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Development of an urea-based controlled-release nitrogen fertilizer using biochar as support material

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Development of an urea-based controlled-release nitrogen fertilizer using biochar as support material

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"A los que han estado, estuvieron y estarán"

En especial a Mamá

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Abstract

Urea (U) is a low cost solid nitrogen (N) fertilizer. However, the N uptake by crops from urea is often as low as 30~50%, with an environment cost associated to N losses via NH_3 volatilization, NO_3^- leaching and N_2O emission.

In order to mitigate this potential environmental cost and leading to a sustainable agriculture several technologies have been develop highlighting controlled-release fertilizers (CRFs) which play an important role improving fertilizer use efficiency by plants and reducing the frequency of fertilizers application. In this sense, biochar could be considered as a tool to provide carbon sequestration, soil amending properties and support matrix for the development a controlled-release fertilizer.

In this context, the main goal of this research was obtaining an urea-based controlled-release nitrogen fertilizer used as biochar support.

Biochar produced by pyrolysis of oat hull at 300 and 500 °C (BO300 and BO500) and pine bark at 300 and 500 °C (BP300 and BP500) were characterized. Mineralogical and physic-chemical properties; and their N-urea sorption capacity were carried out. The results showed that the mineralogical, structural and physic-chemical properties depend on the raw material and pyrolysis temperature. The specific surface area (BET) increased as the pyrolysis temperature increased. However, BET area for BO300 and BP300 presented lower BET area values of 0.1 and 6.6 $m^2 g^{-1}$, respectively probably by the effect of (or due to) impurities from the pyrolysis process. These impurities contribute to the high variability in the N-urea sorption for same operational conditions, being difficult to replicate the results. For the above, the effect of biochar washing time and particle size on the N-urea sorption onto BO300 were studied, a factorial design methodology was carried out. This study involved the design of three experimental blocks, each block was a different washing process using hexane, hexane (B1) followed by methanol (B2) and methanol (B3) were tested. The results indicated that the experimental block B2 was the most effective washing process. The effect of particle size was the most important followed by the washing time. Finally, the optimal conditions to carry out N-urea sorption experiments were by hexane-

methanol washing for 5 h and a particle size a range between 53-150 μm, these conditions showed a sorption capacity of BO300 of 111 mg N-urea g^{-1}.

After obtaining the washing conditions and particle size of biochar, the sorption kinetics of N-urea at room temperature (25 °C) were evaluated. BO300, BP300, BO500, BP500 and activated carbon (AC) were used. The kinetic model used to describe N-urea sorption in the biochar was the Elovich equation. The results showed that the equilibrium time was 48 h for all the evaluated materials however there were differences between their sorption capacities. The sorption capacity of BO300 and BO500 treatments was 17 and 30 mg N-urea g^{-1}, respectively; for BP300 and BP500 values were 38 and 42 mg N-urea g^{-1}, respectively; whereas for AC it was 72 mg N-urea g^{-1}. The sorption kinetics of N-urea on biochar's samples and activated carbon were well described by the Elovich equation, with R^2 values ranging from 0.91 to 0.98.

Due to the low amount of N-urea sorbed onto biochar, the sorption at room temperature did not meet requirements for the development of a controlled-release nitrogen fertilizer. Therefore, the nitrogen impregnation onto BO300 at 150 °C was evaluated. Furthermore, the use of sodium alginate (SA) as the encapsulating agent and the N-release were assessed. After nitrogen impregnation at 150 °C, BO300 treatment showed the total nitrogen content of approximately 19%. The ammonium release tests performed on pellets developed using SA indicated a release lower than 15% on the third day and not above 75% on the 30th day. This behavior agrees with the standards of slow release fertilizers of the Committee of European Normalization (CEN).

The parameters that influence the nitrogen impregnation, temperature of reaction and particle size, for BO300 and BO500 using a factorial design were evaluated. Furthermore, the use of different polymeric materials for the encapsulated formulation was assessed. The results showed that the maximum impregnation time was 10 min for all the evaluated materials; however, there were differences between their adsorption capacities. For BO300 and BO500 the impregnation capacity was 294 and 290 mg N g^{-1} BO, respectively. In relation to the process of nitrogen impregnation, it was determined that the temperature is a key to the process, followed by the particle size. However, the interaction between these

factors is not relevant in the nitrogen impregnation. Moreover, when the nitrogen proportion increases the impregnation capacity increases significantly. Considering the loading nitrogen content onto biochar, and the added amounts of nitrogen in the impregnation process, it is advisable to work with the ratio 1:0.5:5 (biochar: nitrogen: deionized water). This proportion ensures lowest losses of nitrogen during the impregnation process. On the other hand, according to the obtained results the recommended particle size corresponds to sizes <500 μm where the highest impregnation capacity was observed.

Besides, density and viscosity of polymeric solutions affected the preparation of encapsulates and the properties of CRF. The beads produced by dropping a sodium alginate/biochar into a calcium chloride solution showed a more regular spherical form, unlike the case of acetate cellulose (AC) and ethyl cellulose (EC).

The leaching potential of CRF development in disturbed soil column experiments was studied. The experiment was arranged in a completely randomized design with 10 fertilizer treatments × with crop × without crop × 3 replications ×10 events of water addition. It was observed that the $N\text{-}NH_4^+$ amount in leachates presented a maximum of concentration for all treatments at day 22. The greater proportion of N was observed as $N\text{-}NO_3^-$ form in the leachates. For all treatments (crops and no crop assays) the $N\text{-}NO_3^-$ loss by leaching, excepting for the treatment where ESN (commercial N-CRF) was applied, showed higher values after the first and second event of leaching. After day 29th the $N\text{-}NO_3^-$ content showed a fast diminishing. In this sense, EC 2 showed lower $N\text{-}NO_3^-$ content in leachates in contrast than soil treated with U and with BU (biochar impregnated with nitrogen not encapsulated). The crop yield was negatively affected by all CRFs produced using biochar compared with the traditional fertilization (U) and commercial (ESN). Compared with ESN the grain yield was negatively affected in a 83% for C, 81% for SA 2, 70% for AC 2, 62% for AC 1, 52% for EC 1, 38% for SA 1, 28 % for EC 2, 23% for BU and 13 % for U.

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Chapter 1

General Introduction

General introduction

1.1 Introduction

Urea is a solid nitrogen fertilizer; it is cheaper and is therefore used for agricultural production. After being applied to soil, it can be rapidly hydrolyzed to NH_3 and CO_2 by soil urease (Gioacchiini et al., 2002), followed by NO_3 formation through nitrification. In agriculture, more than half of the N fertilizer applied is urea, and this comprises 40% of the global annual urea consumption (Zhao et al., 2010). In Chile urea is the main product in terms of import value, which represents about 45% of total fertilizer purchased from abroad (ODEPA, 2008).

The N recovery by crops from urea is often as low as 30~40%, with a potentially high environmental cost associated with N losses via NH_3 vitalization, NO_3 leaching and N_2O emissions (Zhou et al., 2003).

The solution to these losses lies in the more frequent contribution of smaller quantities of fertilizer, or in the use of controlled-release fertilizers (CRFs). CRFs plays an important role in improving fertilizer use efficiency by plants and by reducing the frequency of fertilization, thereby mitigating environmental pollution and leading to the development of sustainable agriculture.

The literature describes the CRFs as a granular nutrient core material containing at least one water-soluble fertilizer compound and a substantially water-insoluble coating applied on the core material. The fertilizer composition is structured to provide a Gaussian nutrient release rate curve over time with the maximum occurring between 1 and 18 months after exposure of the fertilizer composition to moisture (Tijsma et al., 2000).

At present, the development of CFRs is focusing mainly on obtaining of system in which a fertilizer granule is encapsulated, i.e., it is coated with an inert layer (Lubkowski and Grzmil, 2007; Basu and Kumar, 2008).

However, the use of coating materials may result in a high production cost and even soil contamination after their release into soil (Song et al., 2003). A promising solution to these problems is to mix common urea with industrial organic wastes and controlled-release

inorganic materials as well as mix inorganic compound fertilizers with N-rich and high-quality organic fertilizer (Wang et al., 2005).

In this context, biochar will be presented as a bifunctional tool to provide both carbon sequestration and soil amending properties. Prior research suggests that biochar is modified before being incorporated into the soil (Magrini-Bair et al., 2009). On example, is the incorporation of a nitrogen source to biochar; this process can be achieved through a thermal reaction, when raw materials, a mixture of oil and urea are pyrolyzed. This approach can improve the properties of biochar as a soil conditioner, besides providing a carbon sink in the long term (Magrini-Bair et al., 2009).

Radlein et al. (1997) incorporated 10% nitrogen in an organic matrix formed by polymerization of biomass to obtain an efficient, biodegradable and controlled-release-rate product. As a result, this fertilizer leached less nitrogen compared to mineral ones, reducing groundwater pollution.

The pyrolysis products are preferably chemically combined with a suitable nitrogen compound containing the (e.g. urea) group–NH_2 by forming a mixture between such products and a suitable nitrogen compound. Preferably, the mixing and heating is carried out at 150-180 °C (Radlein et al., 1997).

Similarly, Magrini-Bair et al. (2009) pyrolyzed peanut shell pellets under mild conditions (400 °C). The resulting char retained nitrogen from the feedstock's high protein content. This char also provided the baseline material for further nutrient addition by reaction of pyrolysis oil with urea to add more bioavailable nitrogen. The reactivity of biochar used in the development of CRFs will depend on the feedstock used in the pyrolysis, as well as the reactor operating conditions.

According to this research, bio-oil has a potential application in the formulation of efficient and biodegradable slow-release nitrogen fertilizers. However, one drawback of using this approach for the development of fertilizers could be the non-desired presence of PAHs and furans in bio-oil, due to thermal decomposition of biomass. Moreover, more research is needed regarding the determination of nitrogen release rates (González et al., 2012).

CRF production has focused mainly on obtaining organic fertilizers of specific particle size and physico-chemical characteristics. In recent years there is a trend towards production of biochar-based fertilizers incorporating nitrogen in a process of direct mixing, encapsulation

or pelletizing (Radlein et al., 1997; Khan et al., 2008; Magrini-Bair et al., 2009; Ding et al., 2010).

Several materials have been proposed for CRF encapsulating or coating. The most important of these include wax and sulfur, and organic polymers such as polyolefins (Kosuge and Tobataku, 1988), polyethylene (Salman, 1989), kraft pine lignin (Garcia et al., 1996), cellulose acetate (Jarosiewicz and Tomaszewska, 2003) and sodium alginate (Liang et al., 2007), among others. The release and dissolution rates depend on the coating materials, hydrophobic/hydrophilic characteristic, coating thickness, solvent agents and degradation rate; which in turn is affected by various factors, such as molecular weight of the polymer, and pH, temperature, ions and microorganisms in soil, soil type, content humus and moisture (Liang et al., 2007; Trenkel, 1997).

Sustainable benefits of using these renewable materials as targeted agricultural fertilizers are (1) eliminating conventional fertilizer nitrate runoff into watersheds (a severe and growing water quality problem), (2) increasing soil organic matter accumulation from enhanced root growth, and (3) sequestering carbon in soils. Taken together, the successful development and deployment of these materials could provide a sustainable approach to agriculture and eventually lead to decreasing CO_2 concentrations in the atmosphere (Day et al., 2005; Lehmann et al., 2006; Magrini-Bair et al., 2009).

1.2 Hypothesis and research objectives

1.2.1 Hypothesis

Considering that

- the nitrogen loss from urea is often as 50~60%, with a potentially high environment cost associated, these losses are the result of many chemical, physical and biological processes, whose magnitude is affected by several factors
- a method to effectively reduce losses of nutrient components is the use of controlled-release fertilizers (the composition comprises a granular nutrient core material including at least one water-soluble fertilizer compound and a substantially water-insoluble coating applied on the surface of the material)
- biochar is used for developing nitrogen controlled-release fertilizers

the working hypothesis of this thesis is established as follows:

Nitrogen suitably adsorbed on biochar coated or encapsulated with polymeric materials will decrease its rate of dissolution in soils, thus increasing fertilizer efficiency whereas minimizing nitrogen losses by leaching, and reducing groundwater contamination problems associated with the use of nitrogen fertilizers.

1.2.2 Research objectives

1.2.2.1 General objective

To obtain an urea-based nitrogen controlled-release fertilizer using biochar as a support/carrier material.

1.2.2.2 Specific objectives

1. To study the adsorption equilibrium and kinetics of urea on biochar produced by pyrolysis of lignocellulosic residues.
2. To evaluate the use of polymers for the production of a controlled-release fertilizer using biochar as support material.
3. To evaluate the release of nitrogen sources from the developed controlled-release fertilizer.

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Chapter 2

Biochar as a renewable matrix for the development of encapsulated and immobilized novel added-value bioproducts

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Biochar as a renewable matrix for the development of encapsulated and immobilized novel added-value bioproducts

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Abstract

The use of biochar has traditionally been focused on agronomic applications. Today it is possible to find a wide range of research devoted to study the use of biochar in the most varied fields. This is mainly due to its properties and the diversity of materials that can be used in their synthesis. Also, the conditions of operation in the process of synthesis (pyrolysis) can be easily modified to obtain a product with the desired features for a given application. Consequently, this review examines the biochar properties that are relevant to its applicability in three areas of high industrial interest; control release and immobilization, where it could serve as alternative, cheap and renewable support material to existing conventional types.

Keywords: biochar, support material, immobilization.

2.1 Introduction

After the Kyoto Protocol, international efforts have been made on reducing greenhouse gas emissions through the use of alternative energy sources and renewable fuels. These alternative sources can help to decrease the dependence on fossil fuel reserves and significantly reduce CO_2 emissions (Lehmann et al., 2006; Mathews, 2008). Additional measures taken to mitigate global warming emissions are based on carbon sequestration from the environment. Recently, several strategies from forestation and reforestation in terrestrial ecosystems to innovative technologies such as underground geological and ocean CO_2 storage have been evaluated (Drange et al., 2001; Matthews, 2008).

A simple idea that in recent years has attracted worldwide interest is the application of biochar as soil amendment. This practice is positioned as a new approach to establish, in long-term, a significant sink for CO_2 in terrestrial ecosystems (Gaunt and Lehmann, 2008; Vanderslice and Marrero, 2009).

This idea stems from observations in the Brazilian Amazon (Lima et al., 2002, Whitman and Lehmann, 2009), where low-fertility forest red land used for grazing and cultivation contain large dark areas known as Terra Preta (Magrini-Bair et al., 2009). These are fertile soils due to high levels of soil organic matter (SOM) and nutrients such as nitrogen, phosphorus, potassium and calcium (Lehmann, 2007; Whitman and Lehmann, 2009). These features and high fertility is attributed in part to its high carbon content, the main reason because Terra Preta tends to be much darker than the color of the adjacent soil (Glaser, 2002).

Recent studies have shown that the origin of Terra Petra is not due to geological processes, but rather to anthropogenic activities (Steiner et al., 2008; Magrini-Bair et al., 2009). Magrini-Bair et

al. (2009) argues that the ancient pre-Columbian civilizations cut the forest, burying the logs to later burn them and produce charcoal (Magrini-Bair et al., 2009). This meant that centuries later, these charcoal deposits continue to be fertile.

Biochar is a carbon-rich material obtained from the pyrolysis of lignocellulosic biomass which can be used as a source of bioenergy, as a mitigation measure of global warming through carbon storage, soil improver, precursor in the manufacture of composite materials and potential support material for applications on control release, bioseparations and biocatalysis, among others (Chen, 1967; Goldberg, 1985; Lehmann et al., 2006).

Biochar is considered a source of carbon whose chemical, physical and biological properties can remain stable; therefore, its presence in nature can extend itself over time making an excellent source of organic matter to soil and support material for several applications (Glaser, 2002; Lehmann, 2007). Commonly, biochar has been well studied under the presumption of having positive effects in soil fertility (promoted by a pH increase, the addition of free bases such as calcium (Ca), potassium (K) and magnesium (Mg), and improvement of the cationic exchange capacity). However there is little information about the use of biochar for new applications related to high value-added products. In this respect, biochar could be used in a large number of applications because of its chemical and physical nature, similar to synthetic support materials. Depending on the pyrolysis conditions of different types of biomass, the resulting biochar may be characterized by several functional groups and an adequate porous structure available for environmental and catalytic processes, where conventional support materials are used.

As reported by several researchers, the main application of biochar is spreading and incorporation in soils. The use of biochar to produce bioenergy could be also of great interest in the near future. A study about profitability of biochar produced from crop residues stated that under current

conditions and numerous assumptions (feedstock cost, transportation and storage costs of biochar, fixed and operating costs of the facility) both pyrolysis processes (fast and slow) are unprofitable (McCarl et al., 2009). Nevertheless, Brown et al. (2011) reported that especially slow pyrolysis remain unprofitable assuming feedstock (biomass) cost of US$ 0.08 kg^{-1}. Therefore, it is necessary to find new potential applications for biochar with an increased added value and by this way improving profitability of biochar economy (Brown et al., 2011).

A new possible use of biochar is as novel support material for different applications. In Table 2.1, the market prices of some conventional support materials for biomolecules immobilization are compared with biochar produced from crop residues.

Table 2.1 Cost comparison between conventional support materials and biochar.

Support material	**Price (US$/kg)**	**Reference**
Activated carbon	1.0-3.0	(Babel and Kurniawan, 2003)
Zeolites	0.03-0.12	(Mineral Commodity, 2005)
Montmorillonite	28	(Sorbent Systems)
Alumina	131	(Price list)
Celite	86	(Filter Aid Accessories-Celite 545)
Kaolin	0.11	(Mineral Commodity, 2005)
Graphite	0.15	(Mineral Commodity, 2005)
Biochar	0.02	(Brown et al., 2011)

The values of Table 2.1 evidence a potential application of biochar as support material regarding only the market prices, observing similar prices between kaolin, graphite and biochar.

Therefore, in this work, the potential use of biochar as support material for environmental and catalytic applications is reviewed. Moreover, the influence of the production variables of biochar

(type of raw material and pyrolysis process conditions) on its chemical and physical properties will be discussed and reviewed.

2.2 Raw materials used for the production of biochar

According to the International Biochar Initiative (IBI), biochar should be produced by using waste-derived biomass. Thus, biochar production should not compete for land that can be destined to agriculture or any other activity. Waste-derived biomass may include agricultural and forestry wastes, as well as sludge from wastewater treatment plants and animal manure (Yaman, 2004). However, an important factor to consider when choosing the raw materials for biochar production is the safety of the biomass. For example, conversion of biomass to biochar can result in an accumulation of contaminants such as heavy metals if the original biomass contains high quantities of these species (e.g sludge from wastewater treatment plants) when the derived final products are applied into soil.

Currently, agricultural and forestry wastes are burned or decayed, releasing CO_2 and CH_4 to the atmosphere contributing to global warming, affecting soil biodiversity and, also, contaminating soils, surface and ground water. Therefore, the use of these materials for the production of biochar will prevent negative environmental impacts.

In terms of biochar production, it is of great interest to know the main components of the raw materials derived from agro-forestry activities (lignin, cellulose and hemi-cellulose), since they determine carbon volatility relationship and performance of the pyrolysis products (biochar, bio-oil and syngas). Thus, when biomass with high lignin content is pyrolyzed at low temperatures (e.g. between 250 and 500 °C), it produces biochar yields close to 50% (Demirbas, 2004; Demirbas, 2006) due to the high stability of lignin against thermal degradation. However, other

pyrolysis conditions, such as reactor temperature, heating rate, initial particle size and initial temperature, influence the biochar yields (Yaman, 2004).

Basically, the thermal decomposition of agro-forestry biomass occurs in three main stages. Thermo-gravimetric analyses (TGA) have shown that a first weight loss occurs up to a maximum of 200 °C. This stage is commonly termed, the pre-heating phase since the loss primarily due to moisture removal (Zabaniotoua et al., 2008; Mani et al., 2010). After this stage, primary devolatilization (stage 2) occurs which is characterized by the degradation of cellulose and hemi-cellulose. Weight loss occurs here as volatiles are being displaced and decomposed between 200 and 370 °C (Mok, 1992; Lapuerta et al., 2004). The decomposition range of hemi-cellulose varies between 273 and 285 °C, whereas cellulose decomposition varies between 312 and 332 °C (Mok, 1992).

Secondary devolatilization (stage 3) corresponding to lignin degradation is related to heavier volatiles and is therefore relatively more thermally stable than hemi-cellulose and cellulose, resulting in a overall degradation temperature range between 150 and 900 °C (Mani et al., 2010; Dehkhoda et al., 2010). In Table 2.2 some of the agricultural and forestry wastes with their lignocellulosic contents and biochar yields are shown.

According to Table 2.2 higher lignin content in the feedstocks gives a higher biochar yield in all pyrolysis temperature range. Besides, as pyrolysis temperature increases a lower biochar yield is obtained favoring the production of liquid (bio-oil) and gas products.

Table 2.2 Lignocellulosic materials composition of agricultural and forestry wastes for biochar production.

Feedstock	**Lignocellulosic content (wt %)**			**Pyrolysis conditions**	**Biochar yield (%)**	**Reference**
	Lignin	Cellulose	Hemi-cellulose			
Eucalyptus gummifera	37	38	16	Slow pyrolysis at 500 °C	47.5	Mok (1992)
Eucalyptus saligna	25	45	15		45.2	
Sugar cane bagase	17	36	17		44.4	
Sweet sorghum	16	36	18		41.4	
Luecaena hybrid KX-3	25	43	17		41.2	
Sweet gum	19	40	23		40.6	
Silver maple	22	40	23		40.3	
Populus deltoides	26	39	21		38.7	
Energy cane	15	37	18		38.0	
Corncob	31.7	31.7	3.4	Fast pyrolysis at 500 °C	23	Yanik et al. (2007)
Straw	12.2	34.5	14.2		20	
Oreganum stalk	10.9	33.8	9.3		23	
Olive husk	50.6	25.2	24.2	Slow pyrolysis at 677 °C	35.6	Demirbas (2004)
Corncob	15.5	52.0	32.5		≈ 13	
Tea waste	43.5	33.2	23.3		≈27	
Legume straw	34.0	28.1	34.1	Fast pyrolysis at 800 °C	≈10	Li et al. (2004)
Apricot stone	51.4	22.4	20.8		≈20	

2.2.1 Pyrolysis process

Pyrolysis is defined as the thermal decomposition of organic materials in partial or total absence of oxygen, resulting in three output streams: solid (biochar), bio-oil and gas (synthesis gas). The yield of each product depends on the different reactor configurations (captive sample reactor, fixed bed reactor, etc.) as well as on the temperature and raw materials type (Antal and Gronli, 2003).

Pyrolysis can be classified as fast, intermediate and conventional pyrolysis, depending on the operating conditions that are used. Conventional pyrolysis may also be termed slow pyrolysis. In the older literature slow pyrolysis often referred to as "carbonization" due to the relatively high proportion of carbonaceous material (biochar) (Mohan et al., 2006; Sohi et al., 2009). Table 2.3 summarizes the operating conditions and products conversion yields obtained in different pyrolysis processes.

According to the classification shown in Table 2.3 the pyrolysis process can be separated in fast, intermediate and slow, the slow pyrolysis (≈ 400 ºC) being the most suitable technology to obtain higher biochar yields. Likewise, when liquid fuels are sought fast pyrolysis is especially relevant. In fact, as pyrolysis temperature rises, the proportion of aromatic carbons in biochar rises, favoring liquid formation (I.E.A, 2006; Cao and Harrisb, 2010).

Table 2.3 Pyrolysis operational conditions and products yields obtained (I.E.A. 2006; Brown, 2009).

