar to our presented Boehm titration results in which our investigations suggested a development of the surface oxygen-containing functional groups through air oxidation of biochar.
Figure 4.7 Influence of temperature and feedstock source on total oxygen bound on carbon (O\textsubscript{c}) of PW, PB, and HP chars (measured by XPS).
Table 4.5 shows the change in C and O bonding states on biochar surface after oxidation by air at 250°C. The C1s spectra have been de-convoluted into four components, namely the following: (1) graphitic, aromatic, or aliphatic carbon (285.0-286.0 eV); (2) hydroxyl/ether (286.1-287.1 eV); (3) carbonyl (287.1-288.1 eV); and (4) carboxyl/ester (288.0-289.5 eV). The O1s spectra were also de-convoluted into four component peaks reflecting different chemical states of oxygen. Peaks located at 531.9±0.5, 532.8±0.4, 533.5±0.4, and 535.5±0.2 eV were attributed to the carbonyl/carboxyl, ether/alcohol, ester, and chemisorbed O/H₂O, respectively. These results are in agreement with those reported by Puziy et al. (Puziy et al., 2008) and Valdes et al. (Valdés et al., 2002).

The results shown in table 5 reveal that the amount of oxygen-containing functional groups in unoxidized samples decreased and occasionally disappeared as the pyrolysis temperature increased. This result is mainly due to the removal of oxygenated functional groups from the surface of biochar as the carbonization temperature increased from 350 to 600°C. After oxidation, oxygen functional groups were formed on the surface of the samples, and thus oxidized biochars had higher surface oxygen fraction. In contrast, carbon in graphitic or aromatic surface structures was decreased to approximately 9.277±6.34 at.% for oxidized biochars. This sequence indicated that after oxidation by air, the biochar surface fixed more oxygen complexes and lost carbon atoms from the surface due to burning off (Qian and Chen, 2014).
Table 4.5: The relative amount of C1s and O1s components of oxidized and unoxidized samples

<table>
<thead>
<tr>
<th>Feedstock</th>
<th>Pyrolysis temperature (°C)</th>
<th>Post-Pyrolysis Oxidation</th>
<th>C1s (atom %)</th>
<th>O1s (atom %)</th>
<th>Total OFGs*</th>
<th>=O</th>
<th>-O and -OH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>C-C, C=C or C-H</td>
<td>C-O</td>
<td>C=O</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pine wood biochars (PW)</td>
<td>350</td>
<td>Un-oxidized</td>
<td>46.415</td>
<td>33.878</td>
<td>8.804</td>
<td>2.903</td>
<td>45.585</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>57.152</td>
<td>31.366</td>
<td>9.282</td>
<td>2.200</td>
<td>42.848</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>81.782</td>
<td>15.250</td>
<td>15.250</td>
<td>2.968</td>
<td>33.469</td>
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<td></td>
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<td></td>
<td>87.176</td>
<td>12.159</td>
<td>9.211</td>
<td>3.613</td>
<td>24.984</td>
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<tr>
<td></td>
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<td></td>
<td>70.832</td>
<td>12.871</td>
<td>ND</td>
<td>4.056</td>
<td>24.168</td>
</tr>
<tr>
<td></td>
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<td>Oxidized</td>
<td>77.186</td>
<td>12.866</td>
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<td>3.920</td>
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<td>Pine Bark biochars (PB)</td>
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<td>Un-oxidized</td>
<td>42.694</td>
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<td>15.737</td>
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<td>Oxidized</td>
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</tr>
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<td>76.544</td>
<td>ND</td>
<td>13.900</td>
<td>9.556</td>
<td>23.456</td>
</tr>
<tr>
<td>Poplar wood biochars (HP)</td>
<td>350</td>
<td>Un-oxidized</td>
<td>58.639</td>
<td>38.584</td>
<td>2.777</td>
<td>2.207</td>
<td>43.568</td>
</tr>
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<td>67.182</td>
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<td>6.132</td>
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<td>86.622</td>
<td>13.378</td>
<td>ND</td>
<td>ND</td>
<td>13.378</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Oxidized</td>
<td>58.639</td>
<td>38.584</td>
<td>2.777</td>
<td>2.207</td>
<td>43.568</td>
</tr>
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<td>6.132</td>
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<td>5.854</td>
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<td>86.622</td>
<td>13.378</td>
<td>ND</td>
<td>ND</td>
<td>13.378</td>
</tr>
</tbody>
</table>

*Total OFGs = sum of oxygen-containing functional groups, ND = not determined
Table 4.5 shows that the air oxidation significantly increases the concentrations of total Oxygen Functional Groups (OFGs), particularly carboxylic acid, regardless of feedstock source and pyrolysis temperature. The higher total OFGs content was observed on oxidized pine park (PB) biochar surfaces produced at 350°C (75.544 at.%) compared to pine wood (PW) and poplar wood (HP) biochar surfaces (49.948 and 50.043 at.%, respectively). However, total OFGs content of oxidized PW biochar produced at 600°C was higher (29.644 at.%) than that of PB and HP produced at same temperature (23.812 and 23.456 at.%, respectively). In addition, oxidized PW biochar produced at 450°C had the highest concentration of carboxylic acid group, demonstrating higher gain of oxygenated compounds in PW through the air oxidation process. Thus, by controlling the pyrolysis temperature and feedstock selection, controlled oxidation status of biochar could be achieved by surface modifications using air at 250°C as a post-pyrolysis process. This observation is in line with findings by Dong et al. (Dong et al., 2013) and Li et al. [37].

Figure 4.8 displays the ratio of oxygen (bond on carbon) to carbon, which indicates the degree of surface oxidation. As the pyrolytic temperature increased, the O/C decreased consistently because of the loss of oxygen and carbon species leading to form condensed carbons. After oxidation by air, the O/C ratio increased significantly in low temperature biochars but little improvement were observed in high temperature biochars. This likely due to higher condensed carbon contents, which are difficult to oxidize (Qian and Chen, 2014). Interestingly, our data showed that higher O/C ratio was observed on PB biochar surfaces (0.55 atomic ratio) compared to PW and HP biochar surfaces (0.4 and 3.5, respectively) at 350°C, similarly for O/C ratios at 600°C. This observation is not in agreement with previous findings obtained by titration method, which showed that PW has higher total functional groups than PB. This discrepancy is possibly
because the XPS analysis detects oxygen functional groups located on the upper layer (ca.5-10nm) of biochar surface, mostly near the pore entrances (László and Szűcs, 2001).

**Figure 4.8** Effect of pyrolysis temperature and feedstock source on Oc:C ratio of PW, PB and HP biochars (Oc=oxygen bound on carbon).
3.3.4. Bulk and Surface Oxygen content

Figure 4.9 shows correlations between values obtained from XPS and elemental analysis. These values are strongly correlated indicating that both techniques are in good agreement to explain the alterations of oxygen content during pyrolysis and post-pyrolysis process of various biochar samples. However, values of oxidized groups were shifted toward the XPS O content axial demonstrating that the quantitative results obtained by XPS were particularly useful in characterizing surfaces modified with air oxidation. Importantly, comparing XPS results with those of elemental analysis can give an idea of the homogeneity of the oxygen complexes distribution between the internal and external surface of the carbon particles (Perez-Cadenas et al., 2003). Results shown in figure (11) indicate that all biochar samples exhibited different values from the XPS and elemental analysis for the surface and bulk regions, respectively. This finding suggests that the distribution of oxygen was obviously heterogeneous and the OFGs were primarily fixed on the more external surface of the samples. These results are generally in agreement with the results reported by Oda et al.(2006) and Perez-Cadenas et al. (2003).
Figure 4.9 Correlations between surface O\(_c\) (total oxygen bound on carbon, measured by XPS) and bulk O (total oxygen contents, measured by elemental analysis) of PW, HP, and PB biochars
3.3.5 Cation Exchange Capacity (CEC)

The cation exchange capacity (CEC) can be defined as the total capacity of a biochar to adsorb and exchange positively charged species (Carrier et al., 2012). Figure 4.10 shows the changes in the cation exchange capacity of the resultant biochars before and after oxidation by air at 250°C. Generally, the CEC values were dependent on both feedstock and production temperature. The lowest CEC was observed for the poplar wood and pine bark biochars pyrolyzed at 600 °C, suggesting that biochar CEC decreases with increasing pyrolytic temperature. This decrease could be in part due to the reduction of the content of oxygenated functional groups; changes in CEC have previously been explained by the presence of oxygenated functional groups (Carboxylic, phenolic, and lactonic) (Budai et al., 2014). Our results also showed that CEC has been significantly improved by air oxidation, specifically, for low temperature biochars. The relatively high CEC of low temperature biochars, compared to high temperature biochars, could be attributed to the carboxylic functional groups, which contribute most to the CEC among the acidic functional groups (Singh, 2010; Carrier et al., 2012). This sequence indicated that after oxidation, the biochar gained a larger number of oxygen complexes on the surface. In application point of view, increasing in such these acidic groups is favorable as they impart the cation adsorptive capacity of biochars (Doydora et al., 2011).
Figure 4.10 CEC values for oxidized and unoxidized PW (a), HP(b) and PB (c) biochars.

3.3.6. pH and EC

Table 4.6 shows the values of pH and electric conductivity (EC) of the water in contact with the unoxidized and oxidized biochars. The pH of the biochars studied ranged from 7.9 to 10.4 and increased with production temperature, reaching a plateau at approximately 550°C. pH was also dependent upon the original biomass species, Pine wood biochar had lower pH values (from 8.3 to 8.8) comparable to pine bark and hybrid poplar wood biochars (Mukherjee et al., 2011).
Similarly, the electrical conductivity (EC), total water-soluble ions in the biochars, increased linearly with the pyrolysis temperature and varied very significantly among feedstocks. These results could be related by the content of ash and surface alkalinity of biochars examined. As expected, air oxidation changed pH and EC values due to its effect on O/C ratio; a significant correlation between pH and O/C ratio was observed \((R^2 = 0.894)\) for all biochar samples when pine wood biochars were excluded, due to low ash content. It is important to mention here that the O/C atomic ratio did not usually reflect the acidity of the biochars since the alkalinity is caused by negatively charged functional groups and carbonates on the surface of biochar (Budai et al., 2014).

**Table 4.6** pH and EC of selected oxidized (AO) and unoxidized (UO) biochars

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>pH (H₂O)</th>
<th>EC (H₂O) (ds/m)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UO</td>
<td>AO</td>
</tr>
<tr>
<td>HP-400</td>
<td>10.14 (±0.35)</td>
<td>9.82 (±0.91)</td>
</tr>
<tr>
<td>HP-500</td>
<td>10.54 (±0.10)</td>
<td>10.04 (±0.23)</td>
</tr>
<tr>
<td>HP-600</td>
<td>10.41 (±0.13)</td>
<td>10.04 (±0.08)</td>
</tr>
<tr>
<td>PW-400</td>
<td>8.28 (±0.21)</td>
<td>8.01 (±0.11)</td>
</tr>
<tr>
<td>PW-500</td>
<td>8.85 (±0.35)</td>
<td>8.21 (±0.09)</td>
</tr>
<tr>
<td>PW-600</td>
<td>8.79 (±0.16)</td>
<td>8.27 (±0.16)</td>
</tr>
<tr>
<td>PB-400</td>
<td>7.98 (±0.32)</td>
<td>6.86 (±0.33)</td>
</tr>
<tr>
<td>PB-500</td>
<td>9.88 (±0.13)</td>
<td>8.02 (±0.45)</td>
</tr>
<tr>
<td>PB-600</td>
<td>10.19 (±0.25)</td>
<td>10.05 (±0.12)</td>
</tr>
</tbody>
</table>

Standard deviation are shown in parentheses for analytical replicates (n=3).
3.3.7 Surface charge

Figure 4.11 shows the zeta potential vs equilibrium solution pH plots for primary biochars and oxidized samples. The charge of all biochar samples was strongly dependent on equilibrium solution pH; the $\zeta$-potential curve falls steeply with increasing pH of the electrolyte solution. It could be observed that the lowest $\zeta$-potential values were detected in the alkaline range which likely due to the adsorption of the OH$^-$ ions that also contribute to the potential (Bismarck et al., 1999). However, these negative charges were diminished in the acidic range indicating electrostatic attraction of protons to biochars surface (Qian and Chen, 2014). The presence of dissociable carboxyl groups is also responsible for this effect, since it is well known that dissociation of this group occurs at acidic range, usually around pH of 4 (Harry et al., 2006).

Figure 4.11 also shows a marked difference in $\zeta$-potentials between low and high temperature biochars; $\zeta$-potential values for unoxidized biochars produced at high temperature (e.g. 600°C) were higher than that of low temperature biochars (e.g. 350°C), specifically in neutral and acidic ranges. This phenomenon demonstrated that low temperature biochar surfaces contain acidic groups as well as functionalities, that decreased and eventually disappeared as the pyrolysis temperature increased (Bismarck et al., 1999; Qian and Chen, 2014).

Surface charges of the studied biochar samples dramatically changed after oxidation by air at 250°C; a considerable decrease of positive charge and increase of surface negative charge were clearly observed. The maximum negative $\zeta$-potential values were observed in oxidized low temperature biochars (e.g. -49.5, -49.4, and -38.6 for PW, PB and HP biochars, respectively) which is significantly reduced compared to the original biochar. In general, the $\zeta$-potential values for
oxidized biochars changed to more acidic values; this sequence indicated that after oxidation, the biochar surface fixed a larger number of oxygen complexes. Formation of these complexes provides sites for surface functionalization and surface negative charge by dissociable acidic groups. As the concentration of these dissociable groups at the surface grows, the surface potential also increase giving correspondingly higher $\zeta$-potential values. Similar results of increasing surface negative charge through oxidation were also reported elsewhere (Bismarck et al., 1999; Cheng et al., 2008; Qian and Chen, 2014).
Figure 4.11 Zeta potential of oxidized and unoxidized PW (a), PB (b), and HP(c) biochars produced at different pyrolysis temperatures.
Biochar is amphoteric in nature due to the wide variety of functional groups localized on its surface. Therefore, determination of its zeta potential at zero charge (PZC), at which the biochar becomes uncharged (\(\zeta = 0\)), is always an important parameter required to understand its electrokinetic behavior (Qian and Chen, 2014). Figure 13 illustrates the results for PZCs of biochars before and after being oxidized by air at 250°C. Concomitant to the increase of surface negative charge, the pzc shifted to lower pH values after oxidation. For example, in reference to PW-350 with pH\(_{\text{PZC}}\) of 3, oxidized PW-350 showed lower pH\(_{\text{PZC}}\) by about 1.8 pH units.

The results also showed greater differential pH\(_{\text{PZC}}\) values for the unoxidized biochars and the oxidized samples as a function of production temperature. The pH\(_{\text{PZC}}\) values were dropped by approximately 2 and 2.4 pH units for oxidized PW-450 and oxidized PW-600, respectively, comparable to unoxidized samples. The same pH\(_{\text{PZC}}\) trend was observed in other biochars produced from pine bark and poplar wood, however, the pH\(_{\text{PZC}}\) was also a feedstock dependent parameter. As an example, the pH\(_{\text{PZC}}\) of PW-600 dropped by 2.4 points after oxidation, while the pH\(_{\text{PZC}}\) of PB-600 and HP-600 slightly shifted to 0.7 and 0.8 pH points, respectively. These results can be explained by the formation of oxygenated functional groups on the surface of the char.

Unlike pine bark and poplar wood biochars, the pine wood biochar always exhibited large difference in the pH\(_{\text{PZC}}\) between primary and oxidized sample. These finding is partially in agreement with CEC values of pine wood biochar which yielded a significant amount of CEC. The variation of the pH\(_{\text{PZC}}\) is a function of acidity, in particular the weakly acidic carboxylic functional groups. Our pH\(_{\text{PZC}}\) results are generally in agreement with the results reported by Cheng et al. (Cheng et al., 2008), Qian and Chen (Qian and Chen, 2014), and Qiu et al., (Qiu and Ling, 2006).
Figure 4.12 pH at zero charges of oxidized and unoxidized biochars.
4. Conclusion

The oxidation of biochars with air is a cheap method to add oxygenated functional groups to the surface of these materials. The proximate analyses confirm that the oxygenated functional groups formed contributed to an increase in the volatile content of these materials. Bulk and surface analyses confirm that the biochars produced at low temperatures (350 °C) are easier to oxidize. The increase in the formation of acidic functional groups was more important in biochars produced at low temperature. The oxidation process could lead to a slight decrease or increase of microporous volume and surface area depending on whether the new functional groups block pore entrances or if the oxidation process resulted in the opening of new or existing pores. The changes in surface composition resulted in an increase in the cation exchange capacity of oxidized biochars as well as in the formation of more negatively charged surfaces. The pH at the point of zero charge decreased with oxidation.