Process	Conditions	Bio-oil	Biochar	Syngas
Fast pyrolysis	Moderate temperatures, ~500 °C, short hot vapuor residence time of ~1 s.	75%	12%	13%
Intermediate pyrolysis	Moderate temperatures, ~500 °C, moderate hot vapour residence time of 10-20 s.	50%	20%	30%
Slow pyrolysis (conventional)	Low temperatures ~400 °C, very long vapour residence time (5-30 min).	30%	35%	35%

If biochar is to be used in soil applications, the most suitable technology is slow pyrolysis, since it maximizes the performance of biochar and the final product is more stable[28]. Moreover, during slow pyrolysis nutrients such as phosphorous (P), potassium (K) and sulphur (S) and free bases such as Ca, K and Mg are typically accumulated in biochar in a bio-available form (Hossain et al., 2007). In addition, biochar obtained at low temperatures presents a high cation exchange capacity (Navia and Crowley, 2010).

It is important to highlight that relevant dioxins and polycyclic aromatic hydrocarbons (PAH) concentrations may be expected during pyrolysis (Navia and Crowley, 2010; Verheijenet al., 2010). The occurrence of these compounds in biochar derives from pyrolysis conditions which favor their generation (Verheijenet al., 2010). Pyrolysis temperatures exceeding 700 °C are generally associated to the formation of dioxins and PAHs (Ledesmaet al., 2002; Garcia-Perez,

2008). In the temperature range between 350-600 °C, very small amounts of PAHs may also be formed (Garcia-Perez, 2008).

2.2.2 Properties of biochar

It is important to present an overview of the structural, physical and chemical characteristics of biochar to properly assess its possible use in the development of new products or new support materials.

2.2.2.1 Structural characteristics

The study of structural characteristics of biochar such as specific surface area, pore volume, pore size distribution, texture and density is of high significance for the prediction of potential applications as support material. These characteristics are related to the feedstock's (particle size, moisture and lignocellulose content, among others) and pyrolysis conditions used for biochar synthesis. Therefore, the different structural characteristics are based on the micro-structural rearrangements experienced by the different types of biomass during the pyrolysis process, which mainly depend on the temperature, residence time, heating rate and pressure, among other parameters (Downie et al., 2009). The vascular structure of lignocellulosic biomass contributes to form large pores in biochar, although most of the specific surface area comes from nanopores formed during the heating process (Kumar and Gupta, 1995; Brown, 2009). Therefore controlling these conditions, specific porosity requirements can be achieved for the development of desired biochar products.

A number of studies have demonstrated that pyrolysis temperature is the most important parameter within the operating conditions, as it provokes the main physical changes in biochar structure. In fact, Aguado et al. (2000) studied the generation of a micro-porous structure derived from the pyrolysis of *Pinus insignis* in a conical spouted bed reactor and determined the kinetics of char formation in a temperature range between 350 and 700 °C. They found that below 400 °C micro-pores were generated very slowly and an excessively long residence time was required to obtain a significant change in surface area. They also observed that at 400 °C pores slightly larger than mesopores (between 2 and 20 nm) were developed, independent of the residence time. At higher temperature values (> 450 °C) after 60 seconds an insignificant mesopore formation was observed, while after 180 seconds, mesopores and micropores (< 2 nm) were clearly developed (Aguado et al., 2000).

Kumar and Gupta (1995) studied changes in structural morphology of acacia and eucalyptus species under slow and rapid pyrolysis at temperatures of 250, 400, 600, 800, 1000 and 1200 °C. Their results show that the fibrous structure of wood was conserved during slow pyrolysis even at the highest temperature (1200 °C), while the rapid carbonization, above 600 °C, broke the fibrous structure, showing more presence of micro-pores in the samples. Nevertheless, in both pyrolysis processes a decrease in the pore size of the resultant chars with an increase of carbonization temperature was observed (Kumar and Gupta, 1995).

The effect of different feedstocks and pyrolysis temperature on the development of specific surface area (SSA) in biochar is shown in Table 2.4. Most of the results presented in this table suggest that an increase in the pyrolysis temperature increases SSA, independent of the feedstock used.

Moreover, it is important to compare the techniques used for biochar SSA measurement. As observed in Table 2.4, when CO_2 isotherms are used, SSA is higher compared to N_2 isotherms technique. Yao et al. (2011) stated that SSA measurement by N_2 is an inaccurate procedure for microporous materials (Kwon and Pignatello, 2005; Yao et al., 2011). A possible reason may be the limited N_2 diffusion-controlled rate into small pores at the measurement temperature (usually -196 °C).

2.2.2.2 Chemical composition

Transforming biomass into biochar causes a weight loss, rearrangement of the original sugars to aromatics and formation of a porous and reactive carbon surface. The resulting biochar has a variety of chemical functionalities on its surface that also depends on feedstock type and process conditions. In recent investigations, infrared analysis of biochar showed the presence of alkyl aromatic units that contain hydroxyl functional groups, carboxyl, carbonyl, ether, phenolic, alkyl and polyaromatic hydrocarbons (PAH) (Magrini-Bair et al., 2009; Steinbeiss et al., 2009). Nuclear magnetic resonance spectroscopy (^{13}C-NMR) also corroborates the presence of some of these compounds such as carbonyls, aromatics and alkyl groups (Brewer et al., 2011).

Table 2.4 Effect of temperature and feedstock type on the measured biochar specific surface area**.**

Feedstock	Pyrolysis temperature (°C)	BET surface area		Reference
		N_2 (m^2 g^{-1})	CO_2 (m^2 g^{-1})	
Oak wood	350	-	450.0	Lehmann et al. (2011)
	600	-	642.0	
Corn stover	350	-	293.0	Lehmann et al. (2011)
	600	-	527.0	
Poultry litter	350	-	47.0	
	600	-	94.0	
Corn stover	500	3.1	-	Mullen et al. (2010)
Poultry litter	350	1.1	-	
	700	9.0	-	
Switch grass	250	0.4	-	
	500	62.4	-	Novak et al. (2009)
Pecan shell	350	1.0	-	
	500	222.0	-	
DSTC*	600	336.0	449.0	
STC*	600	2.6	351.0	Yao et al (2011)
Maple wood	400	400.0	711.0	Kwon and Pignatello (2005)

*: DSTC: digested sugar beet tailing biochar; STC: undigested sugar beet tailing biochar

Some of these hydroxyl, carboxyl and carbonyl groups are able to exert several interactions with a large range of biomolecules having usefulness in separation and immobilization processes. However, and depending on the application, highly toxic polyaromatic hydrocarbons must be

carefully removed before biochar use. Total carbon content in biochar has been reported in the range between 17.2 to 90.5%, of which less than 50% corresponds to organic carbon. Total N varies from 1.7 to 56.4 g kg^{-1}, depending on the feedstock. Despite seemingly high, total N content may not be necessarily beneficial to crops, since N is mostly present in an unavailable form, while the available N mineral (NH_4^+-N and NO_3^--N) content for crops is negligible and less than 2 mg kg^{-1} (Chan and Xu, 2009). Analysis of nuclear magnetic resonance (^{15}N-NMR) carried out by Almendros et al. (2003) show that N is mainly found in the form of aromatic and heterocyclic N-containing structures. These compounds occur as a result of biomass heating, converting labile structures into more recalcitrant forms. Carbon nitrogen ratio (C/N) in biochar has been found to vary widely between 7 and 500, with implications for nutrient retention in soils (Chan and Xu, 2009).

When pyrolyzed, concentrated ash could contain considerable quantities of calcium carbonate ($CaCO_3$), bentonite, enrichment in metallic elements as carbonate and oxides species and organic trace species (Van Zwieten et al., 2007). These materials provide valuable liming properties when applied to acid soils, but also toxic elements release could have undesirable effects on plant nutrition and health.

2.3. Current and potential applications of biochar

According to the literature, the most common use of biochar is its application in croplands to increase crop yields and soil fertility, to decrease fertilizer runoff, lime and fertilizer use and to minimize greenhouse gases such as methane and nitrous oxide. Nevertheless, due to the physical, chemical and structural characteristics of biochar, its use in a wide variety of applications ranging from agriculture to medicine is proposed. Therefore this section highlights the use of biochar in

two major fields of new potential applications as renewable support material: control release fertilizers and immobilization support material.

2.3.1 Control release fertilizers (CRFs)

Fertilization is a key operation in crop production (Oliet et al., 1999). However, high levels of fertilizers application may: *i*) reduce the quality of ground water, *ii*) increase adverse health effects (methemoglobinemia and hypoxia), *iii*) alter global N cycle, *iv*) acidify soils, *v*) increase nutrients in estuarine and marine ecosystems, leading to eutrophication, and *vi*) furthermore, raise alarm about greenhouse warming (Frink et al., 1999; Crews and Peoples, 2004). In the case of nitrogen fertilization, it has been estimated that the leaching losses (mainly as NO_3^- and NH_4^+) can reach up to 150 kg N ha^{-1} y^{-1} using sodium nitrate or urea doses of 300 kg N ha^{-1} (Mora et al., 2007).

One way to improve nutrients uptake by plants is the use of controlled-release fertilizers (CRFs) which have been known, since 1962 (Ortli and Lunt, 1962). However, it is still a major research topic, due to the increasing concern towards the excessive release of fertilizers to the environment. CRFs are designed to retain active ingredients and release them gradually, trying to coincide with the nutrients requirement of a plant, ensuring the effectiveness of fertilizing through minimum losses (Wu and Liu, 2008).

CRFs can be formed mainly as: *i*) a granular nutrient core material containing at least one water soluble fertilizer compound, and a substantially water-insoluble coating applied on the core material and *ii*) a mixture of a support material with an adsorbed nutrient on its surface, coated or encapsulated in a polymer matrix. The fertilizer composition is structured to provide nutrient release rate curves which depend on the degradation and/or permeability of the coating material

through the environment by diffusion through the shell. Previous works have determined the release rate occurring between 1 and 18 months after fertilizers exposure to moisture (Tijsma et al., 2000), comparing it with a conventional fertilization processes (e.g. urea). In conventional fertilization processes nutrients release lasts 30–60 days, given a crop growth cycle of 100–120 day, meaning that a conventional fertilizer needs to be applied 2 or 3 times in the same period (Lubkowski and Grzmil, 2007).

In the decade of the 90s, CRFs represented only the 0.15% of the global consumption of fertilizers. Although this was a negligible share of the market, it has been rapidly growing, especially in the USA and Japan, increasing by 76% and 257%, respectively between 1980 and 1995/96. Worldwide, the total slow and controlled-release fertilizer market has grown at an annual rate of 4.5 to 5.0% in the same period (Trenkel, 1997).

The highest consumption and production of CRFs is in the USA, Canada, South Korea, Israel, China, Japan and Europe (Lubkowski and Grzmil, 2007), and the largest proportion of these fertilizers is consumed in non-agricultural markets (e.g., for lawn care, golf courses and landscaping). According to Trenkel (1997) the use of CRFs in agriculture slightly exceeds 10% of the total amount of CRFs in use. Jain (2007) estimated the value of the CRFs is 3-4 times more expensive than conventional fertilizers, which could be the main reason for their limited use in agriculture. However, these costs could be offset by a decrease in operating costs of fertilization, for instance, low-cost support biomaterials whose properties can be similar to those of synthetic ones.

2.3.1.1 Conventional materials used in the generation of CRFs

Few studies report the use of support materials for the development of CRFs. Up-to date research has focused on the use of fertilizer coated granules. In addition, some investigators reported the use of zeolites for the development of CRFs, based on their excellent cationic exchange capacity (CEC) and remarkable cation selectivity (Park et al., 2005). In this regard, Barbarick et al. (1990) tested greenhouse growing systems with sorghum-sudangrass using NH_4^+-saturated clinoptilolite and phosphate rock (dose of 340 mg phosphate kg $soil^{-1}$). The results showed that mixing NH_4^+-saturated clinoptilolite with phosphate rock provided simultaneous slow-release of phosphor and nitrogen. Other authors proved occlusion of KNO_3 and NH_4NO_3 in four natural zeolites at 180 °C for time periods of 2, 4, 6, 8 and 12 h and at 250 °C for 4 h, using a zeolite/nitrogen fertilizer ratio of 1:4 (w/w). Experimental results showed that both NH_4^+-saturated and NH_4NO_3-occluded zeolites, exhibited a similar slowly and steadily NH_4^+ release over 10 days, finding also that the release of NH_4^+ and NO_3^- was dependent on the type of zeolite (Park and Komarneni, 1998).

The use of waste paper for developing an environmentally-friendly slow-release fertilizer by impregnating it with urea was also reported (Khan et al., 2008). The release patterns of N were examined in both batch and continuous conditions in simulated soil solution and distilled water. For both, batch and continuous systems, the release rate was similar being slow and steady in the early stages.

2.3.1.2 The use of biochar in CRFs

The benefits of using renewable materials as targeted agricultural fertilizers are *i*) eliminating conventional fertilizers nitrate runoff into watersheds (a severe and growing water quality

problem), *ii*) increasing soil organic matter accumulation and *iii*) sequestering carbon in soils. The successful development and deployment of these materials could provide a sustainable approach to agriculture and eventually lead to decreasing CO_2 concentrations in the atmosphere (Dayet al., 2005; Lehmann et al., 2006; Magrini-Bair et al., 2009).

Controlled-release fertilizers using biochar as support material with a nitrogen-fertilizer source has so far been less studied (Khan et al., 2007). One of the few studies on this topic deals with the development of a CRF from oak-wood biochar (carbonized at 600 °C) and impregnated by means of a rotary vacuum evaporator with N-P-K fertilizer solution for 24 h at 100 °C. This study evaluated the N, P and K release patterns from impregnated biochar using a simulated soil solution and distilled water as leaching solutions. The experimental results indicated a slow and steady release of N, P and K, detecting a higher content in soil solution than distilled water (Khan et al., 2007). Other studies suggest that the incorporation of a N-source into biochar before its incorporation into soil can improve soil properties, besides providing a carbon sink in the long term (Magrini-Bair et al., 2009). The development of a mixture of biochar/bio-oil/nitrogen has been also proven as an efficient biodegradable slow-release nitrogen fertilizer. These studies suggest that the presence of bio-oil can improve the functionality of the biochar-matrix, providing carboxyl, carbonyl and phenolic groups able to react with active compounds, as a result their incorporation into the biochar-matrix (Radlein et al., 1997; Magrini-Bair et al., 2009). In one of these studies, the biochar-based fertilizer was prepared by a heating treatment of 50 g peanut shell charcoal with 50 g bio-oil and 25 g urea at 200 °C for 1 h. The authors tested different charcoal doses in pots as soil ameliorant (with and without N-P-K fertilizer). After 42 days, experimental results showed that the biochar/bio-oil/N-source mixture supplied the plants with nitrogen, suggesting that the mixture could be considered as a promising CRF (Magrini-Bair et

al., 2009). According to this research, bio-oil has a potential application in the formulation of efficient and biodegradable slow-release nitrogen fertilizers. However, one drawback of using this approach for the development of fertilizers could be the non-desired presence of PAHs and furans in bio-oil, due to thermal decomposition of biomass. Moreover, more research is needed regarding the determination of the nitrogen release rates.

The authors of this review are currently improving the process for developing a granular organic nitrogen controlled release fertilizer. The process is based on the impregnation of a N-fertilizer on agricultural wastes derived biochar from slow pyrolysis and posterior encapsulation using a biodegradable polymer.

The release and dissolution rates of water-soluble fertilizers also depend on factors related to coating materials used in the fabrication of CFRs. After CFR application, water penetrates through a membrane inside the fertilizers granule. Then, nutrients are dissolved and the arising osmotic pressure leads to the release of the active compound which is controlled by diffusion through the coating (Lubkowski and Grzmil, 2007). Therefore, nutrients release is influenced by diverse factors such as coating type, thickness and degradation rate, solvent agents, particle size, shape and surface profile of the substrate onto which the coating is applied, molecular weight of the coating, outside pH, temperature, ions and microorganisms in soil, soil type, humus and moisture content (Trenkel, 1997; Liang et al., 2007). Some of the coating materials used in the development of CFRs are wax, sulphur and organic polymers such as polyolefins (Kosuge et al., 1992), polyethylene (Salman, 1989), kraft pine lignin (Garcia et al., 1996), and polyacrylamide (Rajsekharan and Pillai, 1998). However, the use of coating materials may result in higher production costs and even soil contamination after their release into soil.

Some authors have developed diverse CFRs consisting of urea or KNO_3 mixed with polyacrylamide (Shavit et al., 2003), NPK granular fertilizers coated with polysulfone, polyacrylonitrile and cellulose acetate (Jarosiewicz and Tomaszewska, 2003). Other authors investigated a double-coated, slow-release, and water-retention urea fertilizer (Liang and Liu, 2006), composed of a core of urea granules, a polystyrene shell as inner coating and a cross-linked poly(acrylic acid)-containing urea shell as the outer coating.

2.3.2 The use of biochar as potential biomaterial for the immobilization of bio-molecules and microorganisms

The immobilization of bio-molecules such as proteins, enzymes and microorganisms onto solid supports has attracted much attention due to its scientific importance and application in many areas, such as biology, medicine, biotechnology and food processing. The activity of immobilized bio-molecules depends on the surface area, porosity, chemical nature of the surface and the immobilization methodology, among others (Raman et al., 1991). A variety of materials, including inorganic (clay, silica, alumina, metal oxides) and organic (natural o synthetic polymers) have been used as support material for bio-molecules immobilization (Sheldon, 2007). Even, carbonaceous materials have been used as immobilization supports, showing superior textural properties and higher water stability as compared to silica materials (Quirós et al., 2011). From its characteristics, such as large internal surface area and porosity (Kumar et al., 2009), activated carbon has been widely used in the immobilization of bio-molecules. However, the cost associated with carbon activation increases the overall cost. Consequently, biochar may be considered as a promising immobilization support material for bio-molecules and microorganisms (Cea et al., 2010).

2.3.2.1 Enzyme immobilization

Enzyme immobilization in a suitable matrix is an important practice in commercial and fundamental enzymology. There are several advantages of using immobilized enzymes, such as more convenient handling of the enzyme, it provides a facile separation from other reaction products and facilitates the efficient recovery and reuse of costly enzymes (Sheldon, 2007). There are several mechanisms for enzymes immobilization including adsorption, covalent binding, entrapment, encapsulation and cross-linking (Bickerstaff, 1997). In general, chemical immobilization methods (adsorption, covalent binding and cross-linking) tend to reduce enzyme activity and may disturb enzyme native structure, but may provide a strong and stable enzyme attachment (Duran et al., 2002). Therefore not only enzyme immobilization is required, but also enzyme activity is desired. Different types of matrices have been used in enzyme immobilization studies. However, selection of a support material is the major parameter that affects the immobilization performance. The main properties of an enzyme carrier should be a large surface area, permeability, insolubility, chemical, mechanical and thermal stability, high rigidity, suitable shape and particle size, regenerability and resistance to microbial attachment (Öztürk, 2001). In this context, previous studies have evaluated the use of activated carbon as carrier or support material for enzyme immobilization due to its high surface area and porosity, which provides enough surface to host enzymes and allow an easy transport of substrates into its active sites, and more importantly to create an environment most favorable for the expression of enzyme activity. Regarding the last statement, it has been reported that papain, amyloglucosidase and acid protease can be immobilized by physical adsorption onto charcoal (Rani et al., 2000; Kumar et al., 2009; Duttaa et al., 2009). All immobilized enzymes tested presented specific enzymatic

activities (SEA) between 50 and 90 % of native (free) enzyme. Moreover, for papain enzyme (Duttaa et al., 2009) an immobilization yield onto charcoal of about 4.7 g g^{-1} was reported, whereas for acid protease the reported yield was 150 mg g^{-1} (Rani et al., 2000).

Only few reports regarding the use of biochar as support material for enzyme immobilization are available in the literature. As standard biochar is not activated, its structural properties and capabilities are more moderated compared to activated carbon. Conventionally biochar has less surface area, porosity and functionalization, however, it has been applied in immobilization processes with promising results. In fact, Farag and Hassan (2004) studied the immobilization of keratinase enzyme, isolated and purified from a feather-degrading culture of *Aspergillus oryzae*, using some carriers such as charcoal and sintered glass beads. The immobilized enzyme prepared by physical adsorption showed an activity of 34.2 U g^{-1} charcoal and an immobilization yield of 63.57%, only surpassed by sintered glass (Farag and Hassan, 2004). In addition, preliminary studies conducted by Cea et al. (2010) have shown biochar as a promising support material for lipases immobilization. In this study the obtained biocatalyst presented catalytic activity quite similar to a widely used immobilized commercial lipase (Novozym 435).

An important parameter to consider in the selection of the support material for enzymes immobilization is the metals content. The presence of these elements can cause enzyme activation or inhibition. Studies carried out by Farag and Hassan (2004) on the effect of metal ions on the purified keratinase activity from *Aspergillus oryzae* showed that the presence of Ca^{2+}, Ba^{2+}, Cu^{2+}, Na^{+}, K^{+} and Mg^{2+} activates the enzyme. On the other hand, in the presence of Zr^{2+}, Hg^{2+}, Cd^{2+} and Pb^{2+} enzyme activity was inhibited (Farag and Hassan, 2004). Moreover, studies conducted by Kumar et al. (2005) showed that Al^{3+}, Co^{2+}, Mn^{2+} and Zn^{2+} inhibited lipase enzyme activity extracted from *Bacillus coagulans* BTS-3, while the presence of K^{+}, Fe^{3+}, Hg^{2+} and Mg^{2+}

ions enhanced enzyme activity. In the case of Na^+, no effect in enzyme activity was observed (Kumar et al., 2005). However, enzyme activation or inhibition may depend on the type of enzyme.

Usually, in studies dealing with biochar characterization, the presence of metals is expressed as total metals content (Chan and Xu, 2009; Amonette et al., 2009; Yao et al., 2011), and no information about the release and corresponding species distribution of metals are given. Therefore, it is recommended to perform standard leaching tests and to analyze species distribution with chemical speciation models, to correlate the presence of these ions (mobile fraction) with the enzymes immobilization yield.

2.3.2.2 Microorganism immobilization

Immobilized microorganisms cover a wide area of applications and are essential components of many biotechnological processes. Immobilization of cells in support materials can be carried out in general by entrapment of the microorganisms and/or binding of organic or inorganic functional groups by covalent or ionic interactions (Klein and Ziehr, 1990).

Thus, previous reports have studied the main interactions and mechanisms for attaching different microorganisms on the surface of biochar and activated carbon. This attachment can provide a highly suitable habitat for microbes colonization, growth and reproduction, particularly for actinomycetes and arbuscular mycorrhizal fungi, as well as *Escherichia coli* (Rivera-Utrilla et al., 2001; Thies and Rillig, 2009). Ribera-Utrilla et al. (2001) determined the adsorption capacity of *Escherichia coli* on different activated carbons. Their study showed that the adsorption capacity of *E. coli* by the evaluated activated carbons increased with their hydrophobicity and macropore volume. Also, it was demonstrated that the number of bacteria adsorbed on demineralized activated carbon was negligible. Therefore, the mineral matter present in carbon played an important role in the adsorption of *E. coli.* Moreover, in the presence of electrolytes the

adsorption capacity markedly increased, from virtually negligible (in the absence of electrolytes) to 87.8% (Fe^{3+}), 54.7% (Ca^{2+}) and 24.8% (Mg^{2+}) of the added bacteria. This increase in the adsorption capacity in the presence of electrolytes has been attributed to the reduction in electrostatic free energy and the increase in cell surface hydrophobicity due to the metal bound by some compounds of the cell membrane (Rivera-Utrilla et al., 2001). Therefore, the presence of metal ions in biochar in solution could favor the adsorption capacity of microorganisms.

Related to the entrapment of microorganisms in support materials, pore size and pore size distribution is an important structural property. Hence, some authors have estimated an optimum pore diameter of the support material in the range of 4-5-fold length of the microorganism (Messing and Oppermann, 1979). Sammonin and Elikova (2004) used pore sizes between 2 and 4 μm for the immobilization of *Bacillus mucilaginosus* and *Acinetobacter* sp. in macroporous materials (according to IUPAC classification). Other authors reported pore sizes between 0.8 and 44 μm for the immobilization of *Paracoccus* sp.KT-5 in bamboo-based activated carbon, also a macroporous structure (Lin et al., 2010). It would therefore seem that, controlling the synthesis conditions of biochar will promote the production of a material with the desired structural properties for microbial immobilization.

Among the applications of microbial immobilization, environmental uses have been also reported for charcoal. Indeed, charcoal has been tested for the immobilization of microbial biomass in a horizontal-flow anaerobic reactor for the degradation of linear alkylbenzene sulfonate. In this study, biochar presented the highest kinetic degradation coefficient compared to other support materials (expanded clay and polyurethane foam) tested for microorganisms immobilization (Lin et al., 2010). It has been reported that *Paracoccus* sp. strain KT-5, a microorganism able to degrade pyridine, was attached well on the surface and pores of bamboo-derived-activated

carbon. Comparison between freely suspended cells and cells attached on bamboo-derived-activated carbon indicated a higher pyridine-degrading rate of the immobilized cells. Additionally, the attached biomass on this activated carbon increased considerably and a higher tolerance of these immobilized bacteria cells to pyridine was detected (Linet al., 2010). Moreover, the results obtained by Lin et al. (2010) demonstrated the feasibility and reusability of immobilized cells for pyridine degradation.

In the field of N-fixation, biochar has been also used for the immobilization of N-fixing bacteria for a subsequent incorporation as soil improver. The findings indicate that biochar is an excellent support material for *Rhizobium* inoculants (Pandher et al., 1993) and *Azotobacter vinelandii* inoculates (Magrini-Bair et al., 2009).

2.4 Conclusions

Biochar can be produced from a wide range of organic feedstocks under different pyrolysis conditions. The suitability of each biomass type for biochar production is dependent on a number of chemical, physical, environmental, as well as economic and logistical factors. In fact, the kind of feedstock used as well as pyrolysis conditions, determine the physical and chemical properties of the biochar produced. Biochar can be used in several applications particularly as support material for the development of control release fertilizers and bio-molecules immobilization. In this sense scientific evidence shows that the use of controlled-release fertilizers based on biochar as support material could improve nutrients uptake by plants, while reducing environmental pollution produced by nutrients volatilization and/or leaching. In this regard, several materials are used in the production of controlled-release fertilizers but this technology has a high cost. Therefore, the use more inexpensive materials and simpler technologies are necessary. Thus,

biochar is presented as a promising material for the development of controlled-release fertilizers, not only acting as a soil conditioner but also promoting global warming mitigation.

In addition, the immobilization of bio-molecules such as proteins, enzymes and microorganisms onto solid supports has attracted much attention due to its scientific importance and application in many areas, such as biology, medicine, biotechnology and food processing. Conventional support materials for this purpose are both of natural and synthetic origin. Among natural supports, biochar have been used in immobilization, showing promising properties compared to synthetic materials which are more expensive in their preparation. Additionally, the synthesis conditions of biochar can be managed to obtain the desired properties such as high functionality, large internal surface area and porosity. In this sense, biochar constitutes a promising immobilization support material for bio-molecules and microorganisms.

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Chapter 3

Biochar derived from agricultural and forestry residual biomass: Characterization and potential application for enzymes immobilization

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Biochar derived from agricultural and forestry residual biomass: Characterization and potential application for enzymes immobilization

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Abstract

Very much attention has been focused on lipases as these enzymes can be used as biocatalysts, allowing a cost effective and environmentally friendly method to efficiently catalyze specific reactions. However, its application at industrial scale is still limited due to several shortcomings including low stability in their native state, inhibition by organic solvents and exhaustion of enzyme activity.

To overcome these problems, lipases has been immobilized by several methods onto various supports. In this context, biochar, a low-cost material derived from the pyrolysis of residual biomass, constitutes a promising immobilization support material for enzymes due its suitable physicochemical and structural properties.

In this study, the use of biochar derived from pyrolyzed agro-forestry residual biomass for lipases immobilization is reported. We present the physico-chemical and mineralogical

characterization of biochar and a preliminary study on the immobilization of *Candida rugosa* lipase using biochar as support matrix.

The results obtained showed that the structural and chemical properties of the biochar depend on the raw materials used and pyrolysis temperature. The specific surface area (BET) presented a similar trend, increasing with an increase in pyrolysis temperature. High enrichment of trace elements such as Ba, Cr, Cu, V and Zn was detected in biochar from pine bark and was discarded for lipase immobilization purposes. The binding efficiency of lipase in oat hull biochar was in the range of 40- 60%, corresponding the higher yields to the low particle size of oat hull biochar suggesting further practical applications of this immobilized lipase.

Keywords: Agro-forestry residues, pyrolysis, biochar, characterization, lipase, immobilization.

3.1 Introduction

Waste management from agro-forestry activities leads to a significant pollution of ground and surface waters. Countries with a high potential of foreign trading with agro-forestry products need new strategies to improve worldwide competition and moreover to increase efficiency and productivity without worsen the environment (Kang et al., 2006).

Reuse of agro-forestry residues could be a potential strategy for saving costs, conserving natural resources and developing new added-value products. Soil application, compost production, biological and thermo-chemical conversion of residues from agro-forestry activities are among the most suitable techniques used up-to-date (Lehmann and Joseph, 2009).

Pyrolysis of agro-forestry residual biomass is one of the most promising strategies. This treatment is generally performed under absence of oxygen to produce three main streams, namely biochar, a fine-grained product, synthesis gas and bio-oil. Biochar is usually known to have a moderate content of essential elements, large surface area, and little biological decay (Novak et al., 2009).

Relevant characteristics of biochar such as pH, surface area, and essential elements are mainly governed by precursor nature and pyrolysis operational conditions such as temperature and heating rate (slow or fast pyrolysis) (Hunt et al., 2010).

In recent years, biochar has positioned itself as 1) a competitive soil amendment, improving health and quality of soils and nutrients retention (Lehmann, 2007; Mchenry, 2009) 2) as a bioenergy source (Lehmann et al., 2006; Brownsort, 2009) to produce heat, power or both combined, 3) as carbon sink due to its recalcitrance in soils (Beesley, 2011) and 4) as a tool for restoration and bioremediation of contaminated soils (Spokas and Reicosky, 2009; Dehkhoda et al., 2010).

New potential applications of biochar have been also reported by Dehkhoda et al. (2010), using biochar as catalyst in biodiesel production and as a promising support for *Candida rugosa* immobilization (Cea et al., 2010).

The immobilization of bio-molecules such as proteins, enzymes and whole cells has been performed using a varied spectrum of materials, including clay, silica, natural or synthetic polymers, alumina and metal oxides, among others (Sheldon, 2007). Carbon materials have been also tested, showing superior textural properties and higher water stability as compared to silica materials (Quirós et al., 2011). Charcoal, a similar material as biochar, has been also used as support material for immobilization purposes and has attracted much attention due to its scientific importance and application in many areas, such as biology, medicine, biotechnology, and food processing (Thomas, 2008; Kawaguchi et al.,2010).

Characteristics such as porosity, high surface area and low content of toxic trace elements makes biochar a potential candidate to be used as enzymes immobilization support material. Preliminary studies conducted by Cea et al. (2010) have shown that biochar could be a promising support for *Candida rugosa* immobilization, since in such study, the biocatalyst obtained presented a high catalytic activity quite similar to a widely used immobilized commercial lipase (Novozym 435).

Among the immobilized lipases studied, *Candida rugosa* lipase, a nonspecific lipase (Öztürk, 2001), has been commonly used in organic solvents due to its high ability to catalyze hydrolysis, esterification, transesterification and aminolysis reactions (Villeneuve

et al., 2000). Depending on the operational conditions and immobilization support type, this enzyme could be used as catalyst for biodiesel synthesis.

Therefore, this work attempts to characterize biochar samples produced from agro-forestry residual biomass and to evaluate their potential use as immobilization support material for *Candida rugosa* lipase.

3.2 Materials and methods

3.2.1 Biochar production

Agro-forestry residual biomass used as raw material for biochar production were oat hulls (O) and dried, crunched pine bark (P). A pilot-scale electric pyrolyzer designed at the University of La Frontera, with a maximum capacity to process 5 kg of raw material per batch was used to produce biochar. The pyrolyzer was fed at full load and then purged with nitrogen gas (to displace air) before starting the process. Carbonization temperatures used for both types of residual biomass were 300 and 500 ºC. The temperature was increased at a rate of 3.6 ºC min^{-1} until the specific temperature was reached and maintained for 1 h. After that, a cooling down procedure until room temperature was carried out. Finally, all biochar samples were gently crunched.

3.2.2 Chemical and physical characterization of biochars

Total organic (TOC) and inorganic carbon (TIC) contents were determined by using an organic carbon analyzer (TOC-V CPH coupled at SSM-5000A). Total Kjeldahl nitrogen was determined using the methodology described in APHA (1995).

Major and trace elements were determined after a special two-step acid digestion method developed for the analysis of trace metals in coal and combustion wastes (Querol et al., 1995). Moreover, two international reference materials, NBS 1633b (coal fly ash) and SARM 19 (coal) were used to check accuracy of the analytical and digestion methods. The concentration of major, minor and trace element in the solutions were measured by means of inductively coupled plasma mass spectrometry (ICP-MS) and inductively plasma atomic emission spectrometry (ICP-AES). Moisture content was determined at 105 ºC during 24h.

Whereas the ash yield was determined by treating the biochar samples at 550 °C for 4 h. Volatile matter (V.M.) and fixed carbon (F.C.) was calculated by the methodology described by Fabbri et al. (2012)

Biochars pH was measured with an Orion 9512 electrode, using a biochar suspension sample/distilled water ratio of 1:5. Total acidity ($Ba(OH)_2$ method) and carboxylic acidity ($Ca(C_2H_3O_2)_2$ method) were determined according to Tan (1996). Phenolic acidity was determined by difference between total and carboxylic acidity.

X-ray diffraction (XRD) analysis was carried out using a Bruker, D8 Advance model diffractometer with a primary Göbel crystal, equipped with a detector based on dispersion of SOL-X energies, with a Cu tube and a wavelength of λ=1.5405 Å, operating at 40 kV and 40 mA.

Fourier Transform Infrared (FTIR) analysis of all biochars and immobilized enzyme were obtained by using Bruker Tensor 27 spectrometer. The sample discs were prepared by mixing oven-dried samples (at 105 °C) with spectroscopic-grade KBr at ambient temperature. Infrared spectra were performed at a resolution of 4 cm^{-1} and cumulating 32 scans.

Specific surface area (Brunauer-Emmett-Teller, BET), pore volume (BJH), and pore size distribution were determined using a NOVA 1000e porosimeter (QUANTACHROME) by adsorbing and desorbing nitrogen at 77 K on samples previously dried and out-gassed at 160 °C for 16 h. Morphology was analyzed using a scanning electron microscope (SEM-EDX, JEOL6400).

3.2.3 Enzyme immobilization

Lipase from *Candida rugosa* (type VII) purchased from Sigma-Aldrich Chemical Co. (USA) was used in the immobilization assays. p-nitrophenol palmitate (p-NP), p-nirophenol palmitate (p-NPP) and bovine serum albumin were purchased from Sigma-Aldrich Chemical Co. (USA). All other chemicals used in this study were of analytical reagent grade.

Immobilization of *Candida rugosa* lipase was studied using biochar BO300, and the experiments were carried out in syringes of 5 mL filled with 2 g of biochar. The columns were eluted with 2 mL buffer phosphate 0.1 M at pH 7 (untreated), 2 mL ethanol 99% and methanol 99%. Then, 10 mL of enzymatic suspension (10 mg *Candida rugosa* enzyme mL^{-1}) were eluted in each column, washed three-times with buffer phosphate 0.1 M at pH 7 and dried at 30°C overnight. The eluates were assayed for protein content to indirectly measure the amount of immobilized enzyme. The activity of immobilized and free enzyme was analyzed spectrophotometrically, measuring the absorption increment at 410 nm promoted by the hydrolysis p-NPP. Molar extinction coefficient was adopted as $1.93\times10^{3}M^{-1}cm^{-1}$ for p-nitrophenol (p-NP), which was determined from the absorbance of standar solution of p-NP in the reaction medium (Chiou and Wu, 2004). Protein content was estimated by the method of Bradford (1976). Bovine serum albumin was used as the standard.

Additionally, a fractionation of BO300 was performed by placing 100 g biochar on nested sieves mounted on a Retsch AS200 Control (Retsch Technology, Düsseldorf, Germany). Sieves were mechanically shaken (amplitude 2.5 mm) for 5 min to separate biochar into the following size classes: < 53, 53-75, 75-90, 90-125, 125-250, 250-500 μm. Fractionated samples were packaged in columns and used to immobilize *Candida rugosa* lipase.

The immobilization efficiency was evaluated in terms of specific activity, protein loading and lipase activity as follows:

(1) Lipase activity (U g^{-1} support)= $\frac{Activity\ of\ immobilized\ lipase}{Amount\ of\ immobilized\ lipase}$

(2) Specific activity (U mg^{-1} protein) = $\frac{Activity\ of\ immobilized\ lipase}{Amount\ of\ protein\ loading}$

(3) Protein loading yield (%) = $\frac{Amount\ of\ protein\ loading}{Amount\ of\ protein\ introduced} x100$

(4) Activity yield (%)= $\frac{Specific\ activity\ of\ immobilized\ enzyme}{Specific\ activity\ of\ free\ lipase} x100$

3. 3Results and discussion

3.3.1 Chemical and physical characteristics of biochars

The general chemical characteristics of biochar obtained are presented in Table 3.1. The carbonization of oat hull and pine bark resulted in the formation of a carbon-rich solid with a total carbon content (C_T) in the range of 58 to 77%, which according to the literature are the product of a series of reactions such as dehydration, condensation, polymerization and aromatization (Lehmann et al., 2011). In this study an increase in C_T with increasing pyrolysis temperature was observed. Similar results were reported by Chen et al. (2008), stating that C_T depends on raw materials and pyrolysis operational conditions. In this sense, a decrease in volatile matter and an increase in carbon content were observed for biochar obtained from both types of biomass. The lower content of C_T and the higher content of volatile matter in BP300 compared to BP500 samples points to incomplete thermal degradation during pyrolysis, attributed to the high lignin content of pine bark. According to the report of Yang et al. (2007), it is more difficult to decompose lignin than other compounds like cellulose and hemicellulose at low temperatures. In fact, hemicelluloses are degraded at 200 °C to 260 °C and cellulose at 240 °C to 350 °C, while lignin can be degraded between 280 °C and 500 °C (Sjöström, 1993).

The inorganic mineral content (ash) present in the tested raw materials is enriched during pyrolysis process (Grierson et al., 2011). The ash forming constituents in biochar are generally considered as reactive, due to the presence of alkaline-metals occurred as oxides and salts. In pine bark biochar samples higher quantities of major and minor elements were detected, except for Hg that can be loss by volatilization.

All biochar samples exhibited a similar pH trend. pH values increased when pyrolysis temperature increased, except for BP300. Pine bark biochar produced at 300 °C presented a low pH value of 4.73. This pH value confirms a partial thermal decomposition of pine bark at 300 °C. According to Abe et al. (1998) at 300 °C, decomposition of cellulose and hemicellulose produces acids and phenolic substances contributing to a low biochar pH value. Moreover, it has been reported that biochars derived from wood may develop a more acidic character (Mullen et al., 2010).

Table 3.1 Chemical and physical characteristics of feedstocks and their derived biochar samples.

	O	P	BO300	BO500	BP300	BP500		BO300	BO500	BP300	BP500
	%wt						Trace elements	mg kg^{-1}			
Al_2O_3	0.05	6.86	0.03	0.02	3.05	5.11	As	<0.1	<0.1	<0.1	1.12
CaO	0.30	0.58	0.30	0.28	1.03	1.28	B	19.4	7.0	12.6	18.9
Na_2O	0.04	0.20	0.06	0.06	0.25	0.48	Ba	14.2	13.7	57.6	86.6
K_2O	1.36	0.61	1.73	1.94	0.65	0.77	Ce	<0.1	<0.1	7.0	10.2
MgO	0.21	0.30	0.24	0.23	0.44	0.64	Co	<0.1	<0.1	3.7	5.2
MnO	0.02	0.03	0.02	0.01	0.05	0.06	Cr	1.0	1.2	10.0	18.7
SO_3	0.31	0.25	0.13	0.08	0.06	0.05	Cs	<0.1	0.9	<0.1	0.9
P_2O_5	0.45	0.07	0.46	0.34	0.16	0.19	Cu	5.8	26.3	18.6	26.3
Fe_2O_3	0.03	1.26	0.04	0.02	1.40	2.29	Ga	<0.1	5.5	3.3	5.5
TiO_2	<0.01	0.35	<0.01	<0.01	0.17	0.29	Hg	0.03	0.02	0.01	n.d.*
C_T	42.65	46.35	70.13	76.97	57.92	72.39	La	<0.1	<0.1	2.0	2.9
N_T	0.49	0.65	1.03	0.95	0.41	0.37	Li	<0.1	<0.1	2.5	4.4
Ash	6.20	5.6	7.70	9.43	13.76	20.39	Nb	<0.1	<0.1	0.8	1.3
V.M.			73.93	70.80	77.59	59.76	Nd	<0.1	<0.1	2.8	4.2
F.C.			18.37	19.77	8.65	19.85	Ni	<0.1	<0.1	4.4	8.6
Moisture			2.20	1.64	2.06	1.98					
							Pb	<0.1	<0.1	2.3	3.5
pH	3.06	3.94	7.77	9.57	4.73	8.29	Rb	35.6	39.0	13	16.6
$S_{carboxyls}$ (mmol g^{-1})	-	-	0.39	0.11	0.11	0.11	Sr	7.3	7.5	40.8	63.3
$S_{phenolic}$ (mmol g^{-1})	-	-	2.05	3.47	2.52	5.36	V	<0.1	<0.1	27.4	49.4
$S_{Total\ acidity}$ (mmol g^{-1})	-	-	2.44	3.58	2.63	5.47	Zn	42.2	30.3	67.9	84.7
S_{BET} (m^2 g^{-1})	-	-	0.1	6.6	1.9	63.0					
Vp (cm^3 g^{-1})	-	-	0.009	0.012	0.008	0.028					
Dp (nm)	-	-	3.2	2.2	3.1	3.1					

C_T: Total carbon, **N_T:** Total nitrogen, **V.M.:** Volatile matter, **F.C.:** Fixed carbon, **BET:** Brunauer-Emmett-Teller, **Vp:** Pore volumen, **Dp:** Pore diameter, **BO300:** Biochar oat hull pyrolized at 300 °C, **BO500:** Biochar oat hull pyrolized at 500 °C, **BP300:** Biochar pine bark pyrolized at 300 °C, **BP500:** Biochar pine bark pyrolized at 500 °C, **n.d.*:**Not determined. **Note:** The remaining amount (%) in the chemical composition corresponds to unmeasured elements such as hydrogen, oxygen and silicon.

Generally, above 300 °C, the char presents higher ash yield and alkaline metals content, contributing to an increase in pH value up to about 12 (Chan and Xu, 2009). Such behavior was observed for both biochar samples at 500 °C. However, an increase in ash yield also increases heavy metal and salt contents in biochar at high temperatures, especially for forestry biochar samples. Table 3.1 shows that in general, pine bark biochars present a higher trace elements content, being BP500 enriched in Ba (87 mg kg^{-1}), Cr (19 mg kg^{-1}), Cu (26 mg kg^{-1}), V (49 mg kg^{-1}) and Zn (85 mg kg^{-1}).

Some reasons for this trace elements enrichment of pine bark biochar could be the longer rotation period of wood which enforces accumulation, the higher deposition rates in forests and possibly the lower pH value of forest soils. Therefore, in future research, it would be necessary to perform leaching tests of pine bark biochar to assess the potential mobility of these elements and the possible implication in enzyme immobilization.

In fact, different metal ions may have different effects on the activity of microbial lipases. For example Huang et al. (2004) found that monovalent ions, such as Na^+ and K^+, enhanced lipase activity from *G. marinum* by 17 and 16% at 6 mM, but the same ions decreased the activity when its concentration was increased to 500 mM (Huang et al., 2004). The same authors also demonstrated that divalent ions affected lipase activity differently. In fact, Ca^{2+} and Mg^{2+} increased lipase activity, whereas Co^{2+} had no effect on the activity and $Fe^{2+}$and Mn^{2+} inhibited the activity almost in a 50%. Additionally, Kumar et al. (2005) found that Fe^{3+} and Hg^{2+} ions enhanced enzymatic activity, while Al^{3+}, Co^{2+}, Mn^{2+}, and Zn^{2+} ions inhibited lipase activity from *B. coagulans*. In addition, no effect of Na^+ was observed on enzyme activity. Lipases activation or inhibition by ions can occur, but according to Fadiloğlu and Söylemez (1997), this phenomenon will depend on the substrate, enzyme and assay conditions. Therefore, we need to consider a possible enzyme inhibition or activation provoked by the different ions present in biochars, ions that may be potentially available during the immobilization process or when the immobilized enzyme is used. Fadiloğlu and Söylemez (1997) demonstrated that *C. rugosa* lipase can be stimulated by Ca^{2+} ions by the formation of calcium salts of fatty acid products in an emulsion containing olive oil as

substrate. On the contrary, in a non-emulsified system, Ca^{2+} had no effect on *C. rugosa* lipase activity when olive oil was used as substrate.

Mishra et al. (2009) demonstrated that Lecitase®Ultra, a phospholipase manufactured and marketed by Novozymes, which was studied after purification by ultrafiltration, was completely inhibited by the presence of heavy metal ions such as Cu^{2+} and Ni^{2+} at concentrations of 1 mM.

Mineralogical characterization was also performed to the studied raw materials and biochar samples. Figure 3.1 shows the X-ray diffraction (XRD) patterns of the raw materials (pine bark and oat hull) and biochar samples. Both raw materials (Figure 3.1a and b) exhibited significant differences in the main crystalline mineral phases. However, they exhibit a predominance of amorphous mineral phases with a high XRD background halo between 16° and 20° 2θ.The pine bark XRD-spectrum (Figure 3.1b) showed quartz (SiO_2), anorthite ($CaAl_2Si_2O_8$) and magnetite (Fe_3O_4) as the main crystalline mineral phases. In both diffractograms, broad peaks were observed, indicating that the crystalline degree and crystals size are quite low (Bourke et al., 2007).

Biochar samples derived from oat hull (Figure 3.1c and d) showed low intensity peaks associated to sylvite (KCl). Detected peaks were more intense at 500 °C (Figure 3.1d) than 300 °C, suggesting that an increase in the temperature increases concentration of minerals by reducing organic weight of biochar. Sylvite has been also detected in biochar derived from canola straw, when pyrolysis performance occurred at the temperature range between 300 and 500 °C (Yuan et al., 2011).

The XRD spectra of pine bark biochar samples showed similar crystalline mineral phases as the raw material, without finding any marked difference between both biochars. In Figure 3.1e and f, an increase in pyrolysis temperature attenuated the high background of the diffractogram patterns, especially for pine bark biochars. In both pine bark biochar was detected quartz, calcite ($CaCO_3$) and anorthite, whereas in BP500 was also detected sylvite and tridymite (SiO_2). The detection of calcite in these pine bark biochar samples suggests that alkalinity could be higher than in biochar samples derived from oat hull.