5. Acknowledgements

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6. References


CHAPTER 5. TOWARDS THE QUANTIFICATION OF THE
EFFECTS OF BIOCHAR OXIDATION AND PYROLYSIS
TEMPERATURE ON THE TRANSPORT OF
PATHOGENIC AND NON PATHOGENIC
E. coli IN BIOCHAR-AMENDED
SAND COLUMNS

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(Paper to be submitted to “Environmental Science and Technology”)

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Abstract

A detailed understanding of the transport of *E. coli* O157:H7 within the soil-groundwater system is critical to the protection of public health. Although incorporation of biochar, a carbon-rich porous material, into soils has a potential for reducing the leaching of manure-borne pathogens, knowledge concerning the impact of biochar surface functionality on the retention and transport of *E. coli* O157:H7 is still nonexistent. The main objective of this research was to evaluate whether the addition of unoxidized and oxidized biochar to a sandy soil affects the transport of *E. coli* strains through water-saturated soil columns. We hypothesize that the transport of *E. coli* through biochar-amended soils will vary depending on biochar surface chemistry. We investigated the transport behavior of *E. coli* O157:H7 and *E. coli* K12 in water-saturated column experiments that used a Quincy fine sand (Mixed, mesic Xeric Torripsamments) amended with 20 wt.% pine wood or pine bark biochars produced at 350 and 600°C using a lab scale spoon pyrolysis reactor. Our results showed that (1) Oxidized biochar could enhance the transport of *E. coli* O157:H7 cells due to the surface charge; (2) *E. coli* O157:H7 displayed higher retention then *E. coli* K12 in biochar-amended soil under experimental pH conditions; (3) increased biochar application rates (from 0 to 20%) led to attenuate the transport of both bacterial strains (from 95 to 40%); (4) increased transport was observed for the pine bark biochar produced at 600°C whereas reduced transport was observed for the pine wood biochar produced at the same pyrolysis temperature. Our results suggest that the pine wood biochar produced at 350°C was effective in reducing the transport of *E. coli* in the studied soil.
1. Introduction

The transport of pathogenic bacteria through soil is of scientific and public concern as a significant cause of ground water contamination. It is receiving considerable attention with respect to its role in the outbreaks of waterborne diseases. Among numerous pathogens, *Escherichia coli* O157:H7 is a Gram-negative, facultative anaerobic, enteric bacterium normally excreted in both human and animal feces (Foppen and Schijven, 2006; Kim et al., 2009). *E. coli* O157:H7 was first recognized as a pathogen in 1982 and is a causal agent of bloody diarrhea (hemorrhagic colitis) and renal failure (hemolytic uremic syndrome) in humans (Leclerc et al., 2002; Kim et al., 2009). In the United States alone, this virulent *E. coli* strain causes an average of 75,000 and 61 deaths each year (Wang et al., 2011; Taylor et al., 2014). Massive disease outbreaks have been reported worldwide, the most notable being the Walkerton (Ontario, Canada) tragedy where more than 2,300 persons, of 5,000 people living there, were infected with *E. coli* O157:H7 via drinking contaminated groundwater (Unc and Goss, 2004). Accordingly, the highly pathogenic nature of this organism imposes the necessity to better understand and predict its transport through the subsurface environments in order to assess and mitigate the potential risk to public health.

The modification of the natural filtration capacity of soils (irreversible retention and mobility) may have a strong impact upon the risk of groundwater contamination with pathogenic bacteria (Jean M F Martins et al., 2013). Incorporation of biochar, a carbon-rich porous material, into soils is a potential strategy for reducing the leaching of pathogens. Biochar is receiving growing attention as a soil amendment due to its potential to enhance soil fertility and sequester carbon (Song and Guo, 2012). The production temperature of biochar is the most important parameter affecting surface area, pore volume, pH, volatile matter, oxygen content, and hydrophobicity while
ash content, total carbon, fixed carbon, and mineral concentrations are mainly affected by feedstock properties (Xu et al., 2013), as previously seen in chapters 3 and 4. *E. coli* transport in biochar-amended soils have recently received important attention, especially through laboratory approaches that have evidenced positive effects of biochar on bacterial transport through modifying soil surface area, hydrophobicity, charge, organic carbon content, porosity, or solution chemistry (Abit et al., 2012, 2014; Bolster and Abit, 2012).

Most of biochar studies on bacterial transport were conducted with raw-heterogeneous biochar grains disregarding the role of biochar surface functionality. Indeed, knowledge acquired with only bulk properties of fresh raw-biochars cannot be transferred easily to natural soil environment, where biochars are exposed to biotic and abiotic oxidation processes. There is still a lack of knowledge for understanding the actual role of biochar surface chemistry and porosity for controlling bacterial transport in biochar-amended soils. Such understanding will not just help in developing biochar production ideas and public health strategies but will also improve bacterial transport models by allowing for more precise estimates of bacterial transport vulnerability where biochar additions are applied.

In this study, we compared how two *E. coli* strains behaved with additions of oxidized and unoxidized biochars produced at two pyrolysis temperatures (350 and 600°C) from two commonly used feedstocks (pine wood and pine bark). The aim of this work was to (1) investigate the transport of pathogenic and non-pathogen *E. coli* in biochar-amended sandy soil columns, (2) understand the role of biochar surface functionality on *E. coli* transport using well-characterized air oxidized biochar simulating subsurface natural conditions, and (3) model *E. coli* movement and potential interactions with biochar-soil constituents using a colloid filtration theory.
2. Materials and Methods

2.1 Bacterial Cultures

Pathogenic *Escherichia coli O157:H7* and the nonpathogenic *E. coli K12* were used in this study. The strains were activated by growing for twelve hours at 37°C on a shaker rotating at 150 rpm in Luria Bertani (LB) medium adjusted to a pH of 7.0 using 2N HCl. Following the 12 hours, 1 vol.% of activated culture was transferred into 20 ml of LB medium and grown at the conditions above until the late stationary phase of growth. Cells were harvested when the absorbance at 600 nm reached a value of 0.56 ± 0.02. After growth, the cells were harvested by centrifugation at 5100g for 10 minutes. Pellets were washed three times with deionized water (DIW). Bacterial cells’ pellets were then resuspended in DIW; the concentration of bacterial suspension was set to an optical density of 0.05 at 600 nm using a spectrophotometer (Lambda 25 Perkin Elmer).

2.2 Electrophoretic Mobility Measurements

The electrophoretic mobility of *E. coli* cells harvested at the stationary phase as described above were measured by injecting 2 ml of bacterial cells using a syringe into a nano-Zetasizer 3000 HSA (Malvern Instruments Ltd., Malvern, UK) at room temperature. Prior to measurements, the bacterial cells were harvested at the stationary growth phase as described above and washed twice by centrifugation at 5,100g for 10 min each round. The collected bacterial pellet was then diluted with 0.2 ml filtered DI water and the pH of the suspension with an optical density (λ = 600 nm) of ~ 0.05 was adjusted to the pH value of interest using 0.1 mol/L HCl and 0.1 mol/L NaOH. Electrophoretic mobilities were measured in pH values 1.8, 2.8, 4, 7.7, and 8.5 for *E. coli K12* and
1.7, 2.1, 3.6, 5.1, 7 and 8 for *E. coli* O157:H7. All electrophoretic mobility measurements were performed five times at room temperature.

### 2.3 Biochar preparation and Characterization

Biochar samples were prepared as described previously in Chapter 3 using the Lab scale spoon reactor (figure 3.1). For this study, biochars were produced from Pine Wood and Pine Bark at two pyrolysis temperatures: 350°C and 600°C representative of the lower and upper pyrolysis thresholds for biochar formation. For the oxidation step, air was used as the oxidizing agent to modify the surface of produced biochars. The oxidation procedure used is described in Chapter 4. Here, biochars are denoted as PW for the pine wood feedstock and PW-350, and PW-600 for the resulting unoxidized biochars created at 350 and 600°C, respectively. The oxidized samples are abbreviated as OX referring to air oxidation. Similar abbreviation procedure was applied for the pine bark (PB) batches. All biochars were thoroughly characterized as described in Chapter 3 and Chapter 4. Some of the most important properties of the biochar’ studies and that are relevant for this study are summarized in Table 5.1.
Table 5.1 Selected physico-chemical properties of biochars

<table>
<thead>
<tr>
<th>Property</th>
<th>PW350 UO</th>
<th>PW350 OX</th>
<th>PW600 UO</th>
<th>PW600 OX</th>
<th>PB350 UO</th>
<th>PB350 OX</th>
<th>PB600 UO</th>
<th>PB600 OX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biochar yield (wt. %)</td>
<td>36.0</td>
<td>37.22</td>
<td>16.5</td>
<td>14.96</td>
<td>46.7</td>
<td>41.85</td>
<td>30.9</td>
<td>26.16</td>
</tr>
<tr>
<td>Carbone content (wt. %)</td>
<td>70.50</td>
<td>65.37</td>
<td>87.80</td>
<td>85.08</td>
<td>66.07</td>
<td>63.85</td>
<td>78.11</td>
<td>78.07</td>
</tr>
<tr>
<td>Oxygen content (wt. %)</td>
<td>23.15</td>
<td>29.17</td>
<td>7.06</td>
<td>10.27</td>
<td>23.67</td>
<td>27.11</td>
<td>8.98</td>
<td>10.21</td>
</tr>
<tr>
<td>Nitrogen content (wt. %)</td>
<td>0.26</td>
<td>0.26</td>
<td>0.38</td>
<td>0.32</td>
<td>0.67</td>
<td>0.60</td>
<td>0.75</td>
<td>0.73</td>
</tr>
<tr>
<td>Hydrogen content (wt. %)</td>
<td>5.50</td>
<td>4.79</td>
<td>3.63</td>
<td>3.44</td>
<td>4.88</td>
<td>4.49</td>
<td>3.32</td>
<td>3.29</td>
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<tr>
<td>Fixed carbon content (wt. %)</td>
<td>49.58</td>
<td>48.33</td>
<td>83.15</td>
<td>79.27</td>
<td>51.50</td>
<td>48.71</td>
<td>73.89</td>
<td>70.14</td>
</tr>
<tr>
<td>Volatile matter content (wt. %)</td>
<td>49.82</td>
<td>51.24</td>
<td>15.72</td>
<td>20.84</td>
<td>42.15</td>
<td>47.33</td>
<td>17.81</td>
<td>21.56</td>
</tr>
<tr>
<td>Ash content (wt. %)</td>
<td>0.58</td>
<td>0.42</td>
<td>1.13</td>
<td>0.89</td>
<td>4.72</td>
<td>3.95</td>
<td>8.85</td>
<td>7.70</td>
</tr>
<tr>
<td>Bulk density (g cm(^{-3}))</td>
<td>0.14</td>
<td>0.14</td>
<td>0.24</td>
<td>0.24</td>
<td>0.61</td>
<td>0.61</td>
<td>0.21</td>
<td>0.21</td>
</tr>
<tr>
<td>Particle density (g cm(^{-3}))</td>
<td>0.56</td>
<td>0.56</td>
<td>0.7</td>
<td>0.7</td>
<td>0.45</td>
<td>0.45</td>
<td>0.48</td>
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<tr>
<td>Porosity (%)</td>
<td>71</td>
<td>71</td>
<td>78</td>
<td>78</td>
<td>64</td>
<td>64</td>
<td>68</td>
<td>68</td>
</tr>
<tr>
<td>Moisture content (%)</td>
<td>1.69</td>
<td>2.5</td>
<td>1.71</td>
<td>2.6</td>
<td>3.13</td>
<td>3.4</td>
<td>2.22</td>
<td>2.12</td>
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<tr>
<td>pH(_{\text{H}_2\text{O}})</td>
<td>8.20</td>
<td>7.8</td>
<td>8.79</td>
<td>8.10</td>
<td>7.88</td>
<td>7.5</td>
<td>10.19</td>
<td>9.00</td>
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<td>EC(_{\text{H}_2\text{O}}) (ds m(^{-1}))</td>
<td>0.01</td>
<td>0.02</td>
<td>0.07</td>
<td>0.05</td>
<td>0.07</td>
<td>0.06</td>
<td>0.19</td>
<td>0.08</td>
</tr>
<tr>
<td>CEC (C-mol, kg(^{-1}))</td>
<td>53.96</td>
<td>66.59</td>
<td>52.20</td>
<td>66.90</td>
<td>36.28</td>
<td>56.06</td>
<td>30.37</td>
<td>30.83</td>
</tr>
<tr>
<td>pH(_{\text{BZe}})</td>
<td>3.0</td>
<td>1.2</td>
<td>5.6</td>
<td>3.2</td>
<td>1.6</td>
<td>1.3</td>
<td>4.4</td>
<td>3.7</td>
</tr>
<tr>
<td>Total OFGs (atom %)</td>
<td>45.59</td>
<td>49.95</td>
<td>16.79</td>
<td>29.64</td>
<td>57.31</td>
<td>75.54</td>
<td>9.22</td>
<td>23.46</td>
</tr>
<tr>
<td>Total AFGs (mmol g(^{-1}))</td>
<td>0.15</td>
<td>0.61</td>
<td>0.03</td>
<td>0.13</td>
<td>0.16</td>
<td>0.34</td>
<td>0.02</td>
<td>0.12</td>
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<tr>
<td>Total BFGs (mmol g(^{-1}))</td>
<td>0.01</td>
<td>0.00</td>
<td>0.03</td>
<td>0.02</td>
<td>0.04</td>
<td>0.03</td>
<td>0.11</td>
<td>0.11</td>
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<tr>
<td>(S_{\text{CO}_2}) (m(^2) g(^{-1}))</td>
<td>146</td>
<td>190</td>
<td>500</td>
<td>570</td>
<td>172</td>
<td>187</td>
<td>424</td>
<td>550</td>
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<tr>
<td>Total PV (cm(^3) g(^{-1}))</td>
<td>0.14</td>
<td>0.23</td>
<td>0.57</td>
<td>0.75</td>
<td>0.20</td>
<td>0.24</td>
<td>0.43</td>
<td>0.81</td>
</tr>
</tbody>
</table>


2.4 Soil

Quincy sandy (QS) soil was used in this study because it is present on nearly 285000 hectare in Washington, Oregon, and Idaho, and is an agriculturally important soil in the Pacific Northwest.
region of the US (NRCS, 2013). The sandy soil was collected, air-dried, and sieved through a 2 mm mesh. Some selected physical and chemical characteristics are shown in Table 2. To measure the soil zeta potential, 5 g of the soil was added to 100 ml of deionized water and agitated on an orbital shaker for 6 hours at 25°C. The aliquot of the supernatant was then collected (decanted from the container) and analyzed by Nano-Zetasizer 3000 (Malvern Instruments Ltd., Malvern, UK).