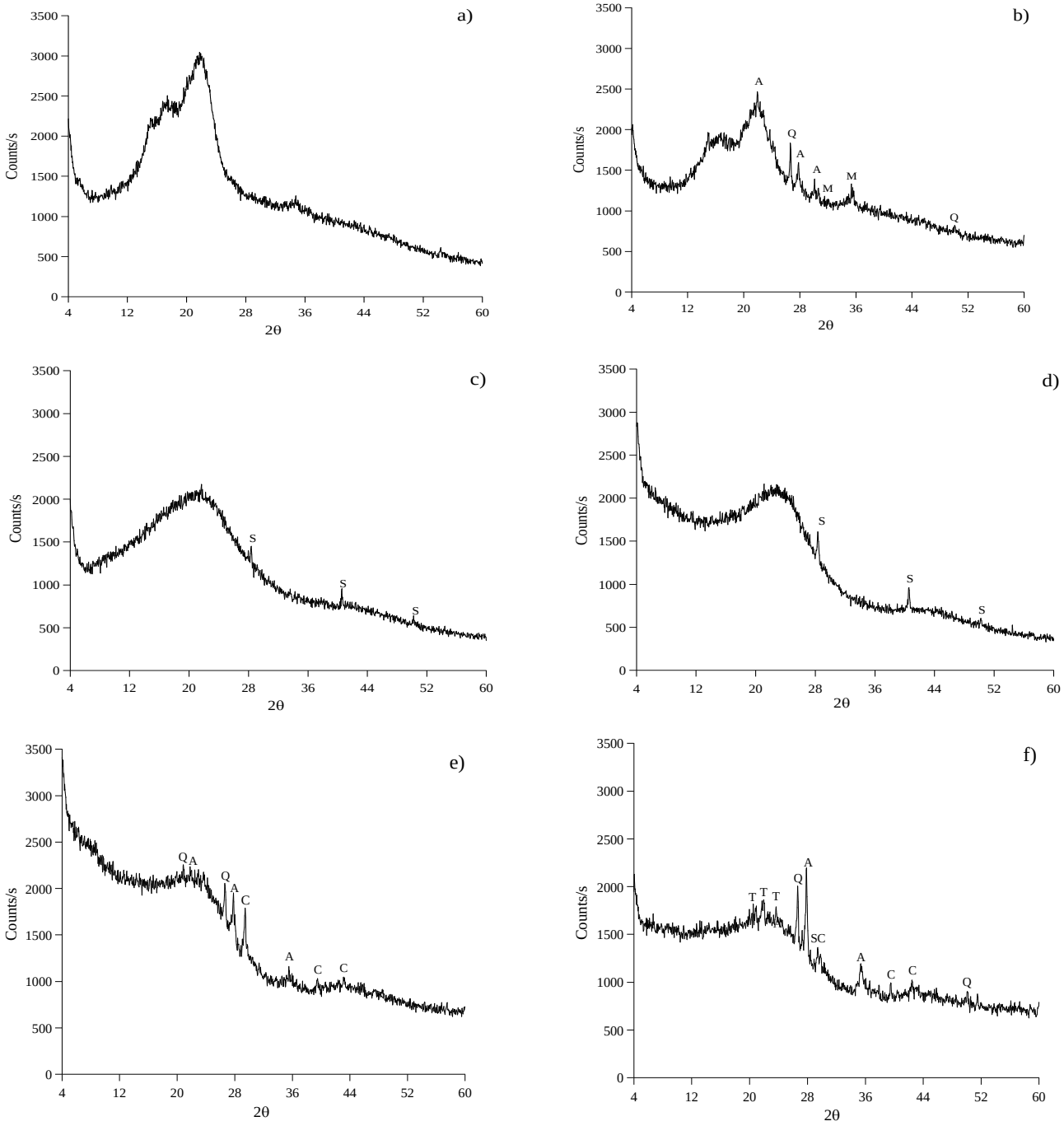


Figure 3.1 X-ray diffraction patterns from (a) oat hull, (b) pine bark, oat hull biochar at 300 °C (c) and 500 °C (d), pine bark biochar at 300 °C (e) and 500 °C (f). S: sylvite, KCl; Q: quartz, SiO_2; A: anorthite, $CaAl_2Si_2O_8$, C: calcite, $CaCO_3$, M: magnetite, Fe_3O_4 and T: tridymite, SiO_2.

In addition, FTIR analysis was performed to elucidate which surface functional groups could be involved in the immobilization of the enzymes. FTIR spectra of biomass types and produced biochars are shown in Fig. 3.2. Different bands in the spectra represent different vibrations of functional groups. The O-H stretching (3400 cm^{-1}), the symmetric CH_3 stretch of the O-CH_3 (2924 and 2855 cm^{-1}) groups that appeared in both original biomass types decreased its intensity when biomass is processed at 300 °C for biochar production. These bands were completely absent in BP500 and with a marked reduction of their intensity in BO500 (Fig. 3.2a, b), indicating that the OH and CH_3 groups were removed or transformed with the temperature. Carboxyl C=O stretching (1736 cm^{-1}) was only present in oat hull biomass and was shifted to a lower energy value (1694 cm^{-1}) in BO300 probably due to the carbonization process. Moreover, its deprotonated form at 1645 cm^{-1} was only present in oat hull biomass and was shifted to a lower energy value (1600 cm^{-1}) in BO300. Both protonated and deprotonated forms of carboxyl groups were completely absent in BO500 (Fig. 3. 2b). Aromatic C=C ring stretching (1618, 1520 and 1440 cm^{-1}) present in pine bark biomass decreased its intensity after a pyrolysis process at 300 °C and are completely absent in BP500. A similar behavior was observed for the O-C stretch (at 1043 cm^{-1}) in the aliphatic ester group. The intensity of the stretch decreased after the pyrolysis of both biomass types.

Surface acidity of BO300 is significantly higher compared to other biochar samples. The high surface acidity of this biochar is mainly related to the carboxylic groups' content, and is confirmed by the FTIR spectrum (Figure 3.2b).

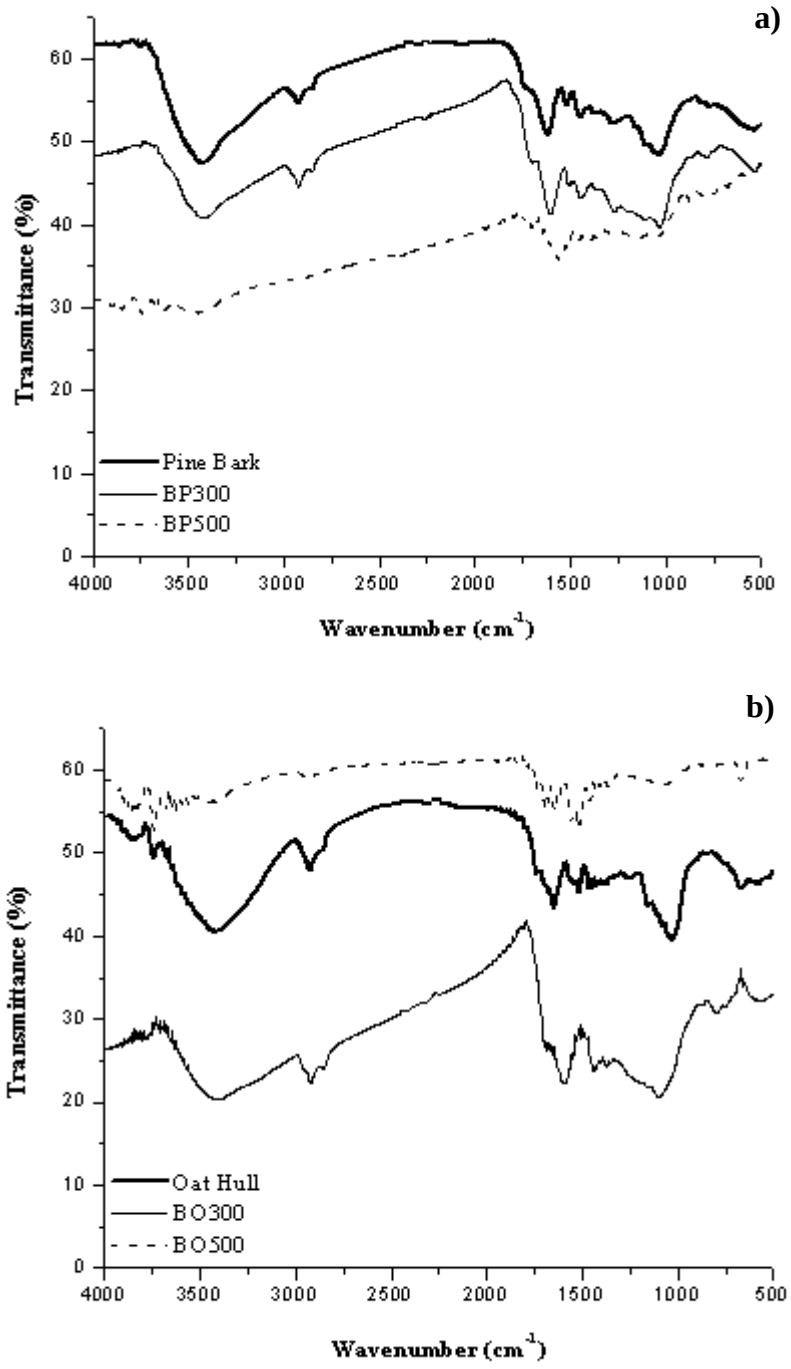
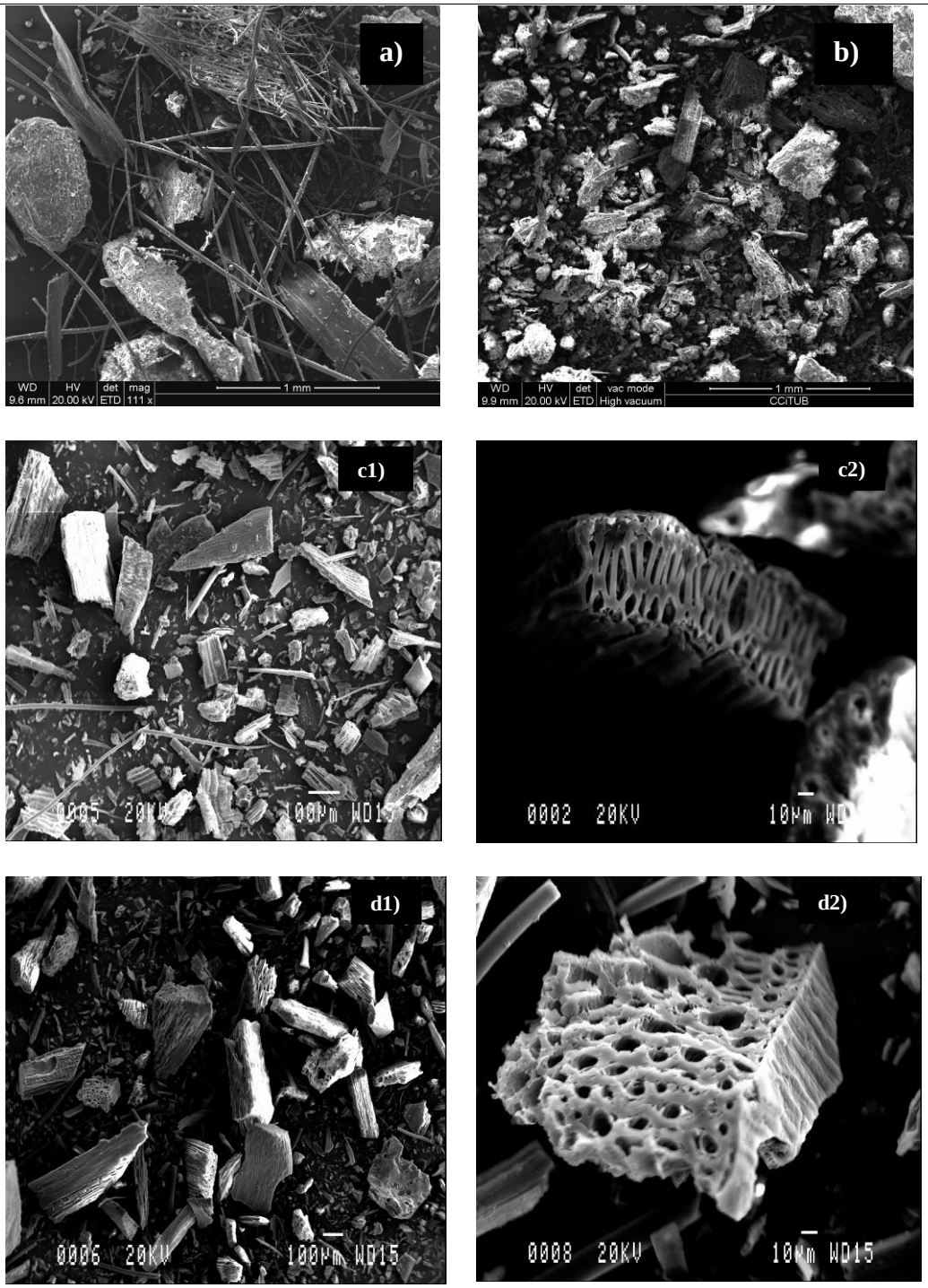


Figure 3.2 FTIR spectrum of (a) pine bark and its derived biochar, (b) oat hull and its derived biochar.

All scanning electron microphotographies (SEM) are displayed in Figure 3.3a large structural difference was found among all biochar samples, especially between agro-forestry residual biomass used and their corresponding biochar.

Raw materials SEM images (Figure 3.3a and b) indicate a predominance of large particles with heterogeneous geometry, especially for oat hull. However, all biochar samples evidenced particles with uneven surface and with certain development of pores, with scarce cases of glasslike surfaces. The search of uneven surfaces on biochar samples is important as pores development may contribute to a higher surface area of biochar.

Oat hull biochar microphotographies showed cracks on biochar surface without the apparently formation of pores, being this fact in agreement with their low specific surface area (Table 3.1). In the case of pine bark biochar, SEM images showed particles with several pores, correlating with their higher specific surface area, as compared to oat hull biochar. No significant morphological differences between biochar samples produced at different temperatures were observed under the SEM-evaluation. Nevertheless, it has been reported that char surface area greatly depends on pyrolysis temperature and raw material. In this study we observed that biochar produced using oat hull as raw material presented a lower specific surface area (SSA) with values ranging between 0.1 and 6.6 m^2 g^{-1} for BO300 and BO500, respectively, while SSA values for pine bark biochars moved between 1.9 and 63 m^2 g^{-1} for BP300 and BP500, respectively. The lower surface area of BO300 can be attributed to the fact that micropores may become filled with tars (condensed volatiles), or less probably to mineral matter which can be occluded in the pores (Novak et al., 2009). Additionally, low-pressure hysteresis in nitrogen adsorption/desorption isotherms has been observed only in oat hull biochar samples. This phenomenon is commonly attributed to diffusion limitations due to constricted pores, which in our study could be explained by the presence of bio-oil onto the surface of oat hull biochar (Brown et al., 2006).


a)
WD 9.6 mm
HV 20.00 kV
det ETD
mag 111 x
1 mm
b)
WD 9.9 mm
HV 20.00 kV
det ETD
vac mode High vacuum
1 mm
CCiTUB
c1)
c2)
0002 20KV
10µm WD
d1)
0006 20KV
100µm WD15
d2)
0008 20KV
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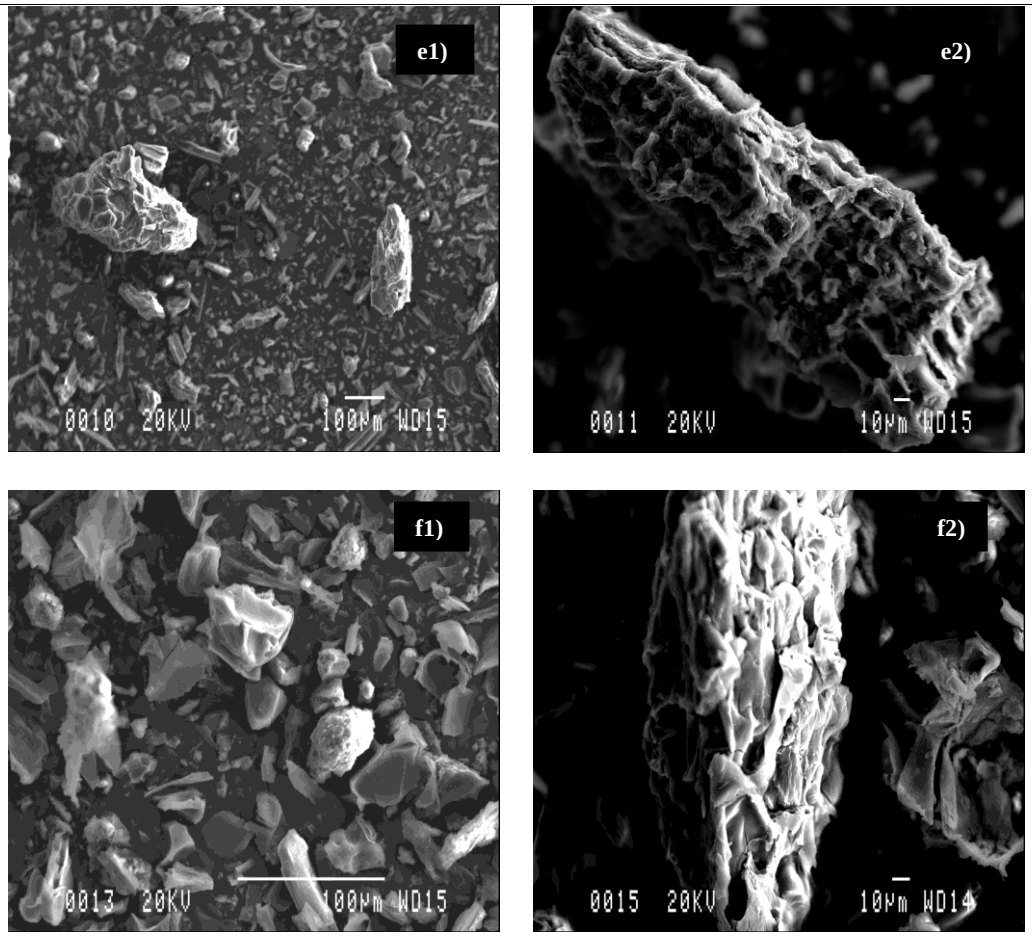

Figure 3.3 SEM of (a) oat hull and pine bark (b), oat hull biochar at 300 °C at different scales (c1, c2) and 500 °C at different scales (d1, d2), pine bark biochar at 300 °C at different scales (e1, e2) and at 500 °C at different scales (f1, f2).

In addition to low SSA values, low pore volumes and average pore diameter were determined in all biochar samples, indicating a predominating presence of micropores (diameter < 2nm), being the presence of mesopores (2 nm < diameter < 50 nm) almost undetectable (Table 3.1). These results suggest that, as the molecular diameter of the *Candida rugosa* lipase to be immobilized is 6.9 nm (de la Casa et al., 1998), the produced biochar samples may present some steric problems to immobilize the enzyme. In fact, according to Li et al. (2010), steric effects of pores may have significant influence on lipase

conformation, leading to changes of enzyme activity and also to diffusion limitations, restricting the contact between lipase and substrate.

3.3.2 Enzyme immobilization

Microcrystalline carbons, such as activated carbons, black carbon, and charcoals, have disordered structures and reactive edge area, which results in a larger propensity for enzymes chemisorptions (Cardosi, 1997). In this sense, the presence of hydroxyl and carboxylic acid groups in biochar surface is particularly useful for the immobilization of *Candida rugosa* lipase through the interaction with the amino groups of the enzyme. The FTIR spectra of biochar samples suggest us that BO300 may be the most suitable candidate to be used as support material for the immobilization of *Candida rugosa* lipase. BO300 presented the highest carboxylic groups content, which can certainly promote enzyme immobilization. In addition, and also according to the FTIR spectrum, BO300 may have a hydrophobic surface, which is a suitable ambient for enzyme immobilization. Furthermore, BO300 presented the lowest metals content that could negatively affect the enzyme activity. All these advantages are of course limited by the low specific surface area and porosity of BO300, however authors assume that functional groups present in BO300 surface may be the key point for assuring an efficient lipase immobilization process.

Protein content in crude lipase supernatant before immobilization on BO300 was determined, being in the range between 320 and 340 μg. After immobilization, protein content in the supernatant ranged between 130 and 200 μg. From the calculation, an immobilization efficiency ranging between 39.7 and 60.9 % was estimated, based on protein concentration in supernatant from *C. rugosa* (Table 3.2). As expected, the highest lipase immobilization capacity was observed by particles with a size between 53 μm and 90 μm. The immobilized enzyme specific activity in the lower biochar size fraction (< 53 μm) was higher than that of the immobilized enzyme in the fraction mentioned above. Even though, the total protein loaded was lower, probably due to the presence of a more homogeneous surface.

Table 3.2 Protein loading yield and activity of the immobilized enzyme onto BO300 and its fractions.

Sample	Protein loading (μg g^{-1} biochar)	Protein loading yield (%)	Lipase activity (U g^{-1} biochar)	Specific activity (U mg^{-1} protein)	Activity yield (%)
BO300 (< 53μm)	87.2	52.8	2109	24186	61.0
BO300 (53-75 μm)	99.4	60.2	2045	20573	51.9
BO300 (75-90 μm)	100.4	60.9	1453	14472	36.5
BO300 (90-125 μm)	89.2	54.0	1145	12836	32.4
BO300 (125-250 μm)	81.8	49.6	372	4547	11.5
BO300 (250- 500 μm)	65.5	39.7	188	2870	7.2
BO300	94.1	57.0	825	8767	22.1
BO300-methanol	93.7	56.5	347	3703	9.3
BO3000-ethanol	91.5	57.1	476	5202	13.1
Free Lipase	-[a]	-	13080[b]	39636	100

[a]Protein content of free lipase solution was 330 μg mL^{-1}.

[b]Activity of the free lipase is expressed for 1 mL.

In Table 3.2 the effect caused by methanol (MeOH) and ethanol (EtOH) used to favor the immobilization process and also to remove tars from the biochar is shown. According to Öztürk (2001), the use of water miscible solvents during the immobilization process improves enzyme adsorption by reducing the solubility of the enzyme in the aqueous phase. However, a negative effect was caused by the alcohols used in this study. Ethanol and methanol diminished the activity yield of the enzyme in 32 and 41% with respect to non-treated BO300. MeOH and EtOH did not show a positive effect on the immobilized enzyme quantity, as shown in Table 3.2, the protein loading was 56.5 to 58% quite similar to the protein loaded by the untreated BO300 (57%). This negative effect could be attributed to a surface hydrophobicity change, favoring bindings other than hydrophobic interaction. Covalent bindings are stronger interactions between enzyme and the support, where the amino group of the enzyme is attached to the surface with the carboxyl, sulfhydryl, hydroxyl or phenolic groups (Öztürk, 2001). It has been demonstrated that covalent immobilization is very strong, and no leakage of the enzymes occurs. In addition, the enzyme becomes more stable, however the structure of the protein is considerably affected leading to a significant loss on the free enzyme initial activity (Villeneuve et al., 2000).

The binding of lipase to biochar derived from the pyrolysis of oat hull at 300 °C was confirmed by FTIR analysis. Figure 3.4 shows the FTIR spectra for the solid-state pure lipase, BO300, and lipase-bound BO300. The characteristic bands at 1659 NH_2 and C-H at 1125 cm^{-1} were present in pure lipase but not in the lipase-bound BO300, confirming the binding of lipase to BO300 surface. This binding occurred through the interaction between the amino groups from the enzyme and carboxyl groups present in the BO300 surface. Moreover, the vibration of BO300 due to the carboxyl C=O stretching (1694 cm^{-1}) decreased its intensity.