**Table 5.2 Selected physicochemical properties of the Quincy sandy soil**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bulk density (g cm(^{-3}))</td>
<td>1.49</td>
</tr>
<tr>
<td>Particle density (g cm(^{-3}))</td>
<td>2.62</td>
</tr>
<tr>
<td>Porosity (vol.%)</td>
<td>43.23</td>
</tr>
<tr>
<td>Moisture content (wt.%)</td>
<td>0.55</td>
</tr>
<tr>
<td>Water holding capacity (wt.%)</td>
<td>16.91</td>
</tr>
<tr>
<td>Organic Matter (wt.%)</td>
<td>0.75</td>
</tr>
<tr>
<td>pH(_{(\text{H}_2\text{O})})(^{(a)})</td>
<td>7.5</td>
</tr>
<tr>
<td>EC(_{(\text{H}_2\text{O})}) (ds m(^{-1})) (^{(a)})</td>
<td>0.08</td>
</tr>
<tr>
<td>CEC (C-mol, kg(^{-1}))</td>
<td>3.5</td>
</tr>
<tr>
<td>NH(_4^+) (mg N kg(^{-1}))</td>
<td>0.035</td>
</tr>
<tr>
<td>NO(_3^-) (mg N kg(^{-1}))</td>
<td>1.583</td>
</tr>
<tr>
<td>Zeta potential at pH 7</td>
<td>-23</td>
</tr>
<tr>
<td>Particle size (texture)</td>
<td></td>
</tr>
<tr>
<td>Sand (wt.%)</td>
<td>95.1</td>
</tr>
<tr>
<td>Silt (wt.%)</td>
<td>3.4</td>
</tr>
<tr>
<td>Clay (wt.%)</td>
<td>1.5</td>
</tr>
</tbody>
</table>

\(^{(a)}\) The pH and EC were measured in a 1:5 (mass/vol) dry-soil: de-ionized water.

---

**2.5 Bacterial Transport in Porous Media.**

Seventy-two column transport experiments were conducted to evaluate the transport behavior of pathogenic and nonpathogenic *E. coli* in biochar-amended soil columns. Three types of porous
media were used: (1) Quincy sandy (QS) soil; (2) mixtures of QS soil and each type of unoxidized biochars (eight types); and (3) mixtures of QS soil and each type of oxidized biochars (eight types). Prior to measurements, the QS soil was cleaned and washed five times in order to remove any residual fine particles that could interfere with measuring the bacterial concentration or that could clog the column tubing. The soil and biochar samples were sieved to uniform sizes with an average diameter of 500 \text{um}. Prior to each experiment, 20 \% of a biochar was mixed thoroughly with the soil and packed dry in columns (column diameter and length are 2.0 \text{ cm}, 10 \text{ cm}, respectively). Packed columns were acclimated to E. pure water (pH=8) for 10 pore volumes (1 PV = 11 \text{ mL}) using a peristaltic pump (Vera Manostat/Barnant). The absorbance of the effluent was measured after equilibration and values of zero further ensured that residuals of fine particles were removed. After equilibrating the packed columns, column experiments were conducted by pumping a bacterial suspension upward at 16 ml/min through each column. We used upward flows in order to eliminate the gravity force effects on transport and to displace air from most pores between biochar and/or soil particles within the column.

The bacterial suspension was allowed to flow through the column for at least 7 PV followed by a 5 PV of bacteria-free solution to elute all cells that were not retained on the grains of porous material. Samples of the column effluent were collected in 3.25-mL fractions using an automated fraction collector (Model CF1, Spectrum Chromatography). Subsequently, the bacterial concentration in the effluent was quantified using a spectrophotometer at 600 nm; any bacteria remaining in the column were assumed to be attached at interfaces within the porous material grains. Bacterial breakthrough curves (BTCs) were presented in a dimensionless form by dividing the outlet concentration (C) by the inlet concentration (C₀) and the C/C₀ fractions were plotted.
against PV values. Each column experiment was repeated at least three times in separate days using different cultures to ensure true replication.

2.6 Mathematical Modelling of the filtration efficiency

The steady-state breakthrough concentration of the bacterial cells was used to estimate the collision efficiency ($\alpha$) for each experiment. The collision efficiency is the fraction of striking bacteria that attach to the collector surfaces. The one-dimensional colloid filtration equation developed by Yao et al. (1971) was used to calculate $\alpha$ as:

$$
\alpha = \frac{-2d_c \ln(C/C_0)}{3(1 - \theta) \eta L}
$$

where $C$ and $C_0$ are the effluent and influent bacterial concentrations, respectively; $\theta$ is the porosity of the media; and $d_c$ is the collector diameter. $\eta$ is collector efficiency, taken as the sum of the physical forces affecting collisions: diffusion, effects of neighboring particles, London-van der Waals forces, interception, and gravitational settling. Hereafter, the single soil/biochar grain is termed as the collector while a bacterial cell is called particle. Table 5.3 summarizes the values of the parameters used in the calculations.
Table 5.3 Summary of parameters used in the models of 1D filtration theory

<table>
<thead>
<tr>
<th>Parameter</th>
<th>QS Soil</th>
<th>QS-PW350</th>
<th>QS-PW600</th>
<th>QS-PB350</th>
<th>QS-PB600</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>UO</td>
<td>OX</td>
<td>UO</td>
<td>OX</td>
</tr>
<tr>
<td>Porosity (θ)</td>
<td>0.452</td>
<td>0.575</td>
<td>0.575</td>
<td>0.587</td>
<td>0.564</td>
</tr>
<tr>
<td>Collector diameter (m)</td>
<td>0.050</td>
<td>0.069</td>
<td>0.068</td>
<td>0.074</td>
<td>0.071</td>
</tr>
<tr>
<td>Column length (m)</td>
<td>0.045</td>
<td>0.050</td>
<td>0.045</td>
<td>0.050</td>
<td>0.046</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value (C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Particle diameter (m) – <em>E. coli</em> O157:H7</td>
<td>1.16x10⁻⁶</td>
</tr>
<tr>
<td>Particle diameter (m) – <em>E. coli</em> K12</td>
<td>1.14x10⁻⁶</td>
</tr>
<tr>
<td>Gravitational constant (m/s²)</td>
<td>9.81</td>
</tr>
<tr>
<td>Approach Velocity (m/s)</td>
<td>52.203x10⁻⁴</td>
</tr>
<tr>
<td>Fluid dynamic viscosity (N.s/m²)</td>
<td>8.94x10⁻⁴</td>
</tr>
<tr>
<td>Fluid density (g/m³)</td>
<td>997.1</td>
</tr>
<tr>
<td>Bacterial cell density (g/m³)</td>
<td>166.5x10⁻³</td>
</tr>
<tr>
<td>Bacterial diffusion coefficient (m²/s)</td>
<td>0.001316872</td>
</tr>
<tr>
<td>Hamaker constant (J)</td>
<td>1x10⁻²⁰</td>
</tr>
<tr>
<td>Poltzmann constant (J/K)</td>
<td>1.38x10⁻²³</td>
</tr>
<tr>
<td>Cross sectional area (m²)</td>
<td>0.0346</td>
</tr>
</tbody>
</table>

(a) UO= unoxidized biochars; (b) OX=oxidized biochar; (C) these values were adapted from (Abu-Lail and Camesano, 2003; Bradford et al., 2006; Tindall et al., 2008; Jean M F Martins et al., 2013)
3. Results

3.1 Zeta potentials of bacteria, soil, and biochars

The ζ-potentials of the bacteria, soil, and biochars under different pH conditions are shown in Figure 5.1. Our results indicate that all were negatively charged under tested experimental conditions (pH between 7.5 and 8.3). Therefore, the repulsive electrostatic forces will be important for all the systems studied. For soil (Fig 5.1A), the ζ-potential decreases linearly with increasing pH. Similarly, the ζ-potential of pine bark-derived biochar produced at 600°C whether oxidized or not (Fig. 5.1F) decreased linearly with increasing pH. In general, the ζ-potentials for almost all the biochars monotonically decrease as the pH of the environment increases. The ζ-potentials of the oxidized biochars were more negatively charged than the un-oxidized ones.

When the bacterial strains are considered, pathogenic *E. coli* O157:H7 was less negatively charged than the non-pathogenic *E. coli* K12. This result is very important because it suggests that for the systems studied the repulsive electrostatic forces will be weaker for the pathogenic *E. coli* O157:H7 and consequently the likelihood to be adsorbed on the surface of the biochars will be higher. In the range of pH from 5.0 to 8.0, ζ-potentials of both strains were negative and generally became more negative as the pH increased (Fig. 5.1B). In dramatic contrast, the ζ-potential of *E. coli* K12 increased sharply in the pH range (<4.5) while the ζ-potentials *E. coli* O157:H7 increased slightly or remained constant at the same range. Our results indicate that the ζ-potentials of the non-pathogenic *E. coli* K12 were more sensitive to pH changes compared to the pathogenic strain *E. coli* O157:H7.
Figure 5.1 Average values (n=3) of zeta potentials for the soil, bacteria, unoxidized (UO) and oxidized (OX) biochars. Error bars representing standard deviation are included for some samples but are too small to be seen on the graph.
3.2 Effect of Biochar concentration on E. coli transport

Figure 5.2 shows breakthrough curves (BTCs) collected for *E. coli* O157:H7 and K12 transport using pine wood chars pyrolyzed at 600°C (PW-600-UO) at concentrations (0, 1, 5, 10, and 20 wt.%). As shown, all BTCs are symmetrical, displaying a relatively sharp breakthrough front and high degree of tailing, except in the case of O157:H7 with 20 wt.% of biochar concentration. In fact, the two bacterial strains exhibited different behaviors to biochar additions. The pathogenic strain (O157:H7) was more susceptible to biochar addition compared to the non-pathogenic strain (K12) (Figure 5.2). This is an indication of stronger interactions between the surface of the biochar and the pathogenic bacteria.

For both strains investigated, steady state was achieved after four pore volumes. For pore volume less than 4, biochar concentration did not have significant effects on bacterial transport. However, at higher pore volumes (PVs between 4 and 9), the addition of only 1 wt.% biochar to sand influenced bacterial transport in the column for both strains. The transport of *E. coli* O157:H7 and K12 were both reduced significantly ($p < 0.05$) when 5 wt.% biochar was added to sand compared to the control (0% biochar). Bacterial transport was very similar for both strains when biochar concentrations $\geq$ 5 wt.. With further increase in biochar concentration (from 5 to 20 wt.%), BTCs obtained for both strains were quite different. For *E. coli* O157:H7, steady state plateaus were significantly different with most reduction in transport when 20 wt.% biochar was used. On the contrary, *E. coli* K12 steady states were independent of biochar concentration in the range of 5, 10 and 20% concentrations ($p > 0.05$).
The steady state values of $C/C_0$ decreased linearly with increased biochar concentrations for *E. coli* O157:H7. However, the steady state values reached a plateau after 5 wt.% biochar for the *E. coli* K12 strain. Throughout the washing phase, $C/C_0$ fell sharply to near zero values in all experiments within 6 and 9 pore volumes for *E. coli* K12 and O157:H7, respectively.
Figure 5.2 Breakthrough curves for *E. coli* O157:H7 (A) and K12 (B) for column study evaluating PW600 biochar application rate effects on bacterial transport. Error bars indicate standard deviation obtained using the triplicate experiments. C) A scatter graph that shows the relationship between the biochar concentration (% wt) and the steady state transport of bacteria (C/C₀) for *E. coli* O157:H7 (black-filled circles) and *E. coli* K12 (white-filled circles). The solid line represents the relationship obtained for *E. coli* O157:H7 was linear with Y=-0.0395X+0.9276, r²=0.9931.
3.3 The effects of low-temperature biochars on bacterial transport

Figure 5.3 shows the breakthrough curves measured for the transport of *E. coli* O157:H7 and *E. coli* K12 through a Quincy sand (QS) mixed with either low-temperature pine wood- or bark-derived biochars at an application rate of at 20 wt.%. As was observed above, using 20 wt.% biochar results in almost complete attenuation of bacterial cells in the sand column. As such, only 20 wt.% biochar concentration will be investigated in this section of the paper.

Three different BTC were collected for each bacterium. The first was with sand alone, the second and third were with oxidized (OX) or un-oxidized (UO) biochars. The shapes of the BTCs varied as a function of conditions used. For example, Figures 2A, 2C and 2D showed symmetrical BTCs with relatively high-degree front and sharp tailing; this shape was most pronounced in the case of *E. coli* O157:H7 in QS soil control or oxidized PW biochar (QS-PW350-OX), and in all cases of *E. coli* K12 transport. Such BTCs indicate that the rates of adsorption and desorption of bacteria from biochar-mixed soils are similar. In comparison, Figure 3B which represents the transport kinetics of *E. coli* O157:H7 pine-bark wood with or without biochar was asymmetrical, exhibiting high to semi-low degrees of tailing. In general, Figure 3B indicates that the adsorption rates of bacteria are smaller than the desorption rates of bacteria. Figure 3A also showed horizontal-like shape; indicating strong and irreversible attachment of *E. coli* O157:H7 bacteria to the sand-UO PW biochar mixture.

Our results revealed that feedstock source and oxidation of low-temperature biochar had a relatively minor influence on the transport of *E. coli* K12 with almost no bacterial retention in the
sand columns. In dramatic contrast, both feedstock source and oxidation status of the biochar significantly altered the transport of *E. coli* O157:H7. The pine wood-derived biochar had highest retention relative to all other treatments. Oxidation of both biochars promoted the transport and decreased attenuation.

**Figure 5.3** Breakthrough curves of *E. coli* O157:H7 and K12 for experiments in columns packed with low temperature biochars [PW350 (A), PB350 (B)] and [PW350 (C), and PB350 (D)], respectively. Error bars indicate standard deviation measured between the triplicate experiments.
3.4 Effects of high temperature biochar on *E. coli* transport

Additional experiments were performed in columns packed with mixtures of Quincy sand soil and pine-wood or pine-bark derived biochars produced at 600°C. As can be seen from Figure 5.3 (A, B, C and D), the attenuation of *E. coli* O157:H7 and *E. coli* K12 increased significantly (*p* < 0.05) when high temperature pine-wood biochars were added. A similar trend of increased attenuation of bacteria was observed with pine-bark derived biochar (QS+PB600-UO) for *E. coli* O157:H7 but not for *E. coli* K12. Figure 5.3 also shows that, for both bacteria, oxidized biochars promoted the transport.
Figure 5.4 Breakthrough curves of *E. coli* O157:H7 and K12 for experiments in columns packed with [PW600 (A), PB600 (B)], and [PW600 (C), and PB600 (D)], respectively. Error bars indicate the standard deviation measured between the triplicate experiments.
3.5 Collision efficiency as affected by biochar type and bacterial strain

To better understanding the influence of biochar addition on bacterial retention, values of the attachment efficiency ($\alpha$) were calculated from the breakthrough curves in Figures 1, 2, and 3, and are presented in Table 4. Comparison of the bacteria attachment efficiencies in table 4 reveals a significant influence of biochar additions and oxidation on the bacterial retention behavior. *E. coli* O157:H7 exhibited the highest attachment efficiency ($\alpha=0.311$) when unoxidized PW350 biochar was added into the soil column, and the lowest value ($\alpha=0.005$) with soil alone. However, the highest attachment efficiency ($\alpha=0.078$) for *E. coli* K12 was observed when oxidized PB350 biochar was added. Overall, the calculated $\alpha$ value was greater for transport of *E. coli* O157:H7 than *E. coli* K12 in the biochar-amended soil columns.

**Table 4.** Values of Steady-state $C/C_0$ and calculated collision efficiencies

<table>
<thead>
<tr>
<th></th>
<th><em>E. coli</em> O157:H7</th>
<th></th>
<th><em>E. coli</em> K12</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SS $C/C_0$</td>
<td>$\alpha$</td>
<td>SS $C/C_0$</td>
</tr>
<tr>
<td>QS Soil alone</td>
<td>0.960</td>
<td>0.005</td>
<td>0.953</td>
</tr>
<tr>
<td>QS-PW350-UO</td>
<td>0.011</td>
<td>0.311</td>
<td>0.857</td>
</tr>
<tr>
<td>QS-PW350-OX</td>
<td>0.882</td>
<td>0.026</td>
<td>0.921</td>
</tr>
<tr>
<td>QS-PW600-UO</td>
<td>0.143</td>
<td>0.148</td>
<td>0.714</td>
</tr>
<tr>
<td>QS-PW600-OX</td>
<td>0.451</td>
<td>0.135</td>
<td>0.777</td>
</tr>
<tr>
<td>QS-PB350-UO</td>
<td>0.139</td>
<td>0.164</td>
<td>0.865</td>
</tr>
<tr>
<td>QS-PB350-OX</td>
<td>0.680</td>
<td>0.094</td>
<td>0.812</td>
</tr>
<tr>
<td>QS-PB600-UO</td>
<td>0.886</td>
<td>0.006</td>
<td>0.837</td>
</tr>
<tr>
<td>QS-PB600-OX</td>
<td>0.279</td>
<td>0.184</td>
<td>0.880</td>
</tr>
</tbody>
</table>

$SS\ C/C_0$: Steady-state $C/C_0$; $\alpha$: Collision efficiency
4. Discussion

The bulk and surface properties of biochar were analyzed in order to explain their potential roles in *E. coli* transport when biochar added to a Quincy sand. Although these properties have been already described in Chapters 1 and 2, the results are briefly summarized in table 5.1. It was observed that the production temperature, feedstock origin, and oxidation of biochar resulted in significant changes in porosity, elemental composition as well as surface charge and functionality. This study aimed to enhance our limited understanding of bacterial transport in biochar-amended sandy soil by providing experimental breakthrough data combined with numerical modelling.