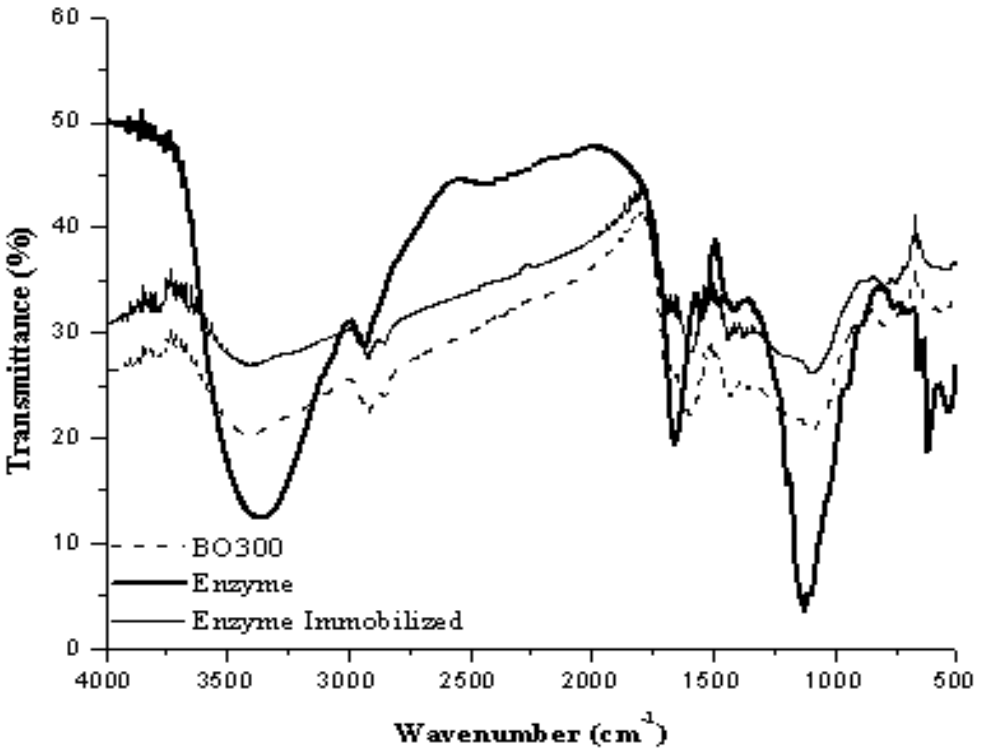

Figure 3.4 FTIR spectrum of free *Candida rugosa* lipase, biochar derived of oat hull combustion at 300 °C and of the immobilized lipase.

3. 4 Conclusions

A physical, chemical and mineralogical characterization of biochar was carried out for the evaluation of different biochar samples to be used as lipases support material.

Biochar from oat hull pyrolysis at 300 °C (BO300) was selected as lipase support material, mainly due to its low heavy metals content and its high carboxylic groups content. The lipase studied was directly bound to the selected biochar via adsorption onto the biochar surface. Through FTIR spectra, the binding of lipase to BO300 was confirmed. The binding efficiency of lipase was in the range between 40-60% depending on biochar particle size, the higher yields corresponding to the low particle size. The reduction in *Candida rugosa* lipase activity yield was attributed to the immobilization mechanisms.

Acknowledgements

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Chapter 4

Controlled-release nitrogen fertilizer using biochar as a renewable support matrix

Patent PCT/IB2012/057245, 2012 (requested)

Controlled-release nitrogen fertilizer using biochar as a renewable support matrix

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Field of the invention

The invention has application in the production of fertilizers, particularly in the production of controlled-release nitrogen fertilizer by urea impregnation onto biochar.

The invention relates to an ecofertilizer comprising a granular urea-based controlled-release nitrogen fertilizer using biochar as a renewable support matrix, and the production process thereof. Biochar is obtained by low temperature pyrolysis at 300 °C using residual biomass as feedstock.

The ecofertilizer of the invention accomplishes with new features regarding nitrogen up-take efficiency for specific cultivars by effectively promoting the slow nitrogen release in up to 30 days. This controlled-nitrogen release from the biochar matrix also increases the production yield of two tested wheat cultivars at field scale in up to 20%.

4.1 Background

Fertilization is one of the key elements of crop production; it can accelerate plant growth, both in its aerial and radical parts. It can also alter the nutritional composition of tissues, with effects on the level of reserves, the ability of attachment and resistance to water and cold stress and diseases, among others.

Regarding to the main fertilizers used in agriculture, the global consumption of nitrogen (N), phosphorus (P) and potassium (K) in 2005/06 was 93.2, 37.1, and 25.8Mt y^{-1}, respectively (IFA, 2007). 55% of the nutrients were used for cereal production, 12% for oilseed crops, 11% for grass land, 11% for commodities, 6% for root crops and only 5% for fruit and vegetable production (Shaviv, 2005).

In this context, nitrogen is the most widely applied plant nutrient. It has often been singled out for its adverse effects on the environment as well as on human and animal health (Keeney, 1997). The estimated worldwide nitrogen fertilizer consumption by agriculture in 2000 was 85.5 Tg (FAO, 2001), of which 60% was destined for cereal production. However, only 33% of the total N applied for cereal production is actually removed in the grain (Raun and Johnson, 1999). This implies that the overall efficiency of N utilization for food production is low. This leads to significant economic losses that become higher and higher due to the continued increase in nitrogen fertilizer prices as a result of the scarcity of fossil fuels (Raun et al., 2002).

Especially, urea is a widely used solid nitrogen fertilizer for agricultural production due to its low cost. After being applied to soil, it can be rapidly hydrolyzed to NH_3 and CO_2 by soil urease (Gioacchiini et al., 2001), followed by NO_3 formation through nitrification. In agriculture, urea is more than half of applied N fertilizer, and this comprise 40% of the global annual urea consumption (Zhao et al., 2009). However, the N recovery by crops from urea is often as low as 30~40%, with a potentially high environment cost associated with N losses via NH_3 volatilization, NO_3 leaching and N_2O emission (Zhou et al., 2003).

These losses are the result of many chemical, physical and biological processes, whose magnitude is affected by several factors such as: temperature, soil pH, cation exchange capacity (CEC), organic matter, and dose coverage and fertilization location.

One way to improve nutrient yield and specifically the efficiency of nitrogen use while reducing the environmental hazards is by using controlled-release fertilizers (CRFs) (Shaviv, 2005). CRFs have been known for several decades. A major focus on CRFs research remains nowadays focused on environmental protection issues. CRFs are designed to release their nutrient contents gradually and to coincide with the nutrient requirement of a plant (Wu and Liu, 2008). This ensures an improved effectiveness of fertilizing through minimizing the losses between application and absorption, thus avoiding the losses by runoff, leaching and N volatilization (Lubkowski and Grzmil, 2007).

The literature describes the CRFs as a granular nutrient core material containing at least one water soluble fertilizer compound, and a substantially water-insoluble coating applied on the core material. The fertilizer composition is structured to provide a Gaussian nutrient release rate curve over time with the maximum of the release rate occurring between 1 and 18 months after exposure of the fertilizer composition to moisture, according to US6139597 (Tijsma et al., 2000).

Moreover, the European Committee for Standardization (CEN) states that a fertilizer can be described as controlled or slow release if the nutrient release, under defined conditions including that of a temperature of 25 °C, meets all the following criteria, a) no more than 15 % in 24 hours, b) no more than 75 % in 28 days and c) at least about 75 % released at the stated release time.

The nutrient release in conventional fertilization (e.g. urea) lasts 30–60 days, which given a 100–120 day long crops growth cycle means that a fertilizers must be applied 2 or 3 times. In comparison, the CRFs release their nutrients slowly and gradually during the whole vegetation season consequently, they need to be applied only once, which reduces greatly both time and energy consumption (Lubkowski and Grzmil, 2007).

Nowadays, CFRs development is an important topic of research, focusing mainly on obtaining a system in which a granule of fertilizer is encapsulated, i.e. it is coated with an inert layer (Lubkowski and Grzmil 2007; Basu and Kumar, 2008). However, the use of coating materials may result in high production cost and even soil contamination after their release into soil. The CRFs value is 3-4 times more expensive than conventional fertilizers, this being the main reason for their limited use. However, these costs can be offset by a decrease in the application and purchase of fertilizers (Shaviv, 2005).

To solve these problems, conventional fertilizers are mixed with agricultural and industrial organic wastes and polymeric materials, forming a mixing with N-rich and high-quality organic fertilizer (Moore, 1995).

Biochar is among these products, it is a carbon-rich material obtained from the incomplete combustion of lignocellulosic biomass in the absence of oxygen and low temperatures. In recent years, the application of biochar as a soil amendment has attracted worldwide interest. This practice is positioned as a new approach to promote a significant carbon dioxide (CO_2) sinks in terrestrial ecosystems, in long-term. In addition, the production of biochar and its subsequent application to the ground would deliver benefits in both soil fertility and crop production (Lehmann et al., 2006; Mathews, 2008; Gaunt and Lehmann, 2008).

Along with the benefits described above, this material exhibits certain characteristic, such as increased capacity to adsorb organic and inorganic pollutants compounds in comparison with other forms of organic matter. It also presents a high cation exchange capacity and negative surface charge. Due to these features, biochar is positioned nowadays as a low-cost adsorbent compared with activated carbon (Crini, 2006).

Despite the benefits that come with the addition of biochar to the soil, some patents and studies suggest the modification of this material before being incorporated into the soil. These modifications include the addition of one or more nutrients either by a direct mixing process, encapsulation and/or pelletizing, among others.

U.S. Patent N°5.676.727 to Radlein et al. (1997) disclosed a process for making organic slow release nitrogenous fertilizers from products obtained of the flash pyrolysis of biomass. They proposed to use a chemical reaction to combine a nitrogen compound containing the NH_2 group with the pyrolysis products. The bio-oil obtained in the process contains high concentrations of carbonyl, carboxyl and phenolic functional groups and it is likely that these groups are largely responsible for the reaction with ammonia. This invention consists of use of bio-oil and charcoal N-rich derived from fast pyrolysis process by the formulation of an efficient biodegradable slow-release nitrogen fertilizer.

Similarly, other works used peanut shell pellets pyrolyzed under mild conditions at 400 °C, for developing a slow-release nitrogen fertilizer. In this study, similar conditions and principles to those propose by Radlein et al. (1997) are used. This charcoal also provided

the baseline material for further nutrient addition by reaction of pyrolysis of oil with urea to add more bioavailable nitrogen. However, the reactivity of products used in the development of CRFs will depend on the feedstock used in the pyrolysis as well as the reactor operating conditions (Magrini-Bair et al., 2009).

WO/2005/054154 to Kotaka (2005) disclosed a method for obtaining nitrogen fertilizers considering the use of charcoal as an adsorbent. The method includes fermentation of organic matter from agricultural waste to produce ammonia gas, which is subsequently adsorbed by the charcoal. The resulting product is used as a nitrogenous fertilizer.

Another method for obtaining a slow release fertilizer 5 considers the mixture of ammoniated superphosphate granules and potassium chloride, water, plaster ($CaSO_4 \bullet 2H_2O$) and charcoal. The plaster gives a high resistance to the product; it also makes possible pelletizing the mixture (Sjogren and Minn, 1987).

Moreover, due to the high surface area presented by charcoal, it tends to smooth the release rate by absorbing extra concentration of fertilizer when the release rate is high and by releasing or desorbing the fertilizer when the release rate is low.

Other forms to obtain nitrogen-enriched charcoals are by chemical modifications of charcoals already formed by thermo-chemical treatment of common raw material. These last procedures involve reactions with various reagents introducing the nitrogen groups, as well as the reaction sequences. For instance, the oxidation of carbon preceding the reaction with ammonia or its derivatives (ammonium carbonate, hydrazine, hydroxylamine and urea) with the carboxyl groups either naturally occurring in charcoal or artificially introduced by performic oxidation, or the nitration of carbon followed by hydrogenation of the nitro groups introduced (Bimer et al., 1998; Coca et al., 1984).

Much attention is being paid to ammoxidation of charcoals, which consists of the direct reaction of active carbons with the mixture of air and ammonia. Depending on the method used, the nitrogen content of the charcoal varies, the same as the chemical nature of nitrogen groups.

This practice has been described in the formulation of slow charcoal-based release nitrogen fertilizer using chemical reaction between a nitrogen source and lignocellulosic matrices (Kim et al.,1981; Coca et al.,1984; Ramírez-Cano et al., 2001). However, this method has

been used nowadays to obtain the activated carbons through nitrogen group introduction (Mangun et al., 2001; Zhuravsky et al., 2012).

Basically, the mechanism of CRF action includes a system in a granule (conventional fertilizers), which is encapsulated or coated. After a fertilizers application, water penetrates through a membrane into a granule. Then, nutrients are dissolved and the arising osmotic pressure leads to a partial rupture of the membrane, which allows the release of active compounds to the soil (Lubkowski and Grzmil, 2007).

In recent years, CRFs production has focused mainly on obtaining organic fertilizers of determined particle size and specific physical-chemical characteristics. Recent studies present a trend towards production of biochar-based fertilizers incorporating nitrogen in a process of direct mixing, encapsulation and/or pelletizing (Khan et al., 2007; Magrini-Bair et al., 2009; Ding et al., 2010).

Various materials were found to be suitable for encapsulating or coating purposes. The most important of these include wax and sulfur and organic polymers such as polyolefins (Kosuge and Tobataku, 1988), polyethylene (Salman, 1989), kraft pine lignin (Garcia, et al., 1996), cellulose acetate (Jarosiewicz and Tomaszewska, 2003), sodium alginate, among others.

4.2 Summary of the invention

This invention is directed to an ecofertilizer comprising a granular organic urea-based controlled-release nitrogen fertilizer using biochar as a renewable support matrix. It also relates to a process for the production of a controlled-release nitrogen fertilizer, such as, the ecofertilizer of this invention.

Biochar is obtained by low temperature pyrolysis at 300 °C of agricultural wastes; and is used as a renewable matrix for nitrogen impregnation. Subsequently, urea impregnated onto biochar is encapsulated using a biodegradable polymer. The encapsulation was carried out by a precipitation method. The controlled-release nitrogen fertilizer developed exhibited substantially complete nitrogen availability as plant nutrient.

4.3 Brief description of the figures

Figure 4.1: Schematics of the process of the present invention.

Figure 4.2: Distribution of field tests (T0-T3, TS) in two experimental sites.

Figure 4.3: Components percentage proportion of encapsulated mixture.

Figure 4.4: Nitrogen stability of encapsulated mixture during 6 months.

Figure 4.5: Ammonium release ($N\text{-}NH_4^+$) concentration into deionized water of encapsulated mixture at 25 °C, 100 rpm and pH 7.46.

Figure 4.6: Ammonium release ($N\text{-}NH_4^+$) percentage into deionized water of encapsulated mixture at 25 °C, 100 rpm and pH 7.46.

Figure 7: Test results of ecofertilizer applications on two different cultivars (Mg ha^{-1}).

4.3.1 Detailed description of figures

Figure 4.1: The schematics illustrates input of biochar with the particle size of less than 5 mm, preferentially of ≤ 2 mm to the marmite (1); input of urea to the marmite (2); input of water to the marmite (3); particles of biochar in suspension (4); granules of urea in suspension (5); a mixer (6) and a thermocouple (7) for monitoring the process temperature between 100 °C and 200 °C, more preferentially at 150 °C, within said marmite. After of a period of time between 1 hour and 12 hours, more preferentially for 8 hours of impregnation process, the solution is removed from marmite (8), and then the biochar impregnated with nitrogen is filtered from the aqueous solution (9). The content of nitrogen in biochar in this process was determined (10), and then biochar impregnated with nitrogen and 5% sodium alginate solution, were mixed (11) at a 3:1 (w/v) mixing ratio. The mixture is transferred to the PVC cylinder with openings (12) of about 4 mm of diameter. The solution is mixed for maintaining the mixture and drip constant.

After that, the sample is precipitated in a 0.5 M $CaCl_2$ (13). The spherical beads were left in the $CaCl_2$ solution for 10 min to ensure complete gelling. Once complete gelling the beads were separated from the $CaCl_2$ solution (14). Finally, beads are filtered and rinse twice with

distilled water (15), and then beads were dried at room temperature overnight to constant weight (16).

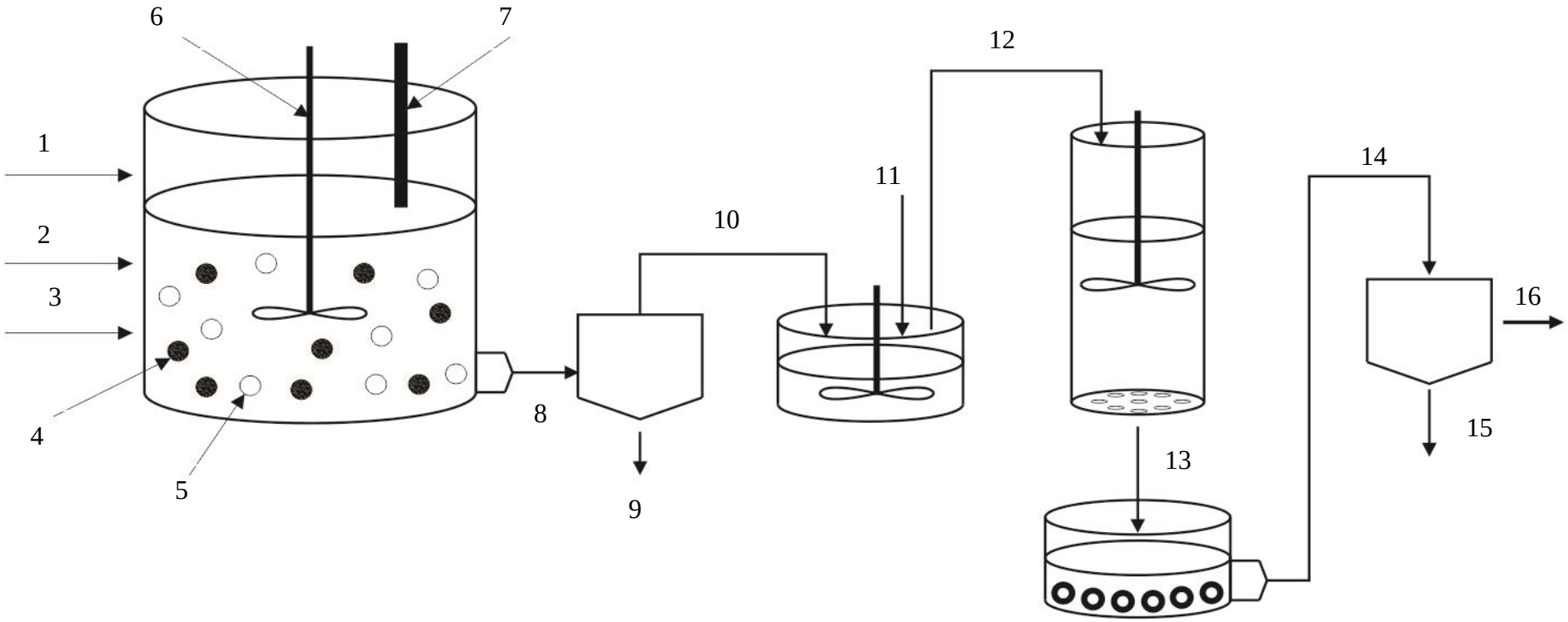

Figure 4.1 Schematics of the process of the present invention.

4.4 Details of the invention

The object of the present invention is the development a granular organic controlled-release nitrogen fertilizer, comprising a source of nitrogen, a support matrix, and a biodegradable polymer coating or encapsulation in a biodegradable polymer.

In a particular embodiment of the invention, the granular organic controlled-release nitrogen fertilizer uses preferentially urea as a nitrogen source, but there are other sources of nitrogen than can be used.

In a further embodiment of the invention, the support matrix is biochar. In a more preferred embodiment, the biochar is in the form of particles of less than 5 mm, more preferentially less than 2 mm.

Other embodiment of the invention considers sodium alginate as a biodegradable polymer coating or encapsulation medium.

The invention also considers the process for the production of the granular organic controlled-release nitrogen fertilizer, wherein said process comprises the following steps:

a) obtaining biochar by slow pyrolysis of a biomass source;

b) impregnating the biochar obtained in the previous step with a nitrogen source, producing biochar particles impregnated with nitrogen;

c) coating or encapsulating the biochar particles impregnated with nitrogen with a biodegradable polymer.

In step a) of the process, the biochar is obtained by low temperature pyrolysis of a biomass source. Preferentially, the temperature of pyrolysis ranges between 300 °C and 600 °C, more preferentially between 300 °C and 500 °C. In a further embodiment, the pyrolysis is carried out for a period of time between 100 and 400 minutes, more preferentially between 120 and 315 minutes. In a further more specific embodiment, the biomass source is oat hull.

In a further embodiment, the particle size of biochar obtained by low temperature pyrolysis is reduced to a size of less than 5 mm, preferentially, less than 2 mm.

In step b) of the process, impregnation of biochar with a nitrogen source is performed in liquid phase. The impregnation reaction is carried out in a suitable reactor at a temperature

between 100 °C and 200 °C, more preferentially at 150 °C. The impregnation reaction is carried out with constant agitation for a period of time between 1 hour and 12 hours, more preferentially for 8 hours. In a more particular embodiment, the solvent used as a liquid phase is a polar solvent. In a more preferred embodiment, the solvent used is water. For the impregnation reaction, the biochar and nitrogen source are present in a weight ratio of biochar:nitrogen source from 2:1 to 1:2, more preferentially 1:1. In a more specific embodiment, the polar solvent is present in a weight ratio of biochar:nitrogen source:polar solvent from 1:1:1 to 1:1:10, more preferentially 1:1:5. Further different ratios are also encompassed in the present invention, such as for example, the weight ratio of biochar:nitrogensource:polar solvent can be from 1:2:10 to 1:2:1, from 2:1:10 to 2:1:1.

Finally, when the period of time of the impregnation reaction has ended, the mixture is left to cool at room temperature and the reaction gases are released. Once the reaction gases have been released, the polar solvent is separated by filtration, obtaining biochar particles impregnated with nitrogen.

In the final step c), the biochar particles impregnated with nitrogen obtained in the previous step are encapsulated or coated with a biodegradable polymer. In a more preferred embodiment, the biodegradable polymer is sodium alginate, although the present invention also encompasses the use of other biodegradable polymers, including cellulose acetate and ethyl acetate (both using formamide as solvent). In a more specific embodiment, the biodegradable polymer is dissolved in a suitable solvent, for example water. The ratio of biodegradable polymer:solvent is from 1:100 (1% in weight) to 1:10 (10% in weight), more preferentially 1:20 (i.e. 5% in weight). The biodegradable polymer/solvent mixture is mixed with the biochar particles impregnated with nitrogen obtained in the previous step, in a ratio of (biochar particles impregnated with nitrogen):(biodegradable polymer/solvent) from 10:1 (weight:volume) to 1:1 (weight:volume), more preferentially 3:1 (weight:volume). The mixture formed is added, dropwise, to a $CaCl_2$ solution allowing the drops to from gellified beads. In a more specific embodiment, the gellified beads have a size between 1 and 5 mm, more preferentially between 2 and 3 mm. Finally, the gellified beads are dried at room temperature overnight.

4.5 Application examples

4.5.1 Biochar production

The process used for obtaining biochar was slow pyrolysis. The carbonization experiment was performed in a pyrolizer with capacity to process 5 kg of raw material. The reactor was purged with N_2 at a flow of 5 L min^{-1}. Oat hull was used for biochar production and the temperature of pyrolysis was of 300 °C, the time to reach T_{max} was 195 min and the processing time for T_{max} was of 120 min and the total time of pyrolysis was 315 min. The mass balance of slow pyrolysis process showed in Table 4.1.