4.1 Potential interaction mechanisms between biochar and *E. coli*

Bacterial adhesion is known as a complex-multifactorial phenomenon that interferes with the properties of surface material and those of the bacteria, and the environment where the adhesion takes place (Desrousseaux et al., 2013).

4.1.1 DLVO and X-DLVO forces-related effect

The observed reduction of *E. coli* transport through biochar-amended columns, compared to the unamended Quincy sand columns, could be attributed to the bacterial attachment processes promoted by physicochemical interactions between bacterial and biochar surfaces. According to the Derjaguin-Landau-Verwey-Overbeek (DLVO) theory (Hermansson, 1999), a particle (i.e. *E. coli* cell) may experience a combination of attractive van der Waal forces and repulsive electrostatic forces during its approach to the collector (i.e. biochar) grains. In biochar-amended columns, considerable increases in *C/C₀* ratios and collision efficiencies (*α*) were observed,
possibly due to an increase in overall attractive forces between the biochar surface groups and bacterial surface components compared with the interactions with the sandy soil. As such, electrostatic interactions between an *E. coli* cell (particle) and collector (biochar) grains are expected to be favorable for attachment onto bare (unoxidized) collectors and unfavorable for attachment onto oxidized collectors.

In contrast to the probable attractive forces between *E. coli* and biochar surfaces, repulsion due to negatively charged surfaces may have occurred. At the pH of these experiments (pH≈ 8), repulsive electrical double-layer interactions are expected when *E. coli* cells come into close proximity to collectors. Under these conditions, biochars with a greater negative charge will experience greater difficulty in retaining *E. coli* cells. To verify this assumption, we evaluated the effect of oxidized biochars, which exhibited higher contents of oxygen functional groups and lower zeta potentials, on the transport behavior of *E. coli*. The results (figures 5.3A, 5.3B, 5.4A, and 5.4B) showed that oxidation diminishes the capacity of the biochar to retain *E. coli* by adding more negatively charged sites on the surface and, thus, creating more repulsive electrostatic interactions. For instance, the enhanced *E. coli* O127:H7 elution upon increasing oxygen content of low-temperature biochars after oxidation is consistent with electrostatic repulsion being important (Kinoshita et al., 1993).

The Lewis acid–base interaction might be another important mechanism for the *E. coli* attachment on biochar surface. Theoretically, the amino group of the *E. coli* cell wall can act as Lewis-base while the oxygen functional groups of biochar serve as Lewis-acids. This concept is somehow reasonable for explaining the interaction between *E. coli* O157:H7 and low temperature biochars (figs., 5.3A and 5.3B), in which the amount of O-containing functionalities was higher
comparable to its high temperature counterparts (Table 1). The presence of the nitrogen atoms in the *E. coli* cell wall and the lone pair of electrons on the oxygen atoms of biochar function groups might be the reason for surface specific interactions between *E. coli* and biochar. However, opposite trends were observed when the same biochars were oxidized and used to attenuate the transport of *E. coli* O157:H7. Here, we postulate that the attractive interactive forces might not be able to exceed the repulsive electrostatic interactions associated with the high negative charges in oxidized biochars. Generally, this assumption is in accordance with the findings obtained by Rivera-Utrilla et al. (2001) and Wang et al. (2012).

### 4.1.2 Non-DLVO forces-related effect

Non-DLVO forces from hydrophobic interactions provide an additionar explanation for the effectiveness of biochar on attenuation of *E. coli* transport through the sand column. It is well known that surface hydrophobicity correlates positively with bacterial attachment (Chen and Walker, 2012). According to Abit et al. (2012) hydrophobic attraction is expected to be much greater between a bacteria and a biochar particle than bacteria and sand. When high-temperature biochars were added to the soil columns, the collision efficiencies were increased and consequently more bacteria were retained comparable to sand alone. It is important to mention here that high temperature biochars exhibited greater hydrophobic characteristics than other biochars, as deducted from their higher carbon content, lower volatile matter and (O + N)/C ratio (table 1). These observations are generally consistent with the results obtained by Foppen and Schijven (2005). In case of figure 5.2, bacterial attachment can also be defined in terms of hydrophobic attraction; the greater collision efficiency at higher concentration of PW600 biochar suggests a hydrophobic effect, due to higher net organic carbon, was of primary importance.
Moreover, bacterial surfaces contain hydrophobic substances such as the lipid-A part of lipopolysaccharides giving them the ability to exhibit hydrophobic interactions (Foppen and Schijven, 2005, 2006)

4.2 Effect of biochar concentrations

As shown above, the mobilized fraction of both *E. coli* strains was smaller in biochar-amended columns compared to the control columns. The collision efficiencies (\(\alpha\)) increased considerably from 1.2x10^{-5} to 6.7x10^{-4} and slightly from 1.5x10^{-5} to 1.2x10^{-4} for *E. coli* O157:H7 and *E. coli* K12, respectively, as biochar concentration increased from 0 to 20% (wt./wt.). Noticeably, at or below 5% of biochar, the collision efficiencies were identical between experiments with varying bacterial strains. This could be linked to the number of attachment sites being limited comparable to particle (i.e., bacterial cell) density used (0.05 OD), thus more bacteria were eluted and recovered. However, when the biochar concentration was increased, additional particles were attached on the collector surface (i.e., biochar grains) due to an increase in the number of available attachment sites. From this point of view, we hypothesize that blocking did not occur in the system even at low biochar concentration, but straining might be happened, especially in case of *E. coli* K12. As described in the literature, blocking occurs when particles attached to the collector surface prevent the retention of additional particles, while straining occurs when particles become physically trapped in pores that are too small to pass through (Syngouna and Chrysikopoulos, 2011). The breakthrough curve shapes are generally consistent with this hypothesis. The straining phenomenon is important for particle size to collector size ratios (\(dp/dc\)) of 0.003 to 0.017 (Bradford et al., 2006). In our study, the \(dp/dc\) ratio is 0.004 and 0.002 for *E. coli* K12 and O157:H7, respectively, suggesting that physical straining of the *E. coli* K12 may contribute to the
collision efficiencies. This finding is consistent with previous column studies of Abit et al., (2012), and Bolster and Abit (2012) suggested straining at intra- or inter-pores of biochar-sand mixtures.

**Figure 5.5** The relationship between the collision efficiency (α) and biochar concentration (wt. %) for *E. coli* O157:H7 (black-filled circles) and *E. coli* K12 (white-filled circles). The solid line represents the relationship obtained for *E. coli* O157:H7.

**4.3 Biochar type-dependent variations in attachment of *E. coli***

Bacterial retention was strongly influenced by biochar preparation conditions including feedstock origin, pyrolysis temperature, and post-pyrolysis oxidation, which affected several characteristics and, therefore, the electrostatic interactions. These characteristics include surface charge, degree of hydrophobicity, Lewis acid-base character, surface roughness, hydrogen-bonding capacity, pH and porous structure; the influence of these characteristics on bacterial
attachment is addressed more specifically elsewhere (Powelson and Mills, 1998; Abu-Lail and Camesano, 2003; Walker et al., 2004; Bolster et al., 2009; Jean M. F. Martins et al., 2013)

4.3.1 Effect of biochar pH

For attachment to occur, the pH of pore water should be an intermediate between the pH\textsubscript{pzc} values of the biochar and \textit{E. coli}. The importance of pH comes from the fact that pH of solution affects the surface charge of both bacteria and carbon material, therefore, their electrostatic interactions (Rivera-Utrilla et al., 2001). As an example, our results showed that addition of unoxidized biochars enhanced the \textit{E. coli} K12 elution upon raising pH from \(\approx 7.5\) to 8.5 (The pH of effluent from the soil alone and unoxidized biochars, respectively; data not shown), which is consistent with electrostatic repulsion being important (Kinoshita et al., 1993). Moreover, it was observed that biochar oxidation resulted in pH decrease due to the deposition of oxygen functional groups on carbon which increased its acidic character (see Chapter 4). From this point of view, the electrostatic repulsion in oxidized biochar columns is expected to be lower than that of unoxidized biochar columns due to their lower pH values (table, 5.1). However, the bacterial retention in the unoxidized biochar columns was greater than in the soil or oxidized biochar columns; in fact, the maximum retention was in accordance with the addition of unoxidized biochars, regardless of bacterial strain. This could be attributed to a complex attachment mechanism involving hydrophobic attraction or other non-DLVO forces.
4.3.2 Effect of biochar mineral content

The accumulation of ash content and mineral elements increased with the increase in pyrolysis temperature and varied in accordance with feedstock origin. According to our findings in Chapter 1, cations, such as Ca$^{2+}$, Mg$^{2+}$, K$^+$, and Na$^+$, were much higher in pine bark biochars than pine wood biochars. The observed reduction of *E. coli* transport through the pine bark biochar columns (figures 5.3B, 5.3D, 5.4B, and 5.4D) may be explained by increased bacterial hydrophobicity and decreased electrostatic repulsive forces promoted by cations or metallic oxides (present in the mineral matter of biochar). Rivera-Utrilla et. al. (2001) observed an apparent bacterial adsorption of the activated carbon that was greater in the presence of Ca$^{2+}$ and Mg$^{2+}$; contrariwise, this adsorptive behavior was reduced considerably after demineralization of carbon. The authors suggest that the presence of aqueous cations enhance bacterial cell surface hydrophobicity which is known to have a positive influence on cell-substrate adsorption. Furthermore, several researchers (Coughlin et al., 1983; Ferris and Beveridge, 1986; Chen and Walker, 2007; Cai et al., 2013; Zhang et al., 2014) found that cations, especially divalent cations, neutralized the anionic charge of a bacterial cell and make its planar charge distribution heterogeneous, thus decreasing repulsive electrostatic forces. Neutralization of surface charge by aqueous cations might have occurred for either bacterial cell or biochar surfaces, which is possibly due to the complexation of divalent cations associated with these surfaces. Our results suggest that bacterial attachment capabilities of pine bark biochars might be linked to their high contents of divalent and monovalent cations comparable to pine wood biochars.
4.3.3 Effect of biochar surface oxygen content

In the case of oxidized biochars, slight increases in collision efficiencies ($\alpha$) were observed, indicating potential interactions between both biochar and E. coli cell surfaces. These interactions might have occurred due to the fact that bacterial cell surfaces are heterogeneous in composition as well as biochar surfaces. This means that some attraction forces could be initiated between certain cellular components and biochar surface groups improving the overall attachment efficiencies. Therefore, when such an interaction is considered as a possible attachment mechanism, the accessibility and quantity of biochar surface functional groups are of paramount importance. The collision efficiencies ($\alpha$) of oxidized high-temperature biochars, specifically, might be linked to the integrated contributions of both biochar surface functionality and internal porosity. Relatively, biochars with low pore volumes and surface areas (i.e., PW350-OX and PB350-OX) did not show improvements in $\alpha$ value after oxidation.

4.3.4 Effect of surface area and pore size

Relative to the Quincy sand (QS) soil, biochar is a highly porous material; the surface area of biochars used ranged between 146 to 570 m$^2$ g$^{-1}$ (for PW350-UN and PW600-OX, respectively) while QS soil surface area is only about 0.032 m$^2$ g$^{-1}$ (Lehrsch and Sojka, 2011). Ideally, addition of 20% biochar by weight has a potential to increase the net surface area by a factor of 2923 and 11406 of QS-PW350-UO and QS-PW600-OX columns, respectively. Nevertheless, this statement
is insufficient since the estimated surface area of biochar corresponds to pores in the nanometer range (micropores), which may not be accessible to *E. coli*. Based on the results obtained by Rivera-Utrilla et al., (2001), it was concluded that only carbon pores with a diameter greater than $3 \times 10^3$ nm (3 micrometers) would be available for bacterial attachment. Based on the micropore size distributions measured in Chapters 1 and 2, biochar micropores are generally smaller than *E. coli* size, regardless the cavities indicated by SEM analysis. Thus, bacterial entrapment into biochar micropores measured by CO$_2$ adsorption isotherms was not significant in our experiments, as indicated by weak correlations between collision efficiency and biochar surface area or micropore volume (table, 5.1). Our results are in accordance with other studies (Rivera-Utrilla et al., 2001; Chen and Walker, 2012).

**4.4 Strain-dependent variations in attachment of *E. coli***

Our results showed that the extent of collision efficiency depends not only on the type of biochar, but also on the bacterial strains. The $a$ values were higher for *E. coli* O157:H7 in comparison to *E. coli* K12, which is in accordance with findings obtained by (Chornewich, 2009a, 2009b). This is likely due to variations in the composition of the strain’s external structure, which in turn affects other cellular surface properties (Pachepsky et al., 2008; Desrousseaux et al., 2013). Figure (figure 5.1) provides an example of the dissimilarities between both pathogenic and non-pathogenic *E. coli* strains. Zeta potentials of *E. coli* K12 were lower and more sensitive to pH changes than those of *E. coli* O157:H7. Unambiguously, *E. coli* K12 exhibited lower zeta potentials than *E. coli* O157:H7 at pH $\geq 3.3$, conversely, *E. coli* O157:H7 had lower zeta potentials than *E. coli* K12 at pH $< 3.0$. According to the available literature (Sherbet, 1978; McLean et al.,
1992; Pachepsky et al., 2008), the functional groups on bacterial cell surfaces exhibit an almost anionic character, metal binding, and buffering capacity over a wide range of pHs. Additionally, these groups are mainly presented in the peptidoglycan, phospholipids, and lipopolysaccharides of Gram-negative bacteria (i.e., *E. coli*). According to other studies, the preferential attachment of *E. coli* strains to collector grains could also be attributed to other bacterial surface characteristics including; hydrophobicity (Kinoshita et al., 1993; Foppen and Schijven, 2005), N:C and O:C ratios (Desrousseaux et al., 2013), lipopolysaccharide content (Gómez-Suárez et al., 2002; Abu-Lail and Camesano, 2003), growth stage (Walker, Hill, et al., 2005; Walker, Redman, et al., 2005) and cell size (Walczak et al., 2011). It is important to mention that most characterization techniques of bacterial surface still depend on averaging of surface properties over population of cells which does not take the influence of localized structure into account (Abu-Lail and Camesano, 2003).

### 4.5 Role of the ζ-potential and isoelectric point

Under tested experimental conditions, ζ-potentials of the soil, bacteria, and biochars were all negative and generally became more negative with greater pH (fig. 5.1). This behavior can be explained by the deprotonation of surface functional groups in the alkaline region, consistent with the existing literature (Wang et al., 2013). For the Quincy sand, the ζ-potential increased slightly with increasing pH indicating that the soil surface does not contain abundant dissociable functional groups. For biochars, the magnitude of the ζ-potential increased with increased pyrolysis temperature and decreased after oxidation. These trends are likely due to the changes in density of carboxylic functional groups (Wang et al., 2013). In addition, oxidized biochars were more negatively charged than the unoxidized counterparts, consistent with increased quantities of oxygenated-functional groups on their surfaces (Chapter 4). For bacteria, ζ-potentials of *E. coli*
K12 were more negative than *E. coli* O157:H7 in the range of pH from 3 to 8.5, indicating that there are more negative charges on *E. coli* K12 than those on *E. coli* O157:H7. Furthermore, this result is in agreement with the published literature (Ferris and Beveridge, 1986; Harter et al., 2008; Pachepsky et al., 2008; Chornewich, 2009; Cai et al., 2013).

The isoelectric point (IEP), where $\zeta = 0$ was determined via multiple measurements of $\zeta$ as a function of equilibrium pH. Trends in $\zeta$-potential for *E. coli* O157:H7 and *E. coli* K12 suggest isoelectric points of 4 and 3.8, respectively, whereas the isoelectric point of the sand occurs at below pH 1. Isoelectric points of altogether biochars were occurred in pH ranges between 5.6 and 1.6 and between 3.7-1.2 for the unoxidized and oxidized biochars, respectively. Oxidation of biochar causes the IEP to shift to a lower pH and the $\zeta$-potential in the alkaline range to decrease; this is due to the increased number of surface acidic groups. As seen in figure 4, biochars, *E. coli* strains, and the Quincy sand were all negatively charged, so theoretically attachment would be prevented by repulsive electrostatic energy giving lower collision efficiencies and higher $C/C_0$ values. However, at the pH of these experiments (pH $\approx 8$), considerable bacterial retention were observed, especially in unoxidized biochar-soil columns. Rivera-Utrilla et al., (2001) reasoned that the increase in bacterial attachment of biochar in comparison to soil alone is probably due to the van der Waal's and non-DLVO’s attractive interactions that may exceed the repulsive electrostatic interaction.
5. Conclusions

Bacterial attachment of biochar is mostly controlled by van de Waal’s forces, non-DLVO’s attractive interactions and the repulsive electrostatic interaction between the negatively charged *E. Coli* and the biochar surfaces. Our studies confirm the importance of the electrostatic interactions controlling *E-coli* adsorption on biochar surfaces. All the materials studied (*E.coli*, biochars and Quincy sand) were negatively charged. The poor retention of the non-pathogenic K-12 E.coli can be explained by their more negatively charged surfaces. The decrease in retention when oxidized biochars were used was explained by the increase of negative charges on the surface of these chars. One of the recommendations of this work is that the development of positively charged biochars could be a viable way to enhance the retention of *E-coli* in soils. The effects of biochar micrometer pore size and the effect of surface chemistry on the non-DLVO’s forces warrants further investigation.

6. Acknowledgments

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7. References


CHAPTER 6. UNDERSTANDING THE ROLE OF BIOCHAR
POROUS STRUCTURE AND SURFACE CHEMISTRY
IN AUGMENTING HYDROLOGIC
PROPERTIES OF A
SANDY SOIL

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Abstract

This paper reports results on the effect of biochar porous structure and surface chemistry on the hydrological properties of a Quincy sand amended with biochars. The biochars studied were produced from Pine Wood and Pine Bark at temperatures of 350°C and 600°C. The resulting materials were then oxidized under air at 250°C to generate oxygenated functional groups on the surface. All biochars were thoroughly characterized (surface and bulk properties) and their hydrological properties measured after addition to a Quincy sand. The soil was collected, air-dried,
and sieved through a 2 mm mesh. The bulk density, porosity, pH, EC, CEC, and particle size distribution were determined. Twenty-seven microcosms were prepared for this study to examine the effect of biochar on hydro-physical properties of the Quincy sand. Each biochar was thoroughly mixed with the soil at 20 g kg$^{-1}$. Bulk density, porosity, organic matter, pH, and EC were determined for biochar-soil mixtures. The field capacity, wilting point and total available soil moisture of the biochar/Quincy sand mixtures were measured for both dry and wet ranges. The soil water potentials and soil water contents were fitted using the model of Van Genuchten. Pearson’s correlation analysis were conducted to identify potential relationships between selected biochar properties and soil water retention characteristics. The biochar produced from PW was more hydrophilic than the biochar produced by PB. The biochars became more hydrophobic as the temperature increased due to the reduction of oxygenated functional groups on the surface. The oxidation increases the content of oxygenated functional groups on the surface and consequently reduces its hydrophobicity. Oxidized biochars exhibited better wettability in comparison with unoxidized biochars. Over a wide range of soil water potentials, oxidized biochar-soil mixtures held significantly more water than the unoxidized biochar-soil mixtures except at the saturation region between -0.1 and -5 kPa of $\psi$. The magnitude of the effect of pyrolysis temperature and feedstock source of biochar was found to vary in some way over the range of soil water potentials. The impact of low pyrolysis temperature biochar was somehow lower on water retention than biochar produced at high temperature, but the difference was not significant. Soil water contents at different matric potentials were significantly inter-correlated (P <0.01) and correlated with bulk densities of biochar-amended soil samples. The bulk density is controlled by the volume occupied by the internal cavities in the biochar. There was significant correlations observed between total acidic functional groups on biochar surface and water contents at different matric potentials.
1. Introduction

Biochar application to soils is not a new concept; Biochar was used successfully by generations of indigenous farmers in the Amazon basin (Maia et al., 2011). Accumulation of bulky quantities of biochar increased soil agronomic quality and formed what is known as Amazonian Dark Earths (or *Terra Preta*) which are still highly valued soils for agricultural and horticultural use (Verheijen et al., 2010). Biochar as a term is reserved for any carbon-enriched material that has been chemically and structurally altered through thermo-decomposition via anaerobic pyrolysis and that is used as a soil amendment (Forbes et al., 2006; Lehmann et al., 2006). Biochar properties depend on the original biomass (chemical composition, ash composition, biomass size), the production conditions (temperature, heating rate, residence time, oxidative conditions ... etc.), the pre-treatment procedures (drying, crushing), and the post-treatment processes (activation method) (Copeland et al., 2008; Lehmann and Joseph, 2009).

Use of biochar is a sustainable option to provide long-lasting improvements in soil fertility (Lehmann et al., 2003; J. M. Novak et al., 2009), especially in sandy soils where sustainable agriculture faces large constraints due to low water holding capacity, and high leaching of soil nutrients (Uzoma et al., 2011). Because of its ability to retain nutrients and to improve soil water holding capacity, biochar soil application can be used to overcome some of the limitations faced during sandy land farming (such as the need for large use of artificial fertilizers and intensive irrigation) providing a pioneer soil management option for these conditions. Positive effects of biochar on soil properties and plant growth in sandy soils are well documented (Uzoma et al., 2011; Basso et al., 2013). Recent studies have shown that biochar soil additions increase pH of acidic soils (J. Novak et al., 2009; Sika, 2012; Basso et al., 2013), enhance cation exchange
capacity (CEC) (Liang et al., 2006; Uzoma et al., 2011; Basso et al., 2013), increase soil water-holding capacity (Uzoma et al., 2011; Sika, 2012), modify soil bulk density (Basso et al., 2013), and increase exchangeable basic cations (Sika, 2012).

While greater scientific attention has resulted in an increasing number of biochar publications, the literature pertaining to sandy soil application of biochar is limited. There is currently limited understanding of the influence of biochar on hydrological properties of sandy soils, and in particular the effect of biochar surface chemistry on the capacity of these materials to hold water. Effects of biochar porous structure and surface properties on hydro-physical properties of sandy soils have not been fully studied. Thus, there is a need for further research addressing how the biochar affects some of the limiting factors of agricultural production in sandy soils. Hence, the goal of this study is to evaluate the effect of biochar bulk and surface properties on hydro-physical properties of Quincy sandy soil-biochar blends.

2. Material and Methods

2.1 Biochar and Soil preparation

Biochars were produced, oxidized, and characterized as described in Chapters 3 and 4. Quincy sandy soil was collected and characterized as described in Chapter 5.
2.2 Experimental design:

Twenty-seven microcosms were prepared for this study to examine the effects of biochar on hydro-physical properties of a Quincy sand. Each biochar was thoroughly mixed with the soil at a rate of 20 g kg\(^{-1}\). The mixing rate was calculated by assuming 15 cm and 1.5 g cm\(^{-3}\) for soil depth and bulk density, respectively. Three replicates of 30 g of each mixture were then packed into plastic containers. Bulk density, porosity, organic matter, pH, and EC were determined for biochar-soil mixtures using the methods described for the biochar. These mixtures were abbreviated as QS, QS-HP350-UO, QS-HP600-UO representing Quincy sandy soil alone, soil with unoxidized HP biochar produced at 350\(^\circ\)C (HP350), soil with unoxidized HP biochar produced at 600\(^\circ\)C, respectively. The same abbreviation were followed for soil with unoxidized PW and PB biochars, and for oxidized samples but with an additional OX abbreviation.

2.3 Soil hydraulic measurements:

To evaluate the influence of biochar on the water relations of a sand, consideration must be given to the effect on field capacity, wilting point and total available soil moisture. In the present study, soil water retention was measured on intact soil microcosms for both dry and wet ranges. Tensoimeters T5 (UMS GmbH, Munich, Germany) were used to measure matric potential above -100 kPa, whereas a WP4C dew point potentiometer (Decagon Devices, Inc., Pullman, WA) was used to measure water potential below -100 kPa. For wet range, the soil microcosms were water-saturated from the base, and allowed to reach equilibrium, water potentials and water content were measured. The effects of osmotic potential are generally considered negligible when only liquid
water flow is considered; however, it is an important parameter for soil microbial processes (e.g. Nitrification and ammonification) as well as plant root activity (Low et al., 1997). In this study, osmotic potential was calculated by multiplying EC (dS m$^{-1}$) by -36 (Kpa). Volumetric water content was calculated as follow:

$$\theta = u \frac{\rho_b}{\rho_w} \quad (1)$$

Where $\theta$ is the volumetric water content (cm$^3$ cm$^{-3}$), $u$ is the gravimetric water content (g g$^{-1}$), $\rho_b$ is the soil bulk density (g cm$^{-3}$), and $\rho_w$ is the density of water (assumed to be 1 g cm$^3$).

In order to determine permanent wilting point ($\theta_{PWP}$), a simple indirect method of Decagon Devices, Inc., Pullman, WA was followed using the WP4C. Air-dried samples of the Quincy sand were assumed to have 0.008 g g$^{-1}$ and 0.003 g g$^{-1}$ water contents at -1.5 MPa and air dry condition, respectively. To reach $\theta_{PWP}$, a small amount of water was added to air-dried soil according to the following calculation:

$$M_w = M_{ad} \frac{w-w_{ad}}{1-w_{ad}} \quad (2)$$

Where $M_w$ is mass of water to add (g), $M_{ad}$ is the mass of air dried soil sample (g), $w$ is the assumed sand water content at -1.5 MPa (g g$^{-1}$), and $w_{ad}$ is the assumed mass of water for air dried sand samples(g g$^{-1}$). After equilibration, water potential was measured using WP4C, and water content was determined using the oven dried weight method. The gravimetric water content at -1.5 MPa was computed with the following equation:

$$w_{-1.5} = w_m \frac{\ln\left(-\frac{1000}{-1.5}\right)}{\ln\left(-\frac{100}{\psi_m}\right)} \quad (3)$$
Where \( w_{-1.5} \) is the gravimetric water content at -1.5 MPa (g g\(^{-1}\)), \( w_m \) is the measured mass water content (g g\(^{-1}\)), \( \Psi_m \) is the measured water potential (MPa). Finally, gravimetric water content was converted to volumetric water content by multiplying the gravimetric water content by the soil bulk density.

The water content at the field capacity (\( \Theta_{FC} \)) is defined as the volume of water held at a water potential (\( \Psi \)) between -10 to -30 kPa, while water content at permanent wilting point (\( \Theta_{PWP} \)) is described as the moisture content in soils at which plants begin to wilt and cannot recover in a saturated atmosphere without increasing moisture in the soil. The water content at field capacity (\( \Theta_{FC} \)) was determined by overnight free drainage method (Song and Guo, 2012; de Melo Carvalho et al., 2014): saturated samples were put in plastic containers with meshed bottom, covered by Parafilm to prevent evaporation, and then left 24hrs to drain. The moisture retained was taken as field capacity and measured using the oven-dry method. Available water content (AWC) and readily available water content (RAWC) were calculated by subtracting the volumetric water content at -1500 and -10 kPa, respectively, from the water content at field capacity (Quin et al., 2014). The available water content represents the quantity of water that can be held and would be available to the plant. The water release curves were obtained by plotting the volumetric water content against the matric potential following the method described elsewhere (Rossi and Nimmo, 1994; Stoof et al., 2010; Abel et al., 2013).

### 2.4 Retention modeling and data evaluation

The soil water potentials and soil water contents were fitted using the model of Van Genuchten (1980), which was selected based on the conspicuous higher degree of fitting and the unimodal
behavior of our data. To obtain the soil water retention curve of Van Genuchten model, the SWRC-Fit version 1.3 software (Seki, 2007) was used; it can also be executed directly from the web page (http://purl.org/net/swrc). The Van Genuchten function with the Mualem restriction \((m = 1-1/n)\) (Genuchten, 1980; Mualem, 1986) is given by:

\[
\theta (\psi) = \theta_r + (\theta_s - \theta_r) \left( \frac{1}{1 + (\alpha \psi)^n} \right)^m \tag{4}
\]

Where \(\theta (\psi)\) is the volumetric water content \([\text{cm}^3\text{cm}^{-3}]\) at given matric potential \(\psi\) (kPa), \(\theta_s\) is the saturated water content \([\text{cm}^3\text{cm}^{-3}]\) when \(\psi = 0\) kPa, \(\theta_r\) is the residual water content \([\text{cm}^3\text{cm}^{-3}]\) at \(\psi \geq -1500\) kPa, and \(\alpha, n, m\) are shape parameters. The Mualem constraint \((m=1-1/n)\) was adopted to increase model parsimony (de Melo Carvalho et al., 2014).

A completely randomized design was used for this study; 12 treatments were randomly assigned to soil containers within the same constant-temperature room. The main goal of this analysis was to compare the effect of two biochar characteristics on measured soil hydro-physical properties. A two-way analysis of variance was used to determine significance of the biochar produced from 3 parent feedstocks at 2 pyrolysis temperatures. All statistical analyses were conducted using Minitab (version 17, Minitab, Inc., State College, PA).

3. Results and Discussion

3.1 Hydro-physical of Biochar

The biochars used were comprehensively characterized before and after oxidation by air at 250°C. The results can be found in chapters 3 and 4.
3.1.1 Particle size distribution

Figure 6.1 shows the particle size distribution for the Quincy sand and biochars used in this study. The sand had an approximately Gaussian distribution centered near 229 µm with particles generally constricted to 10-500 µm. Biochars, except the PB-350, are somewhat larger, with mean particle sizes near 350-470 µm. These samples also show a strong asymmetric distribution towards lower particle sizes, i.e. PB-350. The distribution occurs over a range of 10-175 µm for both chars.

Figure 6.1 Particle size distribution of soil and biochars.
3.1.2 Porosity and functionality of biochar

As seen in chapters 3 and 4, the pyrolysis temperature and air oxidation of biochar resulted in significant changes in the internal porosity and surface functionality. A side-by-side comparison of low- and high-temperature biochars shows that microporous structure and surface chemistry were both altered significantly when pyrolysis temperature reached its highest degrees. At 600°C, the surface area and pore volume increased significantly due to the formation of micropores on the biochar surface. Moreover, SEM analysis was conducted to visualize the topographic features and to calculate large pore (cavities) sizes. Generally, all biochars contained cavities between 0.5 and 200 micron in diameter, which would have an important role in holding quantities of water.

The surface functionality, determined by the content of surface acidic functional groups, was significantly decreased due to the removal of oxygenated functional groups from the surface of biochar. Air oxidation increased surface functionalities due to the increase of total oxygenate functional groups, which are responsible for the formation of negatively charged surfaces. After oxidation, although the general microporous characteristic was preserved, oxidized biochars had increased surface area and a slight change in micropore volume. The SEM analysis did not show any significant effects of oxidation by air at 250°C on surface roughness or cavities size in micro-ranges, implying that oxidation effects occurred at only the nano-range of scale.