Table 4.1 Mass balance of slow pyrolysis process of oat hull pyrolyzed at 300 °C.

Products	%
Biochar	41
Bio-oil	24
Synthesis gas*	35

* by difference

The biochar obtained from slow pyrolysis of oat hull at 300 °C (BO300) was characterized physically and chemically (Table 4.2).

Table 4.2 Physico-chemical characterization of BO300.

Physic characteristic			Chemical characteristic		
Parameter	**Value**	**Unit**	**Parameter**	**Value**	**Unit**
Specific surface area	0.1	m^2g^{-1}	Total carbon	70.13	%
Pore volume	0.034	cm^3g^{-1}	Total nitrogen	1.03	%
Average diameter pore	10.86	Å	Total phosphorous	0.46	%
			Total potassium	1.73	%
			Carboxylic group	0.28	me g^{-1}
			Ash content	8.01	%
			pH (H_2O)	7.7	-

4.5.2 Nitrogen impregnation onto biochar using urea as a nitrogen source

Impregnation process onto biochar (BO300) was carried out in a marmite with a 60 liter capacity (Figure 4.1). In the process urea was used as nitrogen source. Previously to the reaction, the size particle of BO300 was reduced at ≤ 2mm.

The temperature of reaction was of 150 °C and was monitored with a thermocouple. The impregnation of biochar was performed in liquid phase, using water as solvent; the proportions used were 1:1:5 biochar:nitrogen:water, respectively (modified from Bimer et al., 1998). Process parameters are shown in Table 4.3. The reaction was carried out at 150 °C during 8 h with constant agitation. After cooling and releasing the reaction gases, the solvent was separated by filtration.

Table 4.3 Parameters and their ranges used in the urea impregnation process onto biochar (BO300).

Parameter	**Value**	**Unit**
Biochar (BO300)	6	kg
Urea	13	kg
Water	30	L
Reaction time	8	h
Temperature	150	°C

The total nitrogen content of the solid and liquid phase samples were then determined by Kjeldahl method for total nitrogen (APHA, 1995).The moisture content of the solid phase was measured by drying the sample in an oven at 100±5 °C for approximately 24 h.

4.5.3 Encapsulation of the mixture between biochar impregnated with nitrogen and sodium alginate (SA)

The solution of sodium alginate (SA) (Aldrich Chemical) was prepared by dissolution of the solid polymer in distilled water, the concentration of solution was 5 wt%. This was

followed by mixing biochar (undried solid) with the SA. The mixing ratio was 3:1 (w/v) of biochar and SA, respectively.

The resulting mixture was arranged in a cylinder of PVC with openings of about 4 mm of diameter at the bottom, the mixture was stirred vigorously until uniform and then slowly added dropwise to a 0.5 M $CaCl_2$ solution, where the drops turned to white beads immediately because the sodium alginate in the drop was cross-linked by Ca_2^+ at once. The spherical beads were left in the $CaCl_2$ solution for 10 minutes to ensure complete gelling and then separated from the solution. The encapsulated mixture was dried at room temperature overnight.

4.5.4 Stability of nitrogen content in the time of encapsulation

To study the stability of encapsulated nitrogen content at certain time intervals (1, 5, 15 and 30 days and 6 months) a sample of encapsulated mixture was taken and the content of total nitrogen was measured by the Kjeldahl method (APHA, 1995).

4.5.5 Ammonium release of encapsulated mixture in water

To study the release behavior of encapsulated mixture in water, the following experiment was carried out: 1 g of encapsulated mixture was mixed with 200 mL of deionized water and kept in a beaker properly covered and incubated in an orbital shaker at 100 rpm for different periods at 25 °C. All of the following tests were carried out in triplicate, and the average value was taken as the result. At certain time intervals (1, 5, 10, 15, 20, 25 and 30 days), 100 mL of aqueous solution was retired, for nitrogen determination, and an additional 100 mL of water was injected into the bottles to maintain a constant amount of solvent. The amount of N in the aqueous medium was estimated by the Kjeldahl method (APHA, 1995).

4.5.6 Application in field tests

Two different types of soil were selected to perform the field test of the developed ecofertilizer. Figure 4.2 ecofertilizers field tests description is shown.

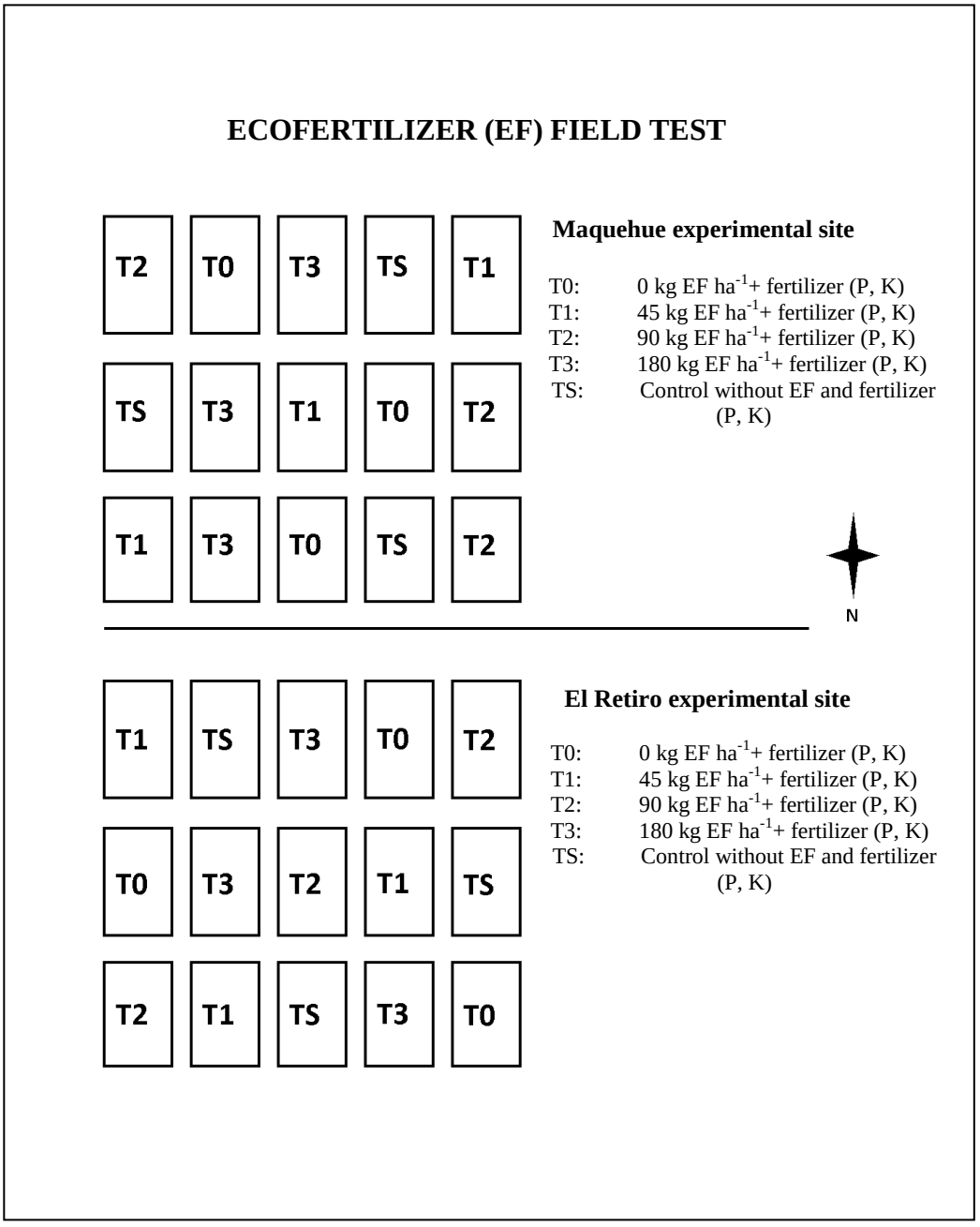

Figure 4.2 Distribution of field tests (T0-T3, TS) in two experimental sites.

4.6 Results

4.6.1 Nitrogen impregnation onto biochar using urea as a nitrogen source

The result of total nitrogen content presented in Table 4.4 shows an increase in total nitrogen content in the BO300 after impregnation process. The process of impregnation at high temperatures (150 °C) showed a significant increase in the content of total nitrogen.

As for the moisture, biochar impregnated with nitrogen was dried at 105±5 °C for approximately 24 h resulting in a 50.43% of moisture.

Table 4.4 Total nitrogen content in the solid and liquid phase after the ammoxidation process.

Phase	**Total nitrogen content (%)**
Solid (BO300)	19±1.8
Liquid	13±1.6
Gaseous*	67.89

*obtaining by difference

4.6.2 Encapsulation of the mixture between biochar impregnated with nitrogen and sodium alginate (SA)

Once the humidity percentage was obtained, it was performed the mixing of biochar impregnated with nitrogen and sodium alginate; the mixing ratio was made 3:1 (w/v).

The resulting mixture was arranged in a cylinder with openings of about 4 mm of diameter and, by means of dropping, the sample was precipitated in a solution of $CaCl_2$ 0.5 M. The spherical beads were left in the $CaCl_2$ solution for 10 min to ensure complete gelling and then separated from the solution, rinsed twice with distilled water, and dried at room temperature overnight.

Finally, the proportion of the components of encapsulate is shown in Figure 4.3. While the average diameters of dry samples were of 2-3 mm.

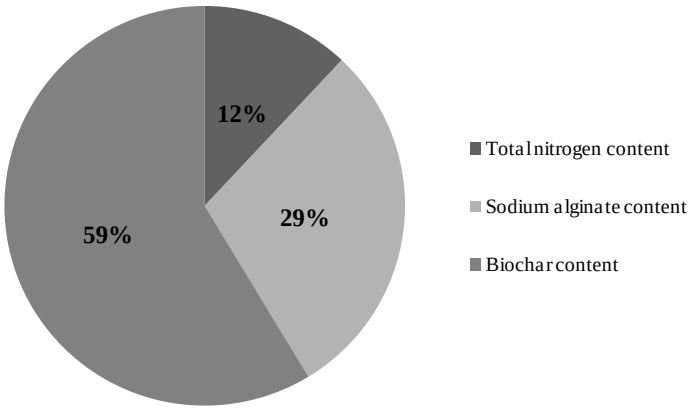

Figure 4.3 Components percentage proportion of encapsulated mixture.

4.6.3 Stability of nitrogen content of encapsulated mixture

The stability of the nitrogen content over time was evaluated, to rule out the loss by NH_3 volatilization of encapsulated mixture. It can be seen from Figure 4.4 that the nitrogen content is constant during the evaluated time. It is observed that first day the total nitrogen content is of 12±1.98% and at the 30th day the nitrogen content remains in 13±1.23%.

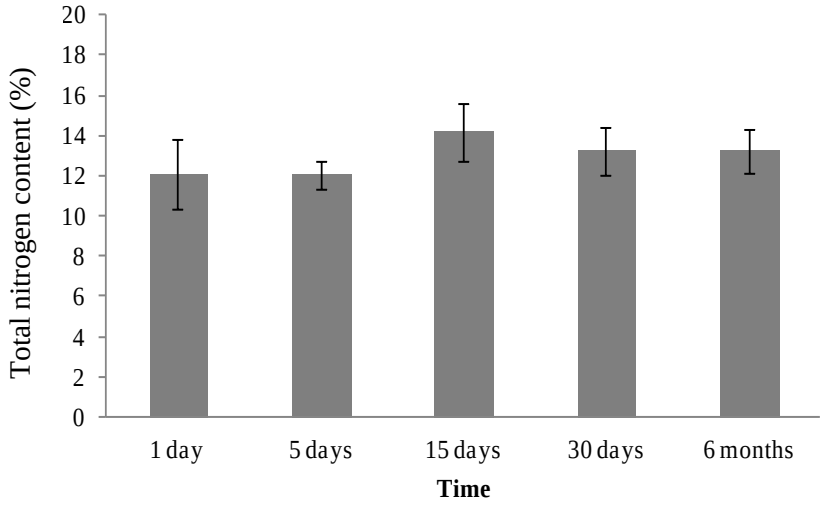

Figure 4.4 Nitrogen stability of encapsulated mixture during 6 months.

4.6.4 Ammonium release test of encapsulated mixture in water

One of the most important characteristics of the encapsulated mixture prepared by the method of this invention was its slow-release property. Figure 4.5 shows the ammonium slow release behaviors of encapsulated mixture in deionized water. It is seen in the figure that the release of $N\text{-}NH_4^+$ during the first 10 days exhibits an exponential behavior and then abruptly decreases. From Figure 4.6 it can be seen that on day 5, 18% of the NH_4^+-N was released, between day 5 and day 15a 37% was released, while at day 30 a 40% NH_4^+-N was released.

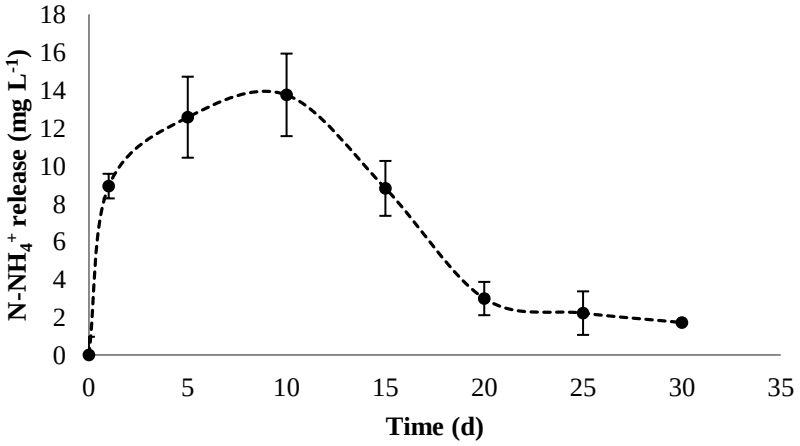

Figure 4.5 Ammonium release ($N\text{-}NH_4^+$) concentration into deionized water of encapsulated mixture at 25 °C, 100 rpm and pH 7.46.

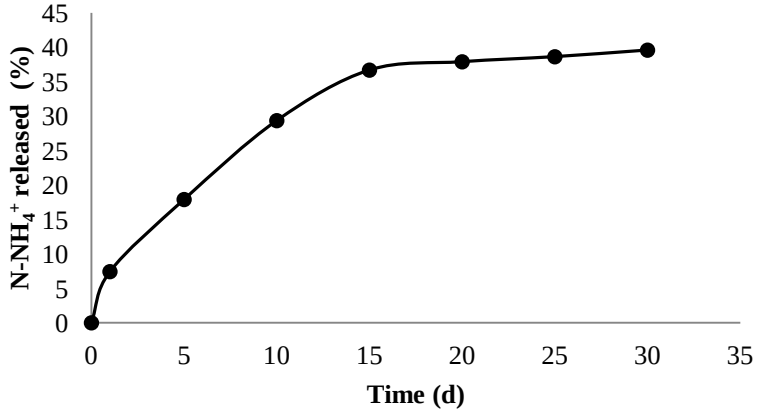

Figure 4.6 Ammonium release ($N\text{-}NH_4^+$) percentage into deionized water of encapsulated mixture at 25 °C, 100 rpm and pH 7.46.

4.7 Detailed discussion of the invention

4.7.1 Nitrogen impregnation onto biochar using urea with nitrogen source

The reaction of biochar with urea at elevated temperature (150 °C) showed highest nitrogen enrichment of biochar (19±1.8). Other works used the same nitrogen source, but at a higher temperature (300 °C), reporting values between 13 and 16.7% in the total nitrogen content in the charcoal.

This increase in nitrogen content could be explained by the presence of urea by-products after thermal decomposition. Nitrogen is likely to be in the form of amides, free NH and NH_2, bonded NH and NH_2, or NH_4^+ species (Adib et al., 2000).

All the findings so far suggest that the chemistry of the reaction of coal with urea is very complex, not only because of the heterogeneity of the charcoal structure but also because of the variety of N-reagents that can arise from urea and can react independently with charcoal (Bimer et al., 1998).

4.7.2 Encapsulation of the mixture between biochar impregnated with nitrogen and sodium alginate (SA)

In recent years, a number of studies have greatly paid attention to the preparation and utilization of polysaccharidic super absorbents because of their biodegradability, biocompatibility, renewability and nontoxicity (Guilherme et al,. 2005; Murthy et al., 2006; Zhang et al., 2007). In particular, sodium alginate (SA) is a renewable and biodegradable natural polymer that is used in a variety of commercial applications because of its capacity for gelatinization (Hua and Wang, 2009).

The technique of precipitation of SA in $CaCl_2$ has been widely described for the obtained of CRF. Basically the method consists in the cross-linking between Na^+ and Ca_2^+ (Liang et al. 2007). The stability of spherical beads depends of SA and Ca_2^+ concentration of solutions. However, a disadvantage in using $CaCl_2$ as precipitation bath is the need to change at certain intervals the $CaCl_2$ solution.

4.7.3 Stability of nitrogen content of encapsulated mixture in time

Before the release of the encapsulated mixture test it was necessary to assess the stability of the nitrogen content in time and rule out the loss of NH_3 such as for example due to volatilization, so knowing the initial concentration of the encapsulated mixture to be used, also gives an idea of the period time that the final product can be stored once produced. According to the values obtained, no difference was seen between the total nitrogen content at first day and 30th day (12±1.98% and 13±1.23%, respectively).

The literature does not report the evaluation of stability of active ingredient in the CRF in time. But it does mention that the viability of CRF depends of storage conditions such as temperature, humidity, among others as well as the polymeric material used in the formulation (Trenkel, 1997).

4.7.4 Ammonium release test of encapsulated mixture in water

CRFs are a granular nutrient core material containing at least one water soluble fertilizer compound, and a substantially water-insoluble coating applied to the core material. The fertilizer composition is structured to provide a Gaussian nutrient release rate curve over time with the maximum of the release rate occurring between 1 and 18 months after exposure of the fertilizer composition to moisture. However, the release time depends on environmental conditions and the properties of the polymers used to formulate of CRF.

According to data obtained for the $N\text{-}NH_4^+$ release of the encapsulated mixture in deionized water at 25 °C, the release does not follow this behavior, since the release of $N\text{-}NH_4^+$ during the first 10 days exhibits an exponential behavior and then abruptly decreases. It is expected that this behavior is maintained in soil, with the difference that release is slower compared to deionized water.

With the sum of $N\text{-}NH_4^+$ release lower than 15% on the 3rd day and not above 75% on the 30th day, this indicated that the slow release character of the encapsulated mixture prepared herein agrees with the standard of slow release fertilizers of the Committee of European Normalization (CEN).

As for the conventional CRF the use of a double-coated in the preparation of slow-release urea has been reported. The fertilizer was prepared by cross-linked poly(acrylic acid)-containing urea (PAAU) (the outer coating), polystyrene (PS) (the inner coating), and urea

granule (the core) with a total nitrogen content of 33.6%, whose $N\text{-}NH_4^+$ release rate was 100% in 18 days in same conditions used in these studies. Note that the stability of the encapsulated mixture in liquid medium and under stirring was approximately 15 days.

4.7.5 Field test

In Figure 4.7, the effect of ecofertilizer application on two different cultivars (Mg ha^{-1}) was tested, namely wheat (*Triticu maestivum)* A) Crac cultivar in the experimental site Maquehue and B) Impulso cultivar in the experimental site El Retiro. The nitrogen content of the encapsulated mixture after the application on field test was 1.62% in average.

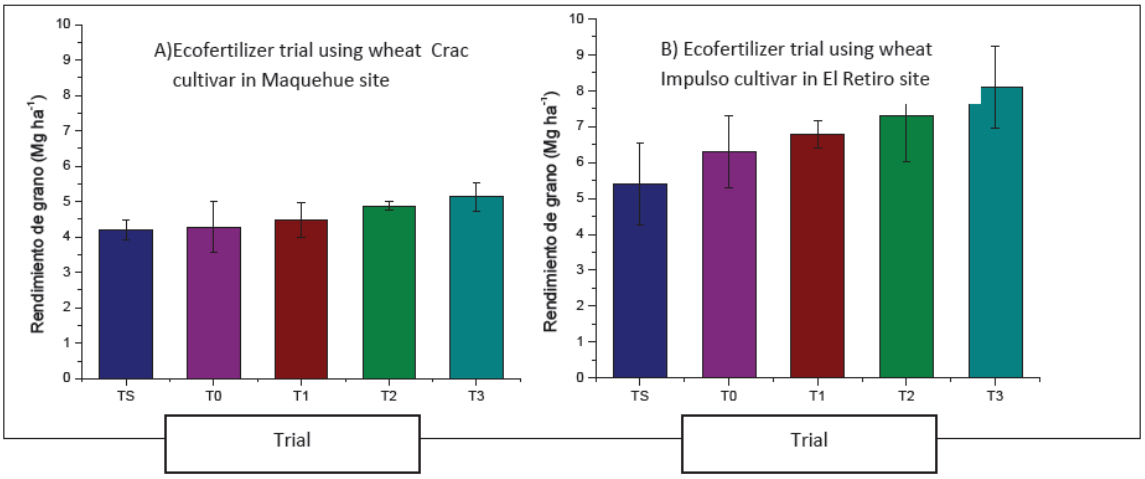

Figure 4.7 Test results of ecofertilizer applications on two different cultivars (Mg ha^{-1}).

4.8 What is claim is:

1. Granular organic controlled-release nitrogen fertilizer comprising a nitrogen source, a support matrix, and a biodegradable polymer coating or encapsulation medium.

2. Granular organic controlled-release nitrogen fertilizer according to claim 1, wherein the nitrogen source is selected among urea.

3. Granular organic controlled-release nitrogen fertilizer according to claim 1, wherein the support matrix is biochar.

4. Granular organic controlled-release nitrogen fertilizer according to claim 3, wherein the biochar matrix is in the form of particles of less than 5 mm.

5. Granular organic controlled-release nitrogen fertilizer according to claim 1, wherein the biodegradable polymer coating is selected among sodium alginate, cellulose acetate and ethyl acetate (both using formamide as solvent).

6. Granular organic controlled-release nitrogen fertilizer according to claims 2 and 3, wherein the nitrogen source is impregnated onto the biochar.

7. Process for the production of a controlled-release nitrogen fertilizer comprising the steps of:

a. obtaining biochar by slow pyrolysis of a biomass source;

b. impregnating the biochar obtained in the previous step with a nitrogen source, producing biochar particles impregnated with nitrogen;

c. coating or encapsulating the biochar particles impregnated with nitrogen with a biodegradable polymer.

8. Process for the production of a controlled-release nitrogen fertilizer according to claim 7, wherein in step a) of the process, the biochar is obtained by low temperature pyrolysis of a biomass source, with the temperature of pyrolysis between 300 °C and 500 °C.

9. Process for the production of a controlled-release nitrogen fertilizer according to claim 7, wherein in step a) of the process, the biochar is obtained by low temperature pyrolysis of a biomass source with the temperature of pyrolysis between 300 °C and 500 °C.

10. Process for the production of a controlled-release nitrogen fertilizer according to claim 7, wherein in step a) of the process, the biochar is obtained by low temperature pyrolysis carried out for a period of time between 100 and 400 minutes.

11. Process for the production of a controlled-release nitrogen fertilizer according to claim 7, wherein in step a) of the process, the biochar is obtained by low temperature pyrolysis carried out for a period of time between 120 and 315 minutes.

12. Process for the production of a controlled-release nitrogen fertilizer according to claim 7, wherein the biomass source is selected among oat hull and pine bark.

13. Process for the production of a controlled-release nitrogen fertilizer according to claim 7, wherein in step a) of the process, the size of the obtained biochar is reduced to less than 5 mm.