3.1.3 Hydrophobicity and Wettability

Table 6.1 shows the hydrophobicity index and water drop penetration time (WDPT) of biochar used in this study. As seen, the unoxidized PW-350 and PW-600 biochars were more hydrophilic
while PB-350 was extremely hydrophobic, implying feedstock-related chemical and physical differences. High temperature biochars were more hydrophilic relative to low temperature biochar, as indicated by MED indexes but not by the WDPT technique. Theoretically, high temperature biochars should be more hydrophobic than low temperature biochars because of the heavily dehydrogenation/dehydroxylation at high pyrolytic temperatures (Kinney et al., 2012). The availability of oxygen-containing groups to bind with water was determined by the polarity and hydrophobicity indexes of biochar surfaces. The polarity indexes [(O+N)/C] of our biochars (Chapter 3 and 4) indicated that oxidized biochars exhibit higher index values and therefore have higher hydrophobicities (table 6.1) relative to unoxidized biochars. According to Bradley et al., (2011) and Kinney et al., (2012), available polar groups, such as C–O/C═O, on biochar surfaces act as water-binding centers and assist the formation of water clusters, and thus, their surfaces should be covered with a layer of water in aqueous solutions.

<table>
<thead>
<tr>
<th>Biochar</th>
<th>Hydrophobicity (MED index)</th>
<th>WDPT (seconds)</th>
</tr>
</thead>
<tbody>
<tr>
<td>QS-PW350 UO</td>
<td>0.7</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>AO</td>
<td>0.0</td>
</tr>
<tr>
<td>QS-PW600 UO</td>
<td>0.0</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>AO</td>
<td>0.0</td>
</tr>
<tr>
<td>QS-PB350 UO</td>
<td>30.0</td>
<td>&gt;60</td>
</tr>
<tr>
<td></td>
<td>AO</td>
<td>10.0</td>
</tr>
<tr>
<td>QS-PB600 UO</td>
<td>1.0</td>
<td>&gt;60</td>
</tr>
<tr>
<td></td>
<td>AO</td>
<td>0.3</td>
</tr>
<tr>
<td>QS-HP350 UO</td>
<td>10.0</td>
<td>&gt;60</td>
</tr>
<tr>
<td></td>
<td>AO</td>
<td>2.5</td>
</tr>
<tr>
<td>QS-HP600 UO</td>
<td>0.9</td>
<td>&gt;60</td>
</tr>
<tr>
<td></td>
<td>AO</td>
<td>0.0</td>
</tr>
</tbody>
</table>
Wettability/dispersibility test was conducted by adding 0.5 grams of biochar into 15 mL of DI water, following the method reported by (Kinney et al., 2012). As seen in Figure 6.2 oxidized biochars exhibited better wettability in comparison with unoxidized biochars, and were more dispersible even after 10 minutes of sonication. Low sedimentation rate of unoxidation biochars indicate lower wettability and higher hydrophobic character.

![Figure 6.2. Wettability/dispensability behavior of (a) PW350, (b) PB350 and (c) HP350 oxidized (with A letter) and unoxidized biochars. The content in each of the bottles is 0.5 mg/15mL and they are sonicated for 10 minutes.](image)

3.2 Characteristics of soil water retention curves (SWRC)

The change in the soil-water retention characteristics from adding biochar is shown in Figure 6.3 Quincy sand without addition of biochar (control) retained a smaller amount of water, even at high soil water potentials, so that water content decreases rapidly with decreasing water potential. The impact of biochar addition on the SWRC was accordingly positive; addition of biochar
increased soil water retention in both high and low potential ranges compared to the control. Two thirds of the water present at saturation was released at $\psi \geq -85$ kPa from the control whereas $\psi \geq -150$ kPa is needed to release the same amount of water from biochar-amended samples. Over a wide range of soil water potentials, oxidized biochar-soil mixtures held significantly more water than the unoxidized biochar-soil mixtures except at saturation region between -0.1 and -5 kPa of $\psi$. It seems that there is a large effect of oxidation on the water retention properties of biochar; this effect can clearly be seen at $\psi$ between -8 and -300 kPa. Soil samples amended with unoxidized biochars, on the other hand, retained less water at $\psi$ from -0.1 to -5 kPa than the control, and contained equivalent amount of water as that of soil control at dry range ($\psi \leq -300$ kPa). The magnitude of the effect of pyrolysis temperature and feedstock source of biochar was found to vary in some way over the range of soil water potentials. Impact of low pyrolysis temperature biochar was somehow lower on water retention than biochar produced at high temperature, but the difference was not significant. Biochar produced at low temperature has more oxygenated functional groups on the surface. Biochars produced at high temperature on the other hand have much higher surface area. Furthermore, biochar-amended soil samples responded differently to feedstock source of biochar at both low and high soil water potential ranges.
Figure 6.3 Predicted soil water retention curves (lines) and measured soil volumetric water contents (symbols) at different matric potentials. Estimates of shape parameters are presented in Table (6.3)
Table 6.2 summarizes the measured and fitted saturated ($\theta_s$), residual ($\theta_r$) water contents, and the shape parameters ($\alpha$, $n$ and $m$) of van Genuchten model (1980). The fitted parameters to the SWRCs had coefficients of determination ($r^2$) close to unit, indicating that the fitting of the proposed interpolation equation to the experimental data was highly acceptable. Application of biochar decreased both the saturated and the residual water contents. Comparable to treatments with unoxidized biochars, the oxidized biochars increased the saturated soil water contents in average by 1.4% and the residual water contents by 0.45%. The $\alpha$, $m$ and $n$ values of biochar-treated soil samples were all smaller than those of soil control. Treatments with oxidized biochar had lower $\alpha$ values than those with unoxidized biochars.

Table 6.2 Fitted and measured values of the residual water content ($\theta_r$) and the saturated water content ($\theta_s$), and the fitted values of parameters ($\alpha$, $n$ and $m$) of the soil water retention model.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>QS</th>
<th>QS-PW350</th>
<th>QS-PW600</th>
<th>QS-PB350</th>
<th>QS-PB600</th>
<th>QS-HP350</th>
<th>QS-HP600</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\theta_s$ ($cm^3 cm^{-3}$)</td>
<td>28.50&lt;sup&gt;a&lt;/sup&gt;</td>
<td>26.06</td>
<td>27.05</td>
<td>26.06</td>
<td>27.06</td>
<td>26.06</td>
<td>27.10</td>
</tr>
<tr>
<td>$\theta_r$ ($cm^3 cm^{-3}$)</td>
<td>6.32&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.15</td>
<td>6.69</td>
<td>6.60</td>
<td>6.60</td>
<td>6.41</td>
<td>6.60</td>
</tr>
<tr>
<td>$n$</td>
<td>9.44</td>
<td>4.46</td>
<td>2.85</td>
<td>2.80</td>
<td>3.72</td>
<td>4.17</td>
<td>4.52</td>
</tr>
<tr>
<td>$m$</td>
<td>0.89</td>
<td>0.78</td>
<td>0.65</td>
<td>0.64</td>
<td>0.73</td>
<td>0.76</td>
<td>0.78</td>
</tr>
<tr>
<td>$r^2$</td>
<td>0.999</td>
<td>0.998</td>
<td>0.996</td>
<td>0.996</td>
<td>0.996</td>
<td>0.996</td>
<td>0.996</td>
</tr>
</tbody>
</table>

Abbreviations: $\theta_s$ = the saturated water content [$cm^3 cm^{-3}$] when $\psi \leq -3$ kPa; $\theta_r$ = the residual water content [$cm^3 cm^{-3}$] at $\psi \leq -1500$ kPa; <sup>a</sup> = The measured values of $\theta_s$ and $\theta_r$; <sup>b</sup> = The fitted values of $\theta_s$ and $\theta_r$; $\alpha$, $n$ and $m$ = shape parameters of the van Genuchten model (1980); UO = unoxidized biochar; AO = air-oxidized biochar. Van Genuchten parameters were fitted to the data between $\approx 1.8$ and $1500$ kPa only. The parameters are therefore only valid for this water potential range.
3.3 Response of soil hydrological variables to biochar porosity and surface chemistry

Table 6.3 shows the effect of the application of biochar on selected physicochemical and hydrological properties of the Quincy sand. An increase of pH was observed after addition of biochar, particularly in the soil samples amended with unoxidized HP350, HP600 and PB600. Biochar application significantly reduced the soil bulk density in all treatments; bulk density in soil samples amended with unoxidized PW350 and PW600 biochars, for example, were 0.22 and 0.21 g.cm\(^{-3}\) higher, respectively, compared with the control. However, comparisons between biochar amended soil samples did not show any significant variation even in oxidized biochar amended samples. The biochars also have an impact on soil water content at field capacity (\(\Theta_{FC}\)); the \(\Theta_{FC}\) of sandy soil was increased by roughly 8.5 % after biochar addition. In case of soil samples amended with oxidized biochars, more water was held and the highest amount of water retained at field capacity (30.22%) was observed in oxidized HP350-amended samples.

The majority of the total water potentially stored was available for plant growth, while in the case of soil without biochar additions, most of this water was easily lost by gravity. A significantly higher available water content (AWC) was observed in the oxidized biochar treatments; the highest percentage was also found in oxidized HP350-amended sample (figure 6.4). Altogether, amended samples show increased water content at the permanent wilting point (PWP) relative to the control. However, water content at permanent wilting point presented weak dependencies on the biochar feedstock source as well as pyrolysis temperature. The osmotic potential is customarily ignored due to the lack of semipermeable membranes in soil water systems. Here, the osmotic potential was calculated as a function of the EC values of biochars just to get an indication about the potential effects of biochar on this parameter. The osmotic potentials appeared highly dependent
on production temperature of biochar, which causes an increase of some inorganic components of biochar.

Table 6.3 Effect of biochar application to sandy soil on bulk density, pH, EC, water contents at field capacity, plant available water contents and osmotic potentials.

<table>
<thead>
<tr>
<th>Property</th>
<th>QS</th>
<th>QS-PW350</th>
<th>QS-PW600</th>
<th>QS-PB350</th>
<th>QS-PB600</th>
<th>QS-HP350</th>
<th>QS-HP600</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>UO</td>
<td>AO</td>
<td>UO</td>
<td>AO</td>
<td>UO</td>
<td>AO</td>
</tr>
<tr>
<td>Bulk density (g cm(^{-3}))</td>
<td>1.49</td>
<td>1.27</td>
<td>1.28</td>
<td>1.26</td>
<td>1.27</td>
<td>1.29</td>
<td>1.27</td>
</tr>
<tr>
<td>pH(_{\text{H}_2\text{O}}) (1:5)</td>
<td>7.5</td>
<td>8.1</td>
<td>7.8</td>
<td>8.12</td>
<td>7.9</td>
<td>8.7</td>
<td>8.2</td>
</tr>
<tr>
<td>EC(_{\text{H}_2\text{O}}) (ds m(^{-1}))</td>
<td>0.08</td>
<td>0.01</td>
<td>0.01</td>
<td>0.02</td>
<td>0.04</td>
<td>0.06</td>
<td>0.06</td>
</tr>
<tr>
<td>(\theta_{\text{FC}}) (%)</td>
<td>16.91</td>
<td>26.55</td>
<td>27.09</td>
<td>25.70</td>
<td>27.50</td>
<td>23.68</td>
<td>25.87</td>
</tr>
<tr>
<td>(\theta_{\text{PWP}}) (%)</td>
<td>5.32</td>
<td>6.15</td>
<td>6.69</td>
<td>6.56</td>
<td>6.56</td>
<td>6.41</td>
<td>6.60</td>
</tr>
<tr>
<td>(\theta_{\text{AWC}}) (%)</td>
<td>11.59</td>
<td>20.40</td>
<td>20.40</td>
<td>19.15</td>
<td>20.94</td>
<td>17.27</td>
<td>19.27</td>
</tr>
<tr>
<td>Osm. (-KPa)</td>
<td>2.88</td>
<td>0.36</td>
<td>0.72</td>
<td>0.72</td>
<td>1.44</td>
<td>0.72</td>
<td>1.8</td>
</tr>
</tbody>
</table>

Abbreviations/notes: WFPS = water filled pore space; \(\theta_{\text{FC}}\) = water content at Field capacity; \(\theta_{\text{AWC}}\) = available water content; \(\theta_{\text{PWP}}\) = water content at Permanent Wilting Point (-1.5 MPa); Osm = osmotic potential. Values are averages over the replicates of the treatments (n = 3).
Our results show that the consistent increase in soil water retention curve (SWRC) is related to an increase in soil moisture at ≤ -30 kPa for unoxidized biochar, which in turn could be linked to their physical properties such large pores (cavities in micro-range), and surface chemistry. This observation is consistent with previous theoretical reasoning of Lehman et al. (2009) suggesting that a net increase in water retention results from the high surface area of biochar compared with the low surface area of un-amended sand. Therefore, biochars with enhanced micro-porosity (higher SA and PV) should ideally have higher water retention capability, as demonstrated by Lei

Figure 6.4 Effect of biochar on available water capacity (AWC). Error bars show standard deviations and treatment means.
This statement confirms findings from other studies that biochar increases soil water retention likely due to its microposity characteristics (Dempster et al., 2012; de Melo Carvalho et al., 2014). Dempster and his colleagues in 2012, for example, found that volumetric soil moisture content at -100 and -1500 kPa increased significantly when a biochar with 273 m$^2$ g$^{-1}$ SA was applied into a sandy soil at 1.8 wt.% application rate. The HP350 biochar used has approximately similar SA to the biochar used by Dempster et al (2012) and a similar significant increase in the water content at -100 kPa, but not at -1500 kPa. The conditions and experimental design is probably the cause for these discrepancies, regardless of biochar production conditions.

Pearson’s correlation analysis were conducted to identify potential relationships between selected biochar properties and soil water retention characteristics obtained from previous SWRCs is shown in Table 6.2. As shown in this table, soil water contents at different matric potentials were significantly inter-correlated (P <0.01) and correlated with bulk densities of biochar-amended soil samples. The bulk density is controlled by the volume occupied by the internal cavities in the biochar. SEM pictures (Chapter 3) demonstrated that all biochars studied contained cavities (large pores) between 0.5 to 300 micron in diameter. These pores should play an important role for increasing soil water retention comparable to soil alone. This postulation is in agreement with (Carrier et al., 2012; Kinney et al., 2012; Abel et al., 2013). We could not find any correlations between biochar surface area and pore volume measured by CO$_2$ adsorption isotherms (chapters 3 and 4). This was expected because internal porosity measured via CO$_2$ isotherms fall within a nano-range scale which may not be accessible to the water (see table, 1). Moreover, there was significant correlations observed between total acidic functional groups on biochar surface and water contents at different matric potentials. Effects of these surface groups on soil water retention became more significant as matrix potentials decrease from 0 kPa to -500kPa (r = 36 and 63 at 0
kPa and -500 kPa, respectively), indicating their capability for adsorbing and holding water molecules at this range. Furthermore, soil water contents were well-correlated with soil bulk density at -10 kPa and -1500 kPa (r = -84 and -73, respectively) after biochar additions, implying that soil water retention mainly at lower matric potentials were mainly affected by biochar cavities (reflected in bulk density measurements) along with surface functionality. The correlation was almost identical in biochars with higher oxygenated functional groups especially in the range of from -10 kPa to -1500 kPa. Generally, our findings are in accordance with those reported elsewhere (Barnes et al., 2014; Yang et al., 2014).

Table 6.4 Pearson correlations between; soil water retention properties, selected biochar properties and bulk densities of biochar-amended QS soil.

<table>
<thead>
<tr>
<th></th>
<th>( \theta_{0kPa} )</th>
<th>( \theta_{-10kPa} )</th>
<th>( \theta_{-33kPa} )</th>
<th>( \theta_{-100kPa} )</th>
<th>( \theta_{-500kPa} )</th>
<th>( \theta_{-1500kPa} )</th>
<th>TC</th>
<th>TAFG</th>
<th>BD</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \theta_{0kPa} )</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \theta_{-10kPa} )</td>
<td>0.25</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \theta_{-33kPa} )</td>
<td>0.04</td>
<td>0.72**</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \theta_{-100kPa} )</td>
<td>0.13</td>
<td>0.55**</td>
<td>0.94**</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \theta_{-500kPa} )</td>
<td>0.04</td>
<td>0.77**</td>
<td>0.80**</td>
<td>0.72**</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \theta_{-1500kPa} )</td>
<td>0.04</td>
<td>0.73**</td>
<td>0.55**</td>
<td>0.43*</td>
<td>0.82**</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TC</td>
<td>0.21</td>
<td>0.19</td>
<td>-0.23</td>
<td>-0.36</td>
<td>-0.23</td>
<td>0.09</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TAFG</td>
<td>0.36*</td>
<td>0.48*</td>
<td>0.51*</td>
<td>0.53*</td>
<td>0.63*</td>
<td>0.28</td>
<td>-0.73**</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>BD</td>
<td>-0.28*</td>
<td>-0.84**</td>
<td>-0.45*</td>
<td>-0.27*</td>
<td>-0.64*</td>
<td>-0.73**</td>
<td>-0.14</td>
<td>-0.46</td>
<td>1.00</td>
</tr>
</tbody>
</table>

0kPa, -10 kPa, -33 kPa, -100 kPa, -500 kPa, -1500 kPa, water contents at 0, -10, -33, -100, -500, and -1500 kPa matric potentials; TC, total carbon content (wt.%); TAFG, total acidic functional groups on biochar surface; BD, bulk density. (* P<0.05, ** P<0.01)
4. Conclusion

Our experimental results suggest that the water retention in the biochar is controlled by the internal cavities (internal porosity) which influence the bulk density of the char and by the surface chemistry of the char that controls the hydrophobicity of the material. Although the water retention on biochars is a function of the initial feedstock, which controls the size and volume of the cavities, the pyrolysis processing conditions also influence this parameter. The biochars produced at high temperature tend to be more hydrophobic due to the removal of oxygenated functional groups from the surface, the oxidation at 250°C induce the formation of more oxygenated groups and consequently enhances the capacity of these chars to hold water.

5. Acknowledgements

The authors would like to acknowledge the financial support by the Washington State Department of Ecology through the Wastes to Fuel program and by the Washington Department of Agriculture for their support through the Appendix A program. The authors are also very thankful to the Agricultural Research Center (NIFA-Hatch-WNP00701) for the financial support provided.
6. References


CHAPTER 7. EFFECT OF BIOCHAR ADDITION ON CO$_2$ AND N$_2$O FLUXES, AND INORGANIC-N CONTENTS IN QUINCY SAND: A SHORT-TERM LABORATORY STUDY

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(Paper to be submitted to “Soil Biology and Biochemistry”)

Abstract

Incorporation of biochar into soil has been advocated as both an environmental tool and a method for C sequestration. Biochar also has the potential to influence the soil N cycle by altering nitrification rates and/or by adsorbing NH$_4^+$ or NH$_3$. A laboratory study was performed to examine
the effect of biochar incorporation into sandy soil on N\textsubscript{2}O and CO\textsubscript{2} emissions, and inorganic-N contents, following the application of N-fertilizer (Urea). Incubation experiments were conducted with unfertilized and fertilized soil amended with different amounts (10, 15 and 20 tons ha\textsuperscript{-1}) of low- and high-temperature biochars (LTB and HTB, respectively). During the first week of incubation, emissions of CO\textsubscript{2} from LTB-amended microcosms were generally higher than from HTB-amended microcosms. This variation was linked to the different quantities of labile carbon between LTB and HTB biochars as well as to the higher surface area available in high temperature biochars for the adsorption of the CO\textsubscript{2} produced. Emissions of N\textsubscript{2}O from the biochar amended microcosms were generally lower than from controls (soil alone) during the first 7 days, but after day 14 there was no significant difference. The inorganic N pool was clearly influenced by biochar addition; NO\textsubscript{3} decreased as biochar doses increased while NH\textsubscript{4}\textsuperscript{+} differed between treatments. In general, the inorganic-N pool available for N\textsubscript{2}O-producing mechanisms was reduced by adding biochar, suggesting a mechanistic link to the observed reduction in N\textsubscript{2}O emissions.
1. Introduction

Biochar application to soil has drawn much attention as a promising option to mitigate climate change through increased carbon storage in soils and decreased nitrous oxide (N$_2$O), ammonia (NH$_3$) and methane (CH$_4$) emissions from agricultural soils (Case et al., 2012; Yoo and Kang, 2012; Ameloot et al., 2013; El-Mahrouky et al., 2015). Laboratory studies have shown that emissions of CH$_4$ and N$_2$O were reduced in biochar-amended soils, and in the same way, ammonia volatilization was also decreased (Zhang et al., 2010; Spokas et al., 2012). The applicability of biochar as a tool for climate change mitigation is limited by our understanding of the mechanisms responsible for the observed changes in greenhouse gas emissions (GHGs) from soils, microbial responses, and soil fertility changes (Yoo and Kang, 2012).

In agricultural ecosystems, nitrous oxide and carbon dioxide are soil-derived greenhouse gases resulting from different biological processes. Major sources of N$_2$O are nitrification and denitrification processes, and as such, emissions are influenced by the inorganic-N supply (Clough et al., 2010; Harter et al., 2014a). Most agricultural N$_2$O emissions are due to increased fertilizer application. A considerable fraction of nitrogen fertilizers are converted to N$_2$O (Harter et al., 2014a).

To date, little is known about the interactions among biochar additions, gaseous emissions, and soil microbial community structure, function and activity. Further research is needed in order to verify reported results and to determine how the surface functionality of different biochars affects the loss of these gases. This research addresses several knowledge gaps. Specifically, the role of biochar properties resulting from variations in production temperature on CO$_2$ and N$_2$O emissions.
2. Material and Methods

2.1 Biochar preparation and Characterization

Two different biochars produced from pine wood at 350 and 600°C were used in this study. The production conditions and characterization were previously described in chapter 3.

2.3 Stability and potential carbon sequestration

The thermal recalcitrance of the biochar produced was studied by the thermogravimetric method described elsewhere (Harvey et al., 2012). Mass loss characteristics associated with the thermal oxidation of biochars were determined using a thermo-gravimetric analyzer (TGA-Mettler Toledo-SDTA851e). In air atmosphere (air flow rate of 100mL min\(^{-1}\)), samples between 5 and 8 mg were heated from 25°C to 105°C at 10°C min\(^{-1}\) and held at this temperature for 15 minutes, and then from 105°C to 950°C at 30°C min\(^{-1}\) and held for 10 minutes. The thermal recalcitrance index (R<sub>50</sub>) and carbon sequestration potential (CSP%) of the biochars produced were estimated using equation (4) and equation (6), respectively.

\[
R_{50, \text{biochar}} = \frac{T_{50, \text{biochar}}}{T_{50, \text{graphite}}} \quad \ldots \ldots (4)
\]

Where \(T_{50, \text{biochar}}\) and \(T_{50, \text{graphite}}\) were the temperature values corresponding to 50% weight loss by oxidation/volatilization of biochar and graphite, respectively. Values for \(T_{50, \text{biochar}}\) and \(T_{50, \text{graphite}}\) were obtained from TG thermo-grams that have been corrected for water and ash content as follows:

\[
W_{i,c} = 100 + \left[ 100 \times \frac{(W_{i,un}-W_{150,un})}{(W_{150,un}-W_{\text{cutoff,un}})} \right] \quad \ldots \ldots (5)
\]

Where \(W_{i,c}\) and \(W_{i,un}\) were the corrected and uncorrected weights, respectively. \(W_{150,un}\) was the weight at 150°C, and \(W_{\text{cutoff,un}}\) was the weight at the temperature when no further oxidation of was apparent.
\[ CSP (%) = \frac{M_f \times \text{yield} \times R_{50} \times C_{\text{biochar}}}{M_f \times C_{\text{feedstock}}} \quad \ldots \ldots (6) \]

Where \( CSP \) was the carbon sequestration potential. \( R_{50} \) was the recalcitrant index of biochars. \( M_f \), \( C_{\text{biochar}} \) and \( C_{\text{feedstock}} \) were the weight of biomass feedstock, carbon contents of biochars and biomass feedstocks, respectively. The values of thermal recalcitrance and C-sequestration potential of the two biochars studied in this paper are shown in Figure 1.

![Figure 7.1](image.png)

**Figure 7.1.** Thermal recalcitrance of resultant biochar; (2)- The potential carbon sequestration (%PCS) of biochars as a function of temperature.

### 2.3 Soil

Details about collection and characterization of soil used in this study can be found in chapter 5.

### 2.4 Experimental design

Two laboratory incubation experiments were carried out in parallel. The first set of incubation experiments was used to measure carbon dioxide (CO\(_2\)) and nitrous oxide (N\(_2\)O) emissions. The second incubation was conducted to determine N-mineralization. In both experiments, sterile
specimen vials of 128-mL volume were used as microcosm for amended and unamended soil treatments. Prior to biochar addition, the vessels were packed with 50 g of air-dried soil at a bulk density of ≈1.5 g cm$^{-3}$ and then moistened with deionized water (H$_2$O) to obtain a 60% water filled pore space (WFPS). The packed vessels were loosely covered with their lids and pre-incubated at 25 °C for 10 days in order to stabilize microbial activity (Thomazini et. al., 2015). After the pre-incubation period, the vessels receiving biochar treatments were amended. The biochar was thoroughly mixed with the soil at rates of 125, 187, and 250 mg 50g$^{-1}$ dry soil by weight, equivalent to 10, 15, and 20 ton ha$^{-1}$, respectively. Application rates higher than 10 ton ha$^{-1}$ are considered unrealistic rates for agronomic soils, however, they could provide measurable impacts of biochar on GHG production (Spokas et al., 2009). The mixing rate was calculated by assuming 15 cm soil depth and 1.5 g cm$^{-3}$ soil bulk density. On the same day, urea ((NH$_2$)$_2$CO) was applied to seven treatments at a dose of 1.28 mg 50 g$^{-1}$ soil, which corresponds to 100 kg N ha$^{-1}$. Two control soils were prepared for all incubation experiments; one contained no applied-N nor added-biochar, and the other contained only applied-N. Deionized water was added at the same time and then at weekly intervals in order to maintain soil moisture at 60% WFPS.

2.4.1 N$_2$O and CO$_2$ emission incubation

Incubations treatments used to measurement CO$_2$ and N$_2$O emissions were conducted in quadruplicate. A total of 60 microcosm vessels were prepared and placed into 500 mL mason jars (1 vessel per jar). The jars were then kept sealed with gas-tight lids to allow accumulation of gas in the jar headspace between sampling times. Jar lids were fitted with a rubber septum to serve as a sampling port. A set of jars containing biochar alone and blanks (ambient) was included at each time interval. Biochar control incubations were conducted to assess the production or consumption
of CO$_2$ from the biochar itself, following Fabbri et al., (2012) and Thomazini et al., (2015). 20 mL of gas was sampled from the headspace of each replicated treatment using a 35 mL syringe at day 1, 3, 7, 14, 21, and 28. Collected gas sample were immediately injected into 12 mL pre-evacuated airtight vials (Labco Exetainer, High Wycombe, UK). After sampling, the jars were opened in a ventilated area for one hour to allow air exchange. A Shimadzu gas chromatograph (model 2014, Shimadzu Corp., Kyoto, Japan) equipped with an electron capture detector was used to measure CO$_2$ and N$_2$O concentrations in the gas samples. The CO$_2$ and N$_2$O emission rates (mg kg$^{-1}$d$^{-1}$) were calculated using the GRACE-Net protocol published by USDA (http://www.ars.usda.gov/research/GRACEnet).

2.4.2 Inorganic-N (NH$_4^{+}$ + NO$_3^{-}$-N)

Treatments in the second incubation experiment were run in triplicate were designed for destructive sampling procedure to collect subsamples for inorganic-N measurements. For measurement of inorganic N content, 42 microcosm vessels were prepared for each time interval for a total of 252 microcosms. Cumulative ammonium and nitrate [(NH$_4^{+}$ + NO$_3^{-}$)-N] concentrations were measured using 100 mL of 1 M KCl extract and a 10 g subsamples from each vessel shaken for 30 min at 180 rpm, following the method described elsewhere (Saunders et al., 2012). The KCl extracts were then filtrated using Whatman No.42 filter paper and stored at -20°C until analysis. A SEAL auto analyzer (SEAL Analytical Inc. Mequon, WI) was used to determine the concentrations of NH$_4^{+}$-N and NO$_3^{-}$-N in each KCl aliquot via the EPA methods number 350.1 and 353.2, respectively.
2.5 Statistical analysis

Statistical analyses were performed using Minitab software version 17, a two-way analysis of variance (ANOVA) was used. We focused on biochar application rate (factor 1) and presence of N-fertilizer (factor 2). All tests of significance were conducted at \( P < 0.05 \). When data were not normally distributed or showed heterogeneity of variances, they were square root or log-transformed before analysis.

3. Results

3.1 Carbon dioxide emission

Figure (7.2) shows the cumulative \( \text{CO}_2 \) emissions from soil (control), low temperature biochar (LTB)-amended soil, and High temperature (HTB)-amended soil before and after N-fertilizer application. The patterns of \( \text{CO}_2 \) emissions were similar for the biochar treatments before and after application of N-fertilizer. The greatest \( \text{CO}_2 \) emissions (up to \( 0.85 \pm 5.0 \) mg \( \text{CO}_2 \)-C kg\(^{-1}\) soil) occurred in the fertilized microcosms (i.e. LTB treatments) during 3 days after the start of the incubation. Simultaneously, the \( \text{CO}_2 \) losses in the control microcosms were lower than in the biochar amended microcosms, but quickly increased to highest levels comparable to the biochar-amended treatments after day 7 of incubation. Excluding day 3, biochar additions generally led to decrease emissions of \( \text{CO}_2 \) throughout the study. Almost in all treatments, significant \( \text{CO}_2 \) peaks were observed when the larger rate of biochar had been applied, compared with the smaller doses (after adjusting for the \( \text{CO}_2 \) emissions from the control). Particularly, the treatments receiving 20 ton ha\(^{-1}\) of biochar emitted, on average, 3.5 times more \( \text{CO}_2 \) than the treatments receiving 10 ton ha\(^{-1}\). Additions of N-fertilizer changed the \( \text{CO}_2 \) emission trends but not significantly (\( P > 0.05 \)). For
example, CO\textsubscript{2} emission increased from 0.78±0.4 mg CO\textsubscript{2}-C kg\textsuperscript{-1} soil to 0.85±5.0 mg CO\textsubscript{2}-C kg\textsuperscript{-1} soil during the first week of incubation in the 20 ton ha\textsuperscript{-1} doses of LTB microcosms after N-fertilizer application.