14. Process for the production of a controlled-release nitrogen fertilizer according to claim 7, wherein in step a) of the process, the size of the obtained biochar is reduced to less than 2 mm.

15. Process for the production of a controlled-release nitrogen fertilizer according to claim 7, wherein in step b) of the process, impregnation of biochar with a nitrogen source is performed in liquid phase.

16. Process for the production of a controlled-release nitrogen fertilizer according to claim 7, wherein in step b) the impregnation reaction is carried out at a temperature between 100 °C and 200 °C.

17. Process for the production of a controlled-release nitrogen fertilizer according to claim 7, wherein in step b) the impregnation reaction is carried out at a temperature of 150 °C.

18. Process for the production of a controlled-release nitrogen fertilizer according to claim 7, wherein in step b) the impregnation reaction is carried with constant agitation for a period of time between 1 hour and 12 hours.

19. Process for the production of a controlled-release nitrogen fertilizer according to claim 7, wherein in step b) in the impregnation reaction, the biochar and nitrogen source are present in a weight ratio of biochar:nitrogen source from 2:1 to 1:2.

20. Process for the production of a controlled-release nitrogen fertilizer according to claim 7, wherein in step b) in the impregnation reaction, the polar solvent is present in a weight ratio of biochar:nitrogen source:polar solvent from 1:1:1 to 1:1:10.

21. Process for the production of a controlled-release nitrogen fertilizer according to claim 7, wherein in step b) in the impregnation reaction the polar solvent is present in a weight ratio of biochar:nitrogen source:polar solvent from 1:2:10 to 1:2:1 or from 2:1:10 to 2:1:1.

22. Process for the production of a controlled-release nitrogen fertilizer according to claim 7, wherein in step b) the mixture is left to cool at room temperature and the reaction gases are released, and once the reaction gases have been released, the polar solvent is separated by filtration, obtaining biochar particles impregnated with nitrogen.

23. Process for the production of a controlled-release nitrogen fertilizer according to claim 7, wherein in step c) the biochar particles impregnated with nitrogen are encapsulated or coated with a biodegradable polymer.

24. Process for the production of a controlled-release nitrogen fertilizer according to claim 23, wherein the biodegradable polymer is dissolved in a suitable solvent from 1% in weight to 10% in weight.

25. Process for the production of a controlled-release nitrogen fertilizer according to claim 23 and 24, wherein the biodegradable polymer is sodium alginate and the solvent is water, and the sodium alginate/water mixture is mixed with the biochar particles impregnated with nitrogen in a ratio of (biochar particles impregnated with nitrogen):(sodium alginate/water) from 10:1 (weight:volume) to 1:1 (weight:volume).

26. Process for the production of a controlled-release nitrogen fertilizer according to claim 25, wherein the mixture of biochar particles impregnated with nitrogen and sodium alginate/water is added, dropwise, to a $CaCl_2$ solution allowing the drops to form gellified beads, and the gellified beads have a size between 1 and 5 mm.

27. Process for the production of a controlled-release nitrogen fertilizer according to claim 26, wherein the gellified beads are dried at room temperature overnight.

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Chapter 5

Evaluation of biodegradable polymers as encapsulating material to develop controlled-release nitrogen fertilizer using biochar as support

Paper in preparation

Evaluation of biodegradable polymers as encapsulating material to develop a controlled-release nitrogen fertilizer using biochar as support

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Abstract

One way to improve nitrogen uptake yield simultaneously reducing the environmental hazards is by using controlled-release fertilizers (CRFs). Biochar constitutes a promising support material for the formulation of CRF due to its physicochemical properties. In this study we evaluated the effect of different polymeric materials as encapsulating agent on nitrogen leaching from N-CRF based on biochar.

Biochar was produced from oat hull pyrolyzed at 300 °C. The N impregnation process was performed in a batch reactor at 150±5 °C for 10 min. The resulting product was encapsulated by using sodium alginate (SA), acetate cellulose (AC) and ethyl cellulose (EC) in different concentrations and ratios.

The leaching potential was studied in disturbed soil column experiments. The experiment was arranged in a completely randomized design with 10 fertilizer treatments × with crop × without crop×3 replications×10 events of water addition.

Leachates were collected on 15, 22, 29, 36, 43, 50, 57, 64, 71 and 78 days after establishment the assay. Nitrate, nitrite, ammonium and urea were measured in leachates. After 90 days, plants were removed from the soil columns and separated into grain, roots and shoots in order to measure biomass and production yield.

It was observed that the $N\text{-}NH_4^+$ amount in leachates shwed a maximum of concentration for all treatments at day 22. The greater proportion of N found in the leachates was in $N\text{-}NO_3^-$ form. For all treatments (assays with and without crop) the $N\text{-}NO_3^-$ loss by leaching, excepting for the treatment where ESN was applied, showed higher values after the first and second event of leaching. After day 29th the $N\text{-}NO_3^-$ content showed a fast diminishing. In this sense, EC 2 showed lower $N\text{-}NO_3^-$ content in leachates than soil treated with U and with BU. The crop yield was negatively affected by all CRFs produced using biochar compared with the traditional fertilization (urea) and commercial (ESN). Compared with ESN the grain yield was negatively affected in a 83% for C, 81% for SA 2, 70% for AC 2, 62% for AC 1, 52% for EC 1, 38% for SA 1, 28 % for EC 2, 23% for BU and 13 % for U.

Keywords: Controlled-release nitrogen fertilizer, biochar, urea, polymers, encapsulated, leaching test.

5.1 Introduction

Fertilization is key to crop production; it can accelerate or retard plant growth, both in its aerial and radical parts. In this context, nitrogen is the most widely applied plant nutrient. It has often been singled out for its adverse effects on the environment as well as on human and animal health. Especially, urea is a widely used solid nitrogen fertilizer for agricultural production due to its low cost. However, N uptake by crops from urea is often as low as 30~40% depending on the culture conditions, with a potentially high environmental cost associated with N losses via NH_3 volatilization, NO_3^- leaching and N_2O emission (Zhou et al., 2003).

One way to improve nutrient yield and specifically the efficiency of nitrogen uptake while reducing the environmental hazards is by using controlled-release fertilizers (CRFs) (Shaviv, 2005).The CRFs are made to release their content of nutrients gradually and if possible to coincide with the nutrient requirements of a plant (Hanafi et al., 2002).These fertilizers are prepared by coating the active soluble component with a membrane that serves as a diffusion barrier (Hanafi et al., 2002). However, the use of coating materials may result in high production cost and even soil contamination after their release into soil if they are not biodegradables.

To solve these problems, conventional fertilizers are mixed with agricultural and industrial organic wastes and biodegradable polymeric materials, forming a mixture with N-rich and high-quality organic fertilizers (Moore, 1995). Biochar is among these products, it is a carbon-rich material obtained from the thermal combustion of lignocellulosic biomass in absence of oxygen at low temperatures. Moreover, in recent years, the biochar application as a soil amendment has attracted worldwide interest.

Despite the benefits obtained with the addition of biochar into the soil, some works suggest the modification of this material before being incorporated. These modifications include the addition of one or more nutrients either by a direct mixing process, encapsulation or pelletizing, among others (Sjogren, 1987; Radlein et al., 1997; Kotaka, 2005; Magrini-Bair et al., 2009).

Several materials have been proposed for CRF encapsulating or coating. The most important of these include wax and sulfur, and organic polymers such as polyolefins (Kosuge and Tobataku, 1988), polyethylene (Salman 1989), kraft pine lignin (Garcia et al., 1996), cellulose acetate (Jarosiewicz and Tomaszewska, 2003) and sodium alginate (Liang et al., 2007), among others. However, the use of polymeric materials has not been reported yet in the formulation of a CRF using biochar as support. Due to these reasons and in this context, the evaluation of release rates and mechanism are essentials for the selection of proper fertilizers for a given set of conditions or toward the development of proper CRF formulations. The evaluation of CRF in the soil is essential in order to establish appropriate soil management and fertilizer application techniques.

A variety of methods have often been used to evaluate the effectiveness of CRF in the laboratory. These methods provide essentially information on nutrient composition under static conditions (Blouin et al., 1971; Savant et al., 1982). In contrast, nutrients availability for plants is controlled by the soil characteristics. Under field conditions, composition of the soil solution is variable due to changes in soil environment i.e., nutrient leaching and nutrient uptake by the plant. The CRF assessment under this condition has usually been examined by measuring the composition of soil solution in relation to plant growth (Yanai et al., 1997).

The main objective of this research was to evaluate the use of different biodegradables polymer as encapsulating material for CRF development using biochar as support. The polymers selection were on base their degradability (SA > AC ≥ EC) and hydrophobicity (SA > AC > EC). Thus, the influence of the type of polymers on the leaching potential of nitrogen from CRF was evaluated.

5.2 Material and methods

5.2.1 Biochar

Biochar was generated by pyrolysis of oat hull at 300 ºC in a reactor with capacity to process 5 kg of raw material per batch. Ones charged with biomass, the reactor was purged with N_2 at a flow of 5 L min^{-1} to remove the oxygen present at the chamber. After process, biochar obtained was crushed using a high speed rotary cutting mill, sieved to obtain the particle size in requested range (≤500μm). The physic-chemical properties of biochar were previously reported in by González et al. (2013).

5.2.2 Nitrogen impregnation onto biochar

Impregnation experiments were carried out in a batch reactor. A mixture of biochar with urea was performed in aqueous media; the proportion of reactants was fixed in 1:0.5:5 biochar, nitrogen (as Urea) and deionized water, respectively according to the methodology of Bimer et al. (1998) partially modified. The impregnation was carried out at constant

temperature of 150 ± 5 °C for 10 min. Then, the nitrogen content of the product was determined by the Kjeldahl method (Sadzawka et al., 2004).

5.2.3 Evaluation of polymers for CRF formulation

The polymers used for the preparation of encapsulates were cellulose acetate (AC) from Acros, ethyl cellulose (EC) from Sigma and sodium alginate (SA) from Sigma. Formamide (F) from Merck as modifying agent for the preparation of CA and EC solutions was used.

The polymer solutions were prepared by dissolution of the solid polymer in an adequate solvent. Acetone was used for CA and EC, whereas distilled water was used for SA. The densities (gravimetric method) and viscosities (digital viscometer VIS-79) of the resulting polymer solutions were measured at room temperature.

Encapsulates were developed from the mixture of polymeric solution and impregnated biochar through the technique of phase inversion for the polymers CA and EC. Biochar in different proportions was gradually added to polymeric solutions and dropped into the precipitation bath (distilled water), where the solvent-nonsolvent exchange proceeded, resulting in the formation of encapsulates (gelation process). The temperature of the precipitation bath was 25 °C. The beads were left in the distilled water for 5 min to ensure complete gelling; then, they were separated and dried.

For the formulation of encapsulates using SA the technique used was cross linking. The mixture between polymer and biochar in different proportions was dropped into the precipitation bath of $CaCl_2$. The spherical beads formed were left in the $CaCl_2$ solution for 5 min to ensure complete gelling and then separated and dried.

Analyses of nitrogen content of the encapsulate samples were determined by Kjeldahl method (Sadzawka et al., 2004). The morphology of selected samples was analyzed using a scanning electron microscope (SEM Quanta 600 FEI from, FEI Inc., Hillsboro, OR, USA) at 20 kV.

Table 5.1 show the composition and physic-chemical properties of the polymer solution and nitrogen content of the CRFs evaluated in the leaching test.

Table 5.1 Composition of the polymer solution and nitrogen content of the CRFs evaluated in the leaching test, where, C: control, U: urea, ESN: commercial N-CRF, BU: Biochar impregnated with nitrogen non-encapsulated, SA 1: Biochar+urea+SA 1% (R:1/1), SA 2: Biochar+urea+SA 2.5% (R:1/5), EC 1: Biochar+urea+EC 10% 10% F (R:1/4), EC 2: Biochar+urea+EC 10% 15%F (R:1/4), AC 1: Biochar+urea+AC 10% 10% F (R:1/2) and AC 2: Biochar+urea+AC 10% 15% F (R:1/6).

Sample	Polymer concentration (wt%)	Solvent concentration (wt%)	Modifying agent concentration (wt%)	Polymer viscosities* (cP)	Polymer density** (kg/m^3)	Proportion biochar/polymer (R)	Nitrogen content (%)
AC 1	10	80	10	127	877	1/2	15.6±0.5
AC 2	10	75	15	150	908	1/6	8.95±0.4
EC 1	10	80	10	321	870	1/4	14.16±0.3
EC 2	10	75	15	379	888	1/4	14.48±0.4
SA 1	1	-	-	202	1006	1/1	15.03±1.1
SA 2	2.5	-	-	43226	1119	1/5	14.66±1.3
BU	-	-	-	-	-	-	25.73±0.4
U	-	-	-	-	-	-	46
ESN	-	-	-	-	-	-	44

* Viscosities were measured by a digital viscometer VIS-79 at room temperature.

** Densities were obtained by gravimetric method at room temperature.

5.2.4 Leaching test

The leaching potential of CRFs was studied in disturbed soil column experiments. A bulk soil sample was taken from 0-20 cm depth of a fallowed field (uncropped for the last 15 years) located on the Central Experimental Farm of Agriculture and Agri-Food Canada in Ottawa, Ontario. The soil correspond to a sandy loam soil with pH 6.6 in water, C content 1.91 % and N content 0.11 % belonging to the Manotick series of the Haplorthods Great Soil Group. The soil was passed through a 2 mm sieve to remove large stones and coarse plant fragments, mixed thoroughly, and stored for 2 to 3 days before transferring it to plastic pots. The experiment was arranged in a completely randomized design with 10 fertilizer treatments (9 treatments shown in Table 5.1 plus a control) × with crop × without crop×3 replications×10 events of water addition. The leaching columns were constructed in PVC tubes, each 20 cm long and 7.6 cm internal diameter. The bottom was fitted to accommodate a filter paper in order to avoid soil loss from the bottom. A glass wool on the soil surface of columns without plants was used to minimize disturbance and reduce the dead volume during leaching. The columns were packed with soil (bulk density of 1.51 kg/m^3), the field capacity was maintained at 75% during the assay. The fertilization with the different CRFs (see characteristics of each CRFs in Table 5.1) was equivalent to 150 kg N ha^{-1} (one addition when starting the assay) and was added to the top 5 cm of the PVC column. Moreover, macro and micronutrients such as: P, K, Mg, Ca, S, Cl, B, Mn, Zn, Cu, Mo in the columns were added in order to maintain a proper fertilization. The assay was conducted in a greenhouse with an average temperature of 25 ± 3 °C. 200 ml of deionized was added in each event of leaching. The leaching process lasted 6 h. Leachates were collected on 15, 22, 29, 36, 43, 50, 57, 64, 71 and 78 days after establishment of AC Barrie wheat. The leachates were filtered with Whatman No. 42 filter paper previously washed with KCl 2 N. Nitrate, nitrite, ammonium, and urea content were measured in the leachates. Initially, 5 seeds of wheat were sown in the columns and after 7 days they were thinned remaining only 2 plants per column (wheat density). After 90 days, plants were removed from the columns and separated into grain, roots and shoots in order to evaluate the productivity.

5.2.5 Analytical methods

5.2.5.1 Chemical analyses

Nitrate, nitrite, and ammonium: Leachate samples were analyzed for ammonium, nitrite and nitrate content using the Lachat QuickChem FIA+ 8000 series. Analysis of ammonium was realized using the Lachat method 12-107-06-2-A, and analyses for nitrite-N and nitrate-N followed the Lachat method 12-107-04-1-B, as described by the equipment manufacturer.

Urea: N-urea was determined by colorimetric method, an aliquot (10 ml) of leachates and 30 ml color reagent were poured into the glass tubes, the content was mixed thoroughly. The tubes were placed into a water bath at 85±0.5 °C. After 27 min, the flasks was removed from the bath, cooled immediately under running water (13-20 °C) for 15 min, the contents were raised up to 50 ml by adding distilled water, and mixed thoroughly. Then, an aliquot was transferred to plastic cuvettes and measured the absorbance at 527 nm. The content of N-urea of each extract was calculated by reference to a calibration graph plotted from the results obtained with standards (Schnitzer, 1982).

5.2.6 Statistical analyses

All experiments and analyses were done in triplicate. Analyses of variance followed by a least significant difference (LSD) and Tukey test at the 0.05 level were used to determine significant differences means between treatments.

5.3 Results and discussion

5.3.1 Nitrogen losses by leaching

The quantity of ammonium in leachates from each formulation for the assays with and without plants is shown in Figure 5.1a and 5.1b, respectively. From Figure 5.1a and b can be observed that at 22 days the $N\text{-}NH_4^+$ leachates showed a maximum for all formulations in both assays (with and without plants). Also, can be observed after day 29th the $N\text{-}NH_4^+$ leaching rate kept stable. The $N\text{-}NH_4^+$ losses in soil columns with was low in comparison with the assays without plants.

The soil columns treated with EC 1 sown with wheat, showed the maximum $N\text{-}NH_4^+$ loss by leaching (0.014 mg $N\text{-}NH_4^+$ kg^{-1} dry soil). In contrast, soil columns without plants and fertilized with AC 1 showed the higher $N\text{-}NH_4^+$ loss by leaching (0.048 mg $N\text{-}NH_4^+$ kg^{-1} dry soil) (Figure 5.1a and 5.1b, respectively).

The results obtained in this study showed a lower $N\text{-}NH_4^+$ loss by leaching in comparison with experiments performed by Fernández-Escobar et al. (2004). The authors studied N losses by leaching for 4 commercial slow-release nitrogen fertilizers (Greenmaster, Basammon, Floranid and Multicote), applied to pots containing 50% of a mixture of river sand and peat (2:1 by volume) and 50% soil. The growth of "Picual" olive trees (*Olea europaea* L.) and N leaching losses were studied.

The authors reported $N\text{-}NH_4^+$ maximum amounts leached between 4-6 mg pot^{-1} aprox, depending of the formulations. Similar to our results, the ammonium leaching was produced within the first month after fertilizer application, with the exception of Basammon which produced ammonium losses over 71 days (Fernández-Escobar et al., 2004).

Paramasivan and Alva, (1997) reported very low amounts of $N\text{-}NH_4^+$ (mg) in leachates of soil columns treated with 3 urea-based controlled-release formulations (Meister, Osmocote and Poly-S).

In general terms, then $N\text{-}NH_4^+$ concentration recovered in the leachates during this study was relatively low for all treatments, fact that could be attributed to a nitrogen loss by

volatilization as NH_3 or due to a fast transformation from NH_4^+ to NO_3^- by nitrification processes (Paramasivan and Alva, 1997; Fernández-Escobar et al., 2004; Merhaut et al., 2006). The occurrence of each one of these processes is affected by $N\text{-}NH_4^+$ concentration, temperature, and soil pH (Peoples et al., 1995; Paramasivan and Alva, 1997).

Overall, $N\text{-}NH_4^+$ leaching losses over a 90-day period in our study were smaller than those reported in other studies carried out by Fernández-Escobar et al. (2004) and Paramasivan and Alva (1997). Differences that can be attributed to the type of fertilizer applied and mainly to the different environments studied, soil in our case and organic mixture for the others researcher.

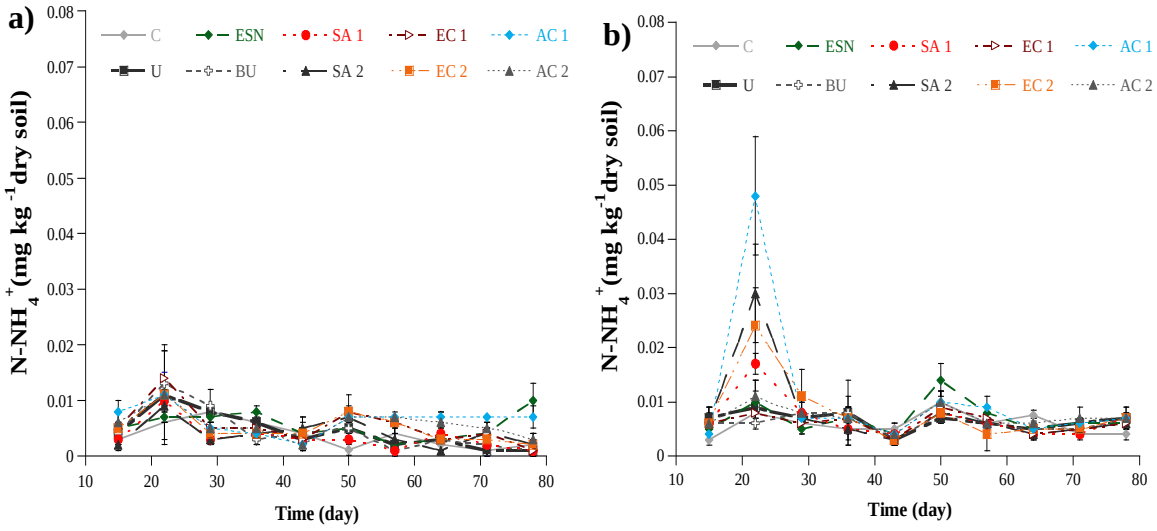

Figure 5.1 Concentration of $N\text{-}NH_4^+$ (mg kg^{-1} dry soil) in the leachates collected weekly in 10 weeks from a) leaching test with plants (AC Barrie wheat) and b) leaching test without plants, where, C: control, U: urea, ESN: commercial N-CRF, BU: Biochar impregnated with nitrogen non-encapsulated, SA 1: Biochar+urea+SA 1% (R:1/1), SA 2: Biochar+urea+SA 2.5% (R:1/5), EC 1: Biochar+urea+EC 10% 10% F (R:1/4), EC 2: Biochar+urea+EC 10% 15%F (R:1/4), AC 1: Biochar+urea+AC 10% 10% F (R:1/2) and AC 2: Biochar+urea+AC 10% 15% F (R:1/6).

*The standard deviation (SD) is the mean of three replicates.

The greatest proportion of N found in the leachates was in nitrate form (Figure 5.2a and 5.2b), regardless of the N formulation. In general terms, the $N\text{-}NO_3^-$ concentration in leachates for all treatments in both assays without and with plants, excepting for soil columns treated with ESN, showed high values after the first and second events. After day 29th the $N\text{-}NO_3^-$ concentration showed a fast decrease. However, for the assays with plants the decrease was more pronounced after 3 leaching event, this behavior can be explained due to the $N\text{-}NO_3^-$ uptake by plants during the first weeks.

The leachates from soil columns containing plants (Figure 5.2a) showed the maximum concentration of $N\text{-}NO_3^-$ (22.57 $N\text{-}NO_3^-$ kg^{-1} dry soil) after the first event of leaching (15th day), the soil columns treated with AC 1 and the soil columns treated with the encapsulated shown values in a range between 7 and 20 $N\text{-}NO_3^-$ kg^{-1} dry soil. In the 22th day SA 1, SA 2, EC 2 and AC 2 increased the $N\text{-}NO_3^-$ concentration in the leachates, especially SA 2 (29.69 $N\text{-}NO_3^-$ kg^{-1} dry soil). After second event of leaching a fast decrease of $N\text{-}NO_3^-$ was showed for all encapsulates.

In the case of the assays without plants, the columns treated with urea and BU showed the higher losses of $N\text{-}NO_3^-$ (26.78 and 22.02 N- NO_3^- kg^{-1} dry soil, respectively) after the first event of leaching. In the 22th day all treatment (except urea and ESN) increased the NO_3^--N concentration in the leachates, especially EC 1 (29.79 $N\text{-}NO_3^-$ kg^{-1} dry soil). After 29th day a fast decrease was showed for all encapsulates. For the case of ENS, the release rate kept stable during the time averaging $N\text{-}NO_3^-$ kg^{-1} dry soil, approximately per event. The fast decrease of $N\text{-}NO_3^-$ for ESN in treatment with plant compared with the treatment without plant can be explained by the action of roots exudates. These compounds produce a change in the rhizosphere pH and increase in the rhizosphere microflora (Marschnera et al., 1987), which could affect the degradation of the polymeric fertilizer layer.