**Figure 7.2** CO\textsubscript{2} emissions following application of LTB biochar (a and b) and HTB biochars (c and d) and before N-fertilization (a and c) and after N-fertilization (b and d). Values are the mean (n = 4) ± SE (bars).
3.2 Nitrous oxide emissions

The N₂O emissions from soil and biochar-amended microcosms are shown in figure (7.3). In contrast to the CO₂ emissions, N-fertilizer additions strongly affected N₂O emissions. The addition of N-fertilizer to the microcosms influenced N₂O emissions: one week following urea application, there was a significant increase in N₂O emissions from all treatments. The highest cumulative N₂O loses were recorded 14 days after N-fertilizer application (0.92 mg N₂O kg⁻¹ soil, for Control). The addition of biochar to the unfertilized microcosms increased the N₂O emissions only on day 3, but had no effect on any particular sampling day after 7 days of the incubation (figures 7.3a and c). In all treatments, N₂O emissions virtually occurred after the first 3 days of incubation, thereafter N₂O loses decreased in unfertilized microcosms while sharply increased in fertilized counterparts. At day 7, N₂O loses were significantly higher in the fertilized-control microcosms without biochar (0.92 mg N₂O kg⁻¹ soil) compared with the fertilized-LTB-amended microcosms (0.53 mg N₂O kg⁻¹ soil in the 20 tons ha⁻¹ biochar-containing and 0.33 mg N₂O kg⁻¹ soil in the 10 tons ha⁻¹ biochar-containing microcosms). The N₂O emissions from the HTB treatments were not significantly different from each other when N₂O emissions were tested over time. N₂O loses decreased strongly to <0.30 mg N₂O kg⁻¹ soil at day 7 in unfertilized microcosms and <0.35 mg N₂O kg⁻¹ soil in fertilized microcosms. From day 14 until the end of the study, N₂O emissions from all treatments stabilized at the level of the control soil.
Figure 7.3  N$_2$O emissions following application of LTB biochar (a and b) and HTB biochars (c and d) and before N-fertilization (a and c) and after N-fertilization (b and d). Values are the mean ($n = 4$) ± SE (bars).
3.3 NH$_4^+$-N

Corresponding to the N$_2$O data, the highest NH$_4^+$-N concentration were recorded during the first week in all treatments, figure (7.4). The mean NH$_4^+$-N concentration for all unfertilized treatments fluctuated between 0.02 and 0.35 mg NH$_4^+$-N kg$^{-1}$ soil during the first seven days of incubation, with the maximum mean NH$_4^+$-N concentrations at day 7 in the LTB-20 treatments (figure 7.4a). Among fertilized microcosms, the control had a significantly larger NH$_4^+$ content at day 3 of incubation compared with biochar treated microcosms (figure 7.4b and d). Statistically, the overall relative difference in NH$_4^+$-N concentrations between the biochar and control treatments was insignificant in unfertilized microcosms (P>0.05). However, addition of biochar to soil significantly affect NH$_4^+$ concentrations (P<0.05) when compared to the control treatment after application of N-fertilizer, and this relative difference was significantly higher at in LTB- than in HTB-amended microcosms. At the end of the experiment (day 28), all treatments showed larger NH$_4^+$-N concentrations compared with previous two time intervals (days 14 and 21).
Figure 7.4  NH$_4^+$-N emissions following application of LTB biochar (a and b) and HTB biochars (c and d) and before N-fertilization (a and c) and after N-fertilization (b and d). Values are the mean ($n = 3$) ± SE (bars).
3.4 NO$_3^-$-N

Figure (7.5) shows NO$_3^-$-N concentrations in soil and biochar treatments before and after N-fertilizer addition. Compared with the NH$_4^+$-N concentrations, NO$_3^-$-N concentrations increased more slowly but constantly with time reaching concentrations of 4.2±0.02 (control) and 2.18±0.1 (20 tons ha$^{-1}$ biochar) mg NO$_3^-$-N kg$^{-1}$ soil at day 28. Only in the unfertilized microcosms, NO$_3^-$-N concentrations were significantly lower at day 3 ($P$<0.05), compared with the initial NO$_3^-$-N concentrations at day 0. The lowest concentration of NO$_3^-$-N was found in microcosms amended with 20 ton ha$^{-1}$ biochar, irrespective production temperature and N-fertilizer additions. Therefore, applying biochar to the soil at rate > 15 tons ha$^{-1}$ reduced NO$_3^-$-N concentrations to the largest extent compared to the control.
Figure 7.5 NO$_3$-N emissions following application of LTB biochar (a and b) and HTB biochars (c and d) and before N-fertilization (a and c) and after N-fertilization (b and d). Values are the mean ($n = 3$) ± SE (bars).
4. Discussion

Biomass pyrolysis at low temperature (i.e. 350°C) is more likely to leave incompletely pyrolyzed fractions (i.e. aliphatic compounds) in the biochar compared with pyrolysis at high temperatures (Bruun et al., 2011). These low temperature chars also have a lower surface area. As previously seen in chapter 3, the low temperature biochar (LTB) contained higher contents of aliphatic and oxidized carbon as compared to biochar produced at high temperature (HTB). Thus, the large CO$_2$ emission from the LTB-amended microcosms during the first week of incubation could be explained by the decomposition of this fraction. This hypothesis is also supported by the observed effects of LTB application rate; as the LTB quantities increase, the CO$_2$ emissions increase (figures 1, a & b). This result is perfectly in line with previous studies (Spokas and Reicosky, 2009; Bruun et al., 2011; Zimmerman et al., 2011; Rutigliano et al., 2014). According to our results, the differences between CO$_2$ emissions from LTB- and HTB-amended microcosms were not significant during the second half of incubation (from day 14 to day 28). Large CO$_2$ emissions were only observed in the first week of incubation. These findings are in accordance with a study carried out by Bruun et al. (2011), who observed greater initial emissions of CO$_2$ during first 10 days of incubation, and linked them to the rapid microbial population growth. The variations in CO$_2$ emissions over time could be linked to the microbial population (Bruun et al., 2011), activity (Rutigliano et al., 2014), composition (Castaldi et al., 2011), and/or variations in the functional state of microorganisms (Degens, 1999).

Temporary enhancement of microbial activity following biochar addition is well known in the literature (Hamer et al., 2004; Wang et al., 2006; Steiner et al., 2007; Kolb et al., 2009; Smith et al., 2010). Such enhancement could stimulate CO$_2$ emissions not only from decomposition of
labile carbon fractions in the biochar but also from the decomposition of native organic soil matter (Bruun et al., 2011). Rogovska et al., (2011) found increased rates of soil organic carbon mineralization after biochar additions. Similarly Troy et al., (2013) reported increased CO₂ emission and accelerated soil organic C mineralization with biochar addition to soil. Since no significant changes in CO₂ production was observed after N-fertilizer additions, the results suggest that the soil microbial community was C-limited rather than N-limited. This could also support the general hypotheses that biochar may promote soil microbial population simply by offering a wider surface for the colonization (Hamer et al., 2004) and/or changing soil properties that favor microbial activities such as aeration (Rogovska et al., 2011), provision of labile C (Bruun et al., 2011), and/or pH (Rutigliano et al., 2014).

Excluding the first week of incubation, biochar additions decrease the emissions of CO₂ throughout the study; this decrease is significant compared with the control microcosms. These findings are in agreement with Smith et al., (2010) who reported that biochar increased microbial respiration only in the short term. In a long term study, Major et al., (2010) found that CO₂ emissions from soil were reduced considerably after one year suggesting that CO₂ emissions would decrease further over time. Thus, the effects of biochar on CO₂ emission from soils require consideration of the time scale of interest (Mukherjee et al., 2014). Moreover, the priming effect of biochar on CO₂ evolution has also been shown to be lower from biochar produced at high temperature compared with biochar produced at low temperature, due to low labile carbon contents (Zimmerman et al., 2011).

Our results showed that nearly all the N₂O emissions in the biochar-amended microcosms occurred within the first 7 days of incubation (Figure 7.3), corresponding with the maximum
CO₂ emission rates (Figure 7.2). After day 3, the N₂O emissions decreased sharply following biochar amendment with varying levels of effect depending on the biochar amendment rates and interactions with N fertilizers. These general observations are in agreement with other studies (Zhang et al., 2010; Bruun et al., 2011). On the third day of incubation, the addition of biochar increased N₂O emissions relative to those from the control soil, which corroborates the findings of Clough et al., (2010), Bruun et al., (2011), Cayuela et al., (2013) and Harter et al., (2014). In our study, the significantly lower N₂O emissions was observed in biochar-containing microcosms 7 days after application of N-fertilizer. This finding is in agreement with recently published field- and laboratory-based studies using different biochars and soils (Yanai et al., 2007; Spokas and Reicosky, 2009; Van Zwieten et al., 2009; Joseph et al., 2010; Bruun et al., 2011; Wang et al., 2012; Harter et al., 2014a).

Since N₂O is emitted from soil during the microbial processes of nitrification and denitrification, the net N₂O formation and release are strongly linked to the abundance and activity of N₂O-reducing bacteria (Saunders et al., 2012; Troy et al., 2013; Harter et al., 2014a). A significant reduction in N₂O emissions was observed by day 7 after N-fertilization. Biochar addition along with nitrogen source availability may change the denitrifier microbial community composition by promoting the growth and activity of N₂O-reducing bacteria (Harter et al., 2014a). Reduced N₂O emissions from the biochar treatment may be caused by (i) direct biochar-induced chemical inhibition of one of the nitrification-denitrification steps (Clough et al., 2010; Bruun et al., 2011), (ii) an enhanced growth and activity of microorganisms capable of complete denitrification (Harter et al., 2014a), (iii) limiting bioavailability of electron donors and acceptors (NO₃⁻ and NH₄⁺) for microbial nitrification and denitrification due to sorption/immobilization.
(Harter et al., 2014a), (iv) increasing the pH (Bruun et al., 2011), and (v) improving soil aeration (Van Zwieten et al., 2009; Cayuela et al., 2015).

For N₂O to form via biological mechanisms, it must involve the inorganic-N pool (Clough et al., 2010). Our results showed that additions of biochar alters the availability and speciation of inorganic nitrogen (NO₃⁻, NH₄⁺) which is known to affect the diversity, abundance and functioning of N₂O-producing microbial communities in soils, and thereby soil N₂O emissions (Harter et al., 2014a). As NH₄⁺ and NO₃⁻ concentrations decreased N₂O fluxes declined in all microcosms because electron donors and acceptors, required for microbial N₂O formation became limiting (Harter et al., 2014a). Inorganic-N concentrations available for N₂O-producing mechanisms were clearly reduced by biochar addition (figures 7.4b, 7.4c, and 7.5a-c), regardless of NH₄⁺ concentration in unfertilized microcosms. Our results revealed that biochars produced at low temperature (LTB) possessed greater effect on reducing soil inorganic-N and N₂O emissions particularly in fertilized microcosms. Such effects may be directly linked to the surface chemistry of the biochars, and availability of cation and anion sites (Clough et al., 2010; Nelissen et al., 2014). Moreover, high-temperature biochar also showed considerable effects on the reductions of inorganic-N and N₂O evolution. Herein, we postulate that effects of HTB on pH and available surface area were the predominant factors controlling changes in HTB-amended microcosms.

In general, biochar treatments reduced inorganic-N contents compared to the control, implying a potential for reduced N₂O emissions. According to the literature, biochar addition causes (i) pH alteration, (ii) biotic and abiotic NO₃⁻ and NH₄⁺ immobilization, (iii) reduced soil organic matter (SOM) mineralization, (iv) suppressed nitrification/denitrification, (v) CEC modification, (Clough et al., 2010; Troy et al., 2013; Nelissen, Ruysschaert, et al., 2014; Nelissen, Saha, et al., 2014).
Further studies are needed to elucidate the N transformations and fluxes that occur when biochar is incorporated into soil receiving N-fertilizer if biochar is to be sequestered into sandy soils.

5. Conclusions

The incorporation of biochar into the soil is a promising approach to sequester Carbon in soils. CO₂ emissions from LTB-amended microcosmos were generally larger than for HTB-amended microcosms. This result was associated to the higher quantities of liable carbon and the lower surface area of these chars. During the first 7 days, the emissions of N₂O from the biochar-amended microcosms were generally lower than from controls (soil alone). The concentrations of NO₃ decreases as the quantity of biochar increases. NH₄⁺ concentrations were found to differ between treatments but variance did not follow a consistent trend. The addition of biochar reduced the inorganic-N pool available for N₂O-production.

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7. References


CHAPTER 8. CONCLUSIONS AND RECOMMENDATIONS

The dissertation reports experimental results to identify the major changes occurred in biochar bulk and surface characteristics due to feedstock source, pyrolysis temperature and/or post-pyrolysis oxidation. Five major studies are presented: (1) effect of feedstock source and pyrolysis temperature on physico-chemical properties of biochar, (2) influence of air oxidation on surface chemistry and functionality of resultant biochars, (3) role of biochar properties in retaining soil water, (4) nature of biochar surface - pathogenic bacteria interactions, and (5) effect of biochar on reducing GHGs emissions from Quincy sandy soil. This chapter briefly summarizes major findings and their research implications.

8.1. Conclusions

1. Pyrolysis temperature and feedstock material are important parameters controlling biochar bulk and surface physico-chemical characteristics. The content of volatiles, oxygen and the ratios of oxygen to carbon (O/C) and hydrogen to carbon (H/C) decreased linearly with pyrolysis temperature suggesting a gradual increase in aromatic structures and thermal recalcitrance. Pine bark biochars had higher ash content than pine wood-derived biochars, and as the pyrolysis temperature increased, the ash content also increased. In general, the concentration of the mineral matter increased with increased pyrolysis temperature leading to greater concentrations of leachable alkalines, increased pH and EC. This work illustrates
the relative importance of feedstock source and pyrolysis temperature on the bulk and surface properties of biochar products.

2. The biochar susceptibility to oxidation was thoroughly examined. The XPS and Boehm titration confirmed that most oxygenated surface functional groups (chiefly carbonyl, carboxyl and hydroxyl groups) are gradually removed as pyrolysis temperature increased. Therefore, biochars produced at low temperatures (such as 350 °C) retained many surface functionalities characteristics of the feedstock. The surface study also showed that the formation of carbonyl and carboxyl groups is easier for biochars produced at low temperature. The formation of these oxygenated functional groups contributes to add negative charges on the surface and consequently the pH at the point of zero charge is always higher for un-oxidized biochars.

3. A detailed study of the effect of unoxidized and oxidized biochars on transport of *E. coli* O157:H7 and *E. coli* K12 in Quincy sandy soil columns provides better understanding of the transport behavior of pathogens within the biochar-amended soil system. Our results showed that *E. coli* O157:H7 displayed higher retention then *E. coli* K12 in biochar-amended soil and increased biochar application rates (from 0 to 20%) is vital for both strains. Bacterial transport was affected by production condition and oxidation status of biochar. The transport through the PB-600 biochar-amended columns was higher than of pine wood biochar produced at the same production temperature. Oxidized biochars enhanced the transport of *E. coli* O157:H7 cells due to their surface negative charges. Our results suggest that that pine wood biochar produced at low temperature was effective in reducing the transport of *E. coli* in the studied soil.
4. The sixth chapter in this dissertation evaluated how oxidation status of biochar could enhance soil water retention on a relatively short time scale and at low application rate (2 wt.%). Results showed that adding biochar to a sandy soil altered hydrological property of the soil. For the biochar itself, it gets more hydrophobic as the temperature increases due to the reduction of oxygenated functional groups on the surface. The oxidation increases the content of oxygenated functional groups on the surface and consequently reduces its hydrophobicity. Application of oxidized biochar to the sandy soil held significantly more water and this is believed to be due to its contents of functional groups. Impact of low pyrolysis temperature biochar was somehow lower on water retention than biochar produced at high temperature, but the difference was not significant (at 95% level of confidence). Soil water contents at different metric potentials were significantly inter-correlated and correlated with bulk densities of biochar-amended soil samples. Adding biochar could have long-term effects on soil water retention not elucidated in this research, but it is likely that higher-rate biochar additions will have more positive effects.

5. Lab incubation experiments were conducted to study the effect of two biochars (PW-350 and PW-600) and their application rates on CO$_2$ and N$_2$O emissions from a sandy soil. At the same time, effects on inorganic nitrogen ($\text{NH}_4^+$ and $\text{NO}_3^+$) were investigated further. Compared to the un-amended soil (control), amendments with biochar significantly reduced cumulative CO$_2$ emissions (at 95% level of confidence). The effect of high temperature biochar was higher than low temperature biochar but not for entire incubation period. Cumulative N$_2$O production within 28 days of the experiments was not affected by biochar addition but low temperature biochar incorporation makes only a minimal
contribution to the suppression of N\textsubscript{2}O emissions at day 28. This study highlights the importance of production conditions for designing biochars for use as soil amendments to sequester carbon and retaining soil inorganic nitrogen.

8.2 Recommendations

The present experimental works conducted were constrained by the time period of study for a PhD degree. There are number of areas of research that still require investigation, thus future research should be focused on the following topics:

1. Future research is necessary to increase the level of confidence regarding the effects of pyrolysis and post-pyrolysis conditions on biochar bulk and surface properties along with the effect on biochar performance as soil amendment and environmental tool.

2. Research to further understanding the interactions between biochar surface and bacterial surface at micro-and nano-levels could be of interest for developing carbon-based biofiltration systems. Therefore, it is recommended to conduct additional research using other techniques such as Atomic Force Microscopy.

3. Studying the influence of the soil physicochemical changes, observed in the present study, on soil microbial dynamics could be of interest for expanding the use of biochar as a soil amendment.
3. Conducting an investigation into the CO$_2$ and N$_2$O- sequestration capability of biochar considered in this PhD study under field conditions, in order to understand more realistic situations which are far more complex than in incubation studies.

4. Although this study provided an insight into the different potential biochar-microorganism and -soil water interactions that can occur during short time of biochar application, further studies would be required to take into account main biotic and abiotic factors that affect field trial analysis. In addition, the time duration of this study limited any possible changes that may have occurred in the interaction effects. It would therefore, be pertinent to conduct field trials over periods of years to enable differences in water retention, microbial structure, and bacterial movement to be observed.