For all controlled-released formulations excepting ESN, a gradual decrease in urea-N concentration after the first leaching event and a steady increase in $N\text{-}NO_3^-$ in the subsequent events, suggest a fast hydrolysis of urea was due to a higher nitrification activity by *Nitrobacter* (Fernández-Escobar et al., 2004).

The continuous supply of a low level of $N\text{-}NO_3^-$ may be beneficial to keep leaves actives because the absorbed $N\text{-}NO_3^-$ tended to be primarily translocated to the leaves and then re-

exported to the other growing parts, due to $N\text{-}NO_3^-$ is the major form of absorbed N by the plants (Ohyama, 1984). However, an excess $N\text{-}NO_3^-$ could cause an inhibitory effect on the root system, which is directly in contact with the soil solution (Harper et al., 1971; Nakano, 1987).

Noteworthy that $N\text{-}NO_2^-$ was not detected during all time for columns under different treatments and with or without plants, suggesting a high and fast nitrification activity of *Nitrobacter* (Ardakani et al., 1975).

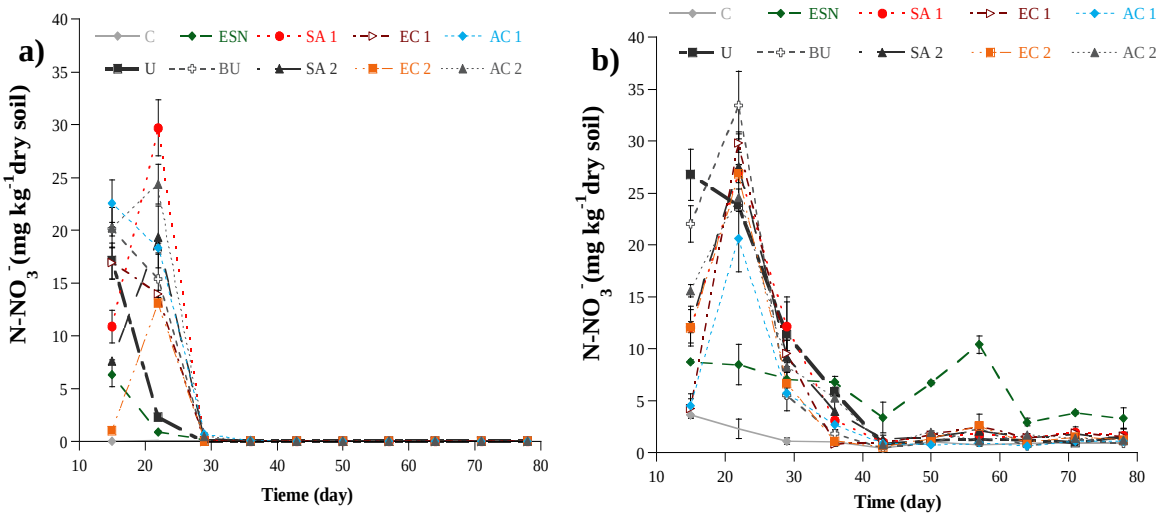

Figure 5.2 Concentration of $N\text{-}NO_3^-$ (mg kg^{-1} dry soil) in the leachates collected weekly in 10 weeks from a) leaching test with plant (AC Barrie wheat) and b) leaching test without plant, where, C: control, U: urea, ESN: commercial N-CRF, BU: Biochar impregnated with nitrogen non-encapsulated, SA 1: Biochar+urea+SA 1% (R:1/1), SA 2: Biochar+urea+SA 2.5% (R:1/5), EC 1: Biochar+urea+EC 10% 10% F (R:1/4), EC 2: Biochar+urea+EC 10% 15%F (R:1/4), AC 1: Biochar+urea+AC 10% 10% F (R:1/2) and AC 2: Biochar+urea+AC 10% 15% F (R:1/6).

*The standard deviation (SD) is the mean of three replicates.

Urea was only detected during the first 4 events of leaching; however, during the first event in both assays with and without plants (Figure 5.3a and 5.3b, respectively) all treatment showed a maximum peak of N-urea concentration. For the assays with plants, ESN showed the maximum concentration of N-urea in the first event of leaching (4.18 mg N-urea kg^{-1}

dry soil) and the minimum concentration was found in the leachates of soil column treated with AC 2 (2.46 mg N-urea kg^{-1} dry soil), at the same period of time.

For the assays without plants, the leachates from the soil column treated with AC 2 showed the higher concentration of N-urea (3.52 mg N-urea kg^{-1} dry soil) after the first event of leaching. To the contrary, the minimum concentration was found in the leachates obtained from the column treated with U (1.52 mg N-urea kg^{-1} dry soil). Although urea have not polymeric cover, these values did not exceed to the obtained from the soil columns treated with encapsulated, suggesting that hydrolysis of the urea occurred during the first days. Studies reported by Paramasivam and Alva (1997) showed that the urea hydrolysis occurs during the first 10 days of a crop establishment. Therefore, the results obtained in this study suggest that the polymeric materials could be able to retard the urea hydrolysis due to the slow release.

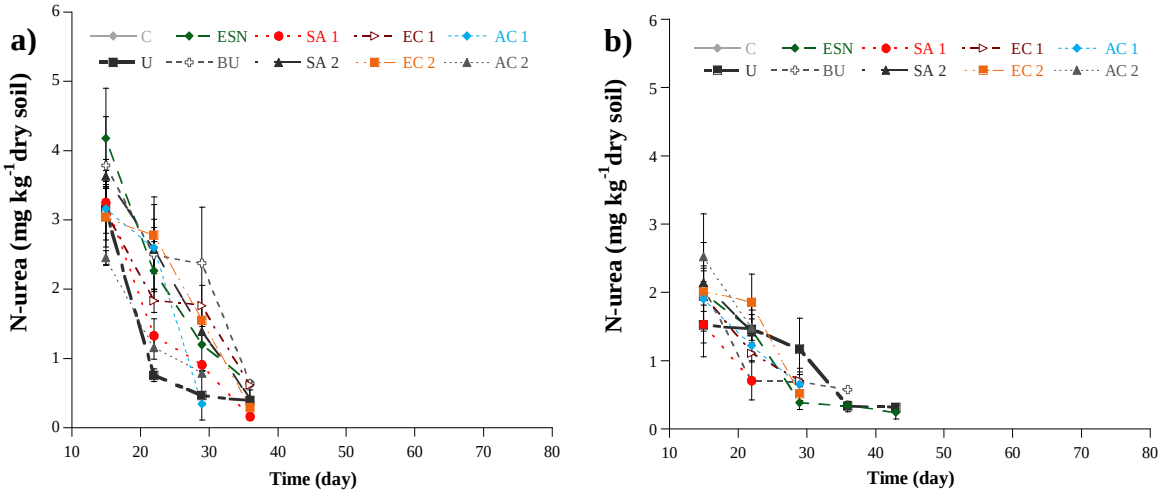

Figure 5.3 Concentration of urea (mg kg^{-1} dry soil) in leachates collected from a) leaching tests with plant (AC Barrie wheat) and b) leaching test without plant, where, C: control, U: urea, ESN: commercial N-CRF, BU: Biochar impregnated with nitrogen non-encapsulated, SA 1: Biochar+urea+SA 1% (R:1/1), SA 2: Biochar+urea+SA 2.5% (R:1/5), EC 1: Biochar+urea+EC 10% 10% F (R:1/4), EC 2: Biochar+urea+EC 10% 15%F (R:1/4), AC 1: Biochar+urea+AC 10% 10% F (R:1/2) and AC 2: Biochar+urea+AC 10% 15% F (R:1/6).

*C: in the control, urea was not detected.

**After of 4 leaching event, in all samples urea not was detected.

***The standard deviation (SD) is the mean of three replicates.

Table 5.2 show the total cumulative amount of $N\text{-}NH_4^+$, $N\text{-}NO_3^-$ and N-urea in leachates after 90 days. In terms of cumulative $N\text{-}NH_4^+$ losses for the assays with plants, was not observed significant differences between applied formulations, only SA 1, EC 1 and AC 1 showed significant differences with respect to control. When the CRFs were applied to soil columns without plants, only AC 1 showed significant differences with regard to the others formulations evaluated; however, all formulation (except BU and EC 1) shown significant differences with respect to the control.

Regarding to cumulative $N\text{-}NO_3^-$ losses from soil columns containing plants, $N\text{-}NO_3^-$ were lower compared with assays without plants due to nitrogen in this form is mainly absorbed by plants. We observed significant differences between CRFs (U, BU, SA 1, SA 2, EC 1, AC 1 and AC 2) applied to the soil columns and the CRFs (ESN and EC 2), where, the last two treatment shown lower cumulative $N\text{-}NO_3^-$ content in leachates.

On another hand, $N\text{-}NO_3^-$ losses, U, BU, SA 1, and AC 2 showed the higher losses after the leaching events, showing significant difference with the other CRFs applied in the assays without plants.

Cumulative N-urea losses showed significant difference between evaluated treatments with and without plant. However, the cumulative N-urea concentration in leachates in assays with plants was higher compared with assays without plants assay. These results suggest a possible inhibition or immobilization of urease. In this sense, plant roots exudates into rhizosphere could have caused urease activity inhibition (Subbarao et al., 2008; Watkins et al., 2009). On the other hand, a possible high microbial density on the rhizosphere could produce an enzyme immobilization (Dharmakeerthi and Thenabadu, 1996).

The difference in the amount of N species containing at the leachates collected from soil columns under various N formulations could be attributed to differential behavior of the polymeric materials with respect to their ability to release nutrients. In this sense, is recommended the use of polymeric materials for the CRF formulation containing biochar, due to the BU showed the highest values of $N\text{-}NO_3^-$ concentration in leachates, similar to urea.

In particular, is recommended the use of ethyl cellulose, due to this treatment show a low $N\text{-}NO_3^-$ losses. Besides, this treatment (EC 2) showed the highest grain yield compared with other developed formulations (Figure 5.4a).

Ethyl cellulose (EC) widely used to prepare slow-release formulations of drugs, herbicides and fertilizer coating due to its controlled-release property (Dailey et al., 1993; Rekhi and Jambhekar, 1995; Pérez-Garcia et al., 2007; Ni et al., 2009).

The microscopic structures of the CRFs developed obtained by using scanning electron microscopy are shown in Figure 5.5. The prepared encapsulate with SA at 1% (Figure 5.5a) does not show a clearly skin layer; by contrast, for the prepared encapsulated with biochar+urea+EC 10% 15%F (R: 1/4) (EC 2) (Figure 5.5c) the image shows an asymmetric layer with a clearly outlined thin skin layer. We can also observe differences in the structure of layer for both polymers (Figure 5.5b and 5.5d). For EC 2, the image shows the outer skin layer is more compact and denser. This layer acts as a barrier which reduces the rate of intragranular diffusion of water, the dissolution of ingredients, and fertilizer transfer out of the granule (Jarosiewicz and Tomaszewska, 2003). In this sense, the studies demonstrated that the release rate of $N\text{-}NO_3^-$ from encapsulates with EC 2 decreases compared with SA encapsulates, showing significant differences between both treatments (Table 5.2).

In terms of biodegradables polymeric materials, sodium alginate (SA), a natural biopolymer can be ionically crosslinked by the addition of divalent cations in aqueous solution (Liu et al., 2008). The capsule of alginate gelatinized by Ca^{+2} has been used as controlled release formulation of pesticides, fertilizer and bacterial fertilizers (Liang et al., 2007; Liu et al., 2008; Singh et al., 2009) due to the mild condition for gelation, no toxicity and high biodegradability (Kurosawa et al., 1989 and Van Elsas et al., 1992). However, SA matrix does not have a strong mechanical strength and is easily destructible in the presence of monovalent cations (Liu et al., 2008). Also, SA is highly soluble in water. Cellulose acetate (AC) is highly biodegradable and hydrophilic; however, it exhibits a low-temperature resistance and is pH-sensitive (Jarosiewicz and Tomaszewska, 2003). The high degradability and hydrophilicity of both polymeric materials (SA and AC) influenced the

losses of nitrogen in the developed formulations, in particular in the $N\text{-}NO_3^-$ losses, showed a high amount in the leachates (Table 5.2).

On the other hand, ethyl cellulose (EC) is an inert, hydrophobic and biodegradable polymer (Ni et al., 2009). Due to its hydrophobic character, the CRF developed using EC as encapsulating agent, produced leachates with low $N\text{-}NO_3^-$ content compared with the formulations developed using SA and AC as encapsulation agents.

Regarding to the use of formamide as modifying agent, Jarosiewicz and Tomaszewska (2003) report that the use of formamide has a role over the coating porosity. High amounts of formamide increase the coating porosity. Thus AC 2 and EC 2 should show higher porosity and thus greater N losses. However, the low $N\text{-}NO_3^-$ amount in the leachates of AC 2 and EC 2 compared with AC 1 and EC 1 could be due to the toxic nature of the formamide that would cause an inhibition of soil microorganisms.

Nitrogen efficiency is shown in Table 5.3. In both assays, with and without plants, all formulations applied to soil columns showed a low percent of nitrogen losses in form of $N\text{-}NH_4^+$. Significant differences were observed between formulations for nitrogen losses in form of $N\text{-}NO_3^-$. The commercial controlled release formulation, ESN showed the minimum nitrogen losses in form of $N\text{-}NO_3^-$ (9.22%) and the maximum nitrogen losses in form of urea (15.84%) for assays with plants. Regarding to the formulations developed EC 2 showed the lowest losses of $N\text{-}NO_3^-$ (22.05%) in the leachates compared with the conventional fertilizer (urea, 36.85%), in the column containing plant. On the other hand, the same formulations showed the highest nitrogen content in the seed, 46.66 and 41.97 N%, respectively.

In assays without plants the major proportion of nitrogen losses was in $N\text{-}NO_3^-$ form. In this since, urea showed high loss of nitrogen in $N\text{-}NO_3^-$ form (88.82%).

Table 5.2 Cumulative for $N\text{-}NH_4^+$, $N\text{-}NO_3^-$ and N-urea in (mg) of leachates from different fertilizers formulations, where, C: control , U: urea, ESN: commercial N-CRF, BU: Biochar impregnated with nitrogen non-encapsulated, SA 1: Biochar+urea+SA 1% (R:1/1), SA 2: Biochar+urea+SA 2.5% (R:1/5), EC 1: Biochar+urea+EC 10% 10% F (R:1/4), EC 2: Biochar+urea+EC 10% 15%F (R:1/4), AC 1: Biochar+urea+AC 10% 10% F (R:1/2) and AC 2: Biochar+urea+AC 10% 15% F (R:1/6).

	With plant			***Without plant***		
Formulations	**$N\text{-}NH_4^+$ (mg)**	**$N\text{-}NO_3^-$ (mg)**	**N-urea* (mg)**	**$N\text{-}NH_4^+$ (mg)**	**$N\text{-}NO_3^-$ (mg)**	**N-urea* (mg)**
C	0.04	0.15	nd	0.04	12.77	nd
U	0.06	38.82	7.58	0.07	73.16	4.93
ESN	0.06	6.42	10.77	0.07	53.98	4.29
BU	0.06	23.69	9.66	0.06	65.70	3.97
SA 1	0.06	43.24	5.99	0.07	62.78	2.41
SA 2	0.07	33.48	4.06	0.08	53.52	3.57
EC 1	0.07	34.65	8.89	0.06	56.89	3.95
EC 2	0.06	15.14	8.35	0.08	56.15	4.26
AC 1	0.08	41.78	6.10	0.13	48.55	3.53
AC 2	0.05	36.59	4.40	0.07	62.07	6.01
$LSD_{0.05}$[a]	0.02	4.87	1.82	0.02	4.87	1.82

* nd: not detected.

* After 4 leaching events (29 days), N-urea was not detected for all treatments.

[a]Least significant difference ($p \leq 0.05$) for the $N\text{-}NH_4^+$, $N\text{-}NO_3^-$, N-urea and plant interaction.

Table 5.3 Nitrogen efficiency for each treatments and formulations testing, where, U: urea, ESN: commercial N-CRF, BU: Biochar impregnated with nitrogen non-encapsulated, SA 1: Biochar+urea+SA 1% (R:1/1), SA 2: Biochar+urea+SA 2.5% (R:1/5), EC 1: Biochar+urea+EC 10% 10% F (R:1/4), EC 2: Biochar+urea+EC 10% 15%F (R:1/4), AC 1: Biochar+urea+AC 10% 10% F (R:1/2) and AC 2: Biochar+urea+AC 10% 15% F (R:1/6).

	With plant						***Without plant***		
Formulations	**N-NH_4^+ (%)**	**N-NO_3^- (%)**	**N-urea (%)**	**Shoot N (%)**	**Root N (%)**	**Seed N (%)**	**N-NH_4^+ (%)**	**N-NO_3^- (%)**	**N-urea (%)**
U	0.09 abc	36.85 c	11.15 abc	5.09 ab	2.93 a	39.64 ab	0.10 c	88.82 a	7.25 ab
ESN	0.09 abc	9.22 e	15.84 a	6.71 ab	3.81 a	46.66 a	0.10 c	60.60 de	6.31 abc
BU	0.09 abc	34.61 c	11.92 ab	7.07 ab	3.52 a	40.93 ab	0.10 c	77.83 ab	5.83 abc
SA 1	0.09 abc	58.86 a	8.80 bc	2.48 b	1.50 b	27.93 c	0.10 c	73.56 bc	3.55 c
SA 2	0.10 ab	49.02 b	5.97 bc	4.27 ab	1.87 b	27.97 c	0.12 bc	59.94 de	5.25 bc
EC 1	0.09 ab	50.74 b	9.08 ab	3.88 ab	0.86 b	35.20 bc	0.09 c	64.89 bd	5.80 abc
EC 2	0.11 a	22.05 d	12.29 ab	6.15 ab	1.90 b	41.97 ab	0.11 bc	63.79 cde	6.26 abc
AC 1	0.07 c	61.23 a	8.97 bc	4.42 ab	2.01 ab	17.48 d	0.18 a	52.62 de	5.19 bc
AC 2	0.07 c	53.59 ab	6.48 c	7.44 a	0.36 b	28.16 c	0.11 bc	72.50 bc	8.85 a

*Different letters indicate significant differences between treatments (Tukey test $P \leq 0.05$).

5.3.2 Effect of different formulation on grain yield and dry matter of shoots and roots

The effect of different formulations on grain yield and dry matter of shoots and roots for AC Barrie (*Triticum aestivum* L.) are shown in the Figure 5.4a. Grain yield of wheat was significant when the soil columns were treated with ESN and urea (19.17 and 16.75 t ha^{-1}, respectively) not showing significant differences between them. Regarding to the encapsulates developed, BU, SA 1 and EC 2 not shown significant difference on grain yield with respect to the soil column treated with urea. However, BU and SA 1 are discarded as potential CRFs due to the high amount of NO_3^--N that is losses by leaching. SA 2 showed the lower grain yield (3.53 t ha^{-1}) similar to the control without N fertilization. Probably this result can be explained for the N up take by the microorganisms due to high carbon content of the encapsulating agent present in the formulation (2.5% SA).

It is difficult to compare the results obtained in this study with the found in literature, due to (i) CRF vary greatly in their effectiveness under different environment and (ii) some forms of CRF and its placement method may not effectively synchronize N release with crop demand (Malhi et al., 2010). Chen et al. (2008), in a review, also found that CRF increased crop production in some but not all studies, and that its effectiveness will depend on crop, fertilization, soil and management factors.

The results for dry matter of shoots and roots are showing in the Figure 5.4b. We can show that N fertilization increased significantly biomass of shoots and roots.

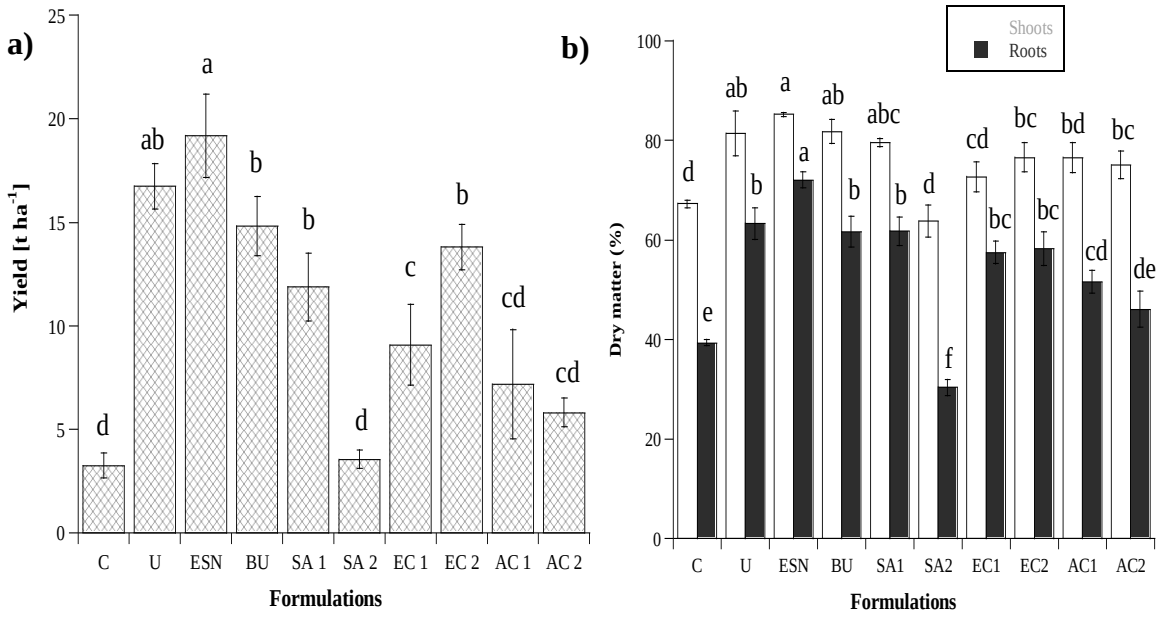

Figure 5.4 Effect of formulations on a) grain yield for AC Barrie wheat in the leaching test and b) dry matter of shoots and roots for AC Barrie after 90 days, where, C: control, U: urea, ESN: commercial N-CRF, BU: Biochar impregnated with nitrogen non-encapsulated, SA 1: Biochar+urea+SA 1% (R:1/1), SA 2: Biochar+urea+SA 2.5% (R:1/5), EC 1: Biochar+urea+EC 10% 10% F (R:1/4), EC 2: Biochar+urea+EC 10% 15%F (R:1/4), AC 1: Biochar+urea+AC 10% 10% F (R:1/2) and AC 2: Biochar+urea+AC 10% 15% F (R:1/6).

*The standard deviation (SD) is the mean of three replicates.

**Different letters indicate significant differences between treatments (Tukey test $P \leq 0.05$).

Table 5.4 shows the shoot and roots dry weight and de root/shoot ratio (R/S) for all treatments. Nitrogen nutrition has significant effects on root and shoots relations (Feng and Liu, 1996; Lioert et al., 1999).

EC 2 and ESN showed the higher R/S ratio (0.42 and 0.41, respectively), not showing significant differences. Cultivars with great R/S usually have a relatively greater water and nutrient uptake capacity, higher yield and greater drought resistance. By contrast, low R/S ratios usually present a low grain yield (SA 2) (Passioura, 1983).

Table 5.4 Mean values for shoots and roots dry weight in (g) (values for two plant) and root/shoot ratio of AC Barrie under different treatments; where, C: control, U: urea, ESN: commercial N-CRF, BU: Biochar impregnated with nitrogen non-encapsulated, SA 1: Biochar+urea+SA 1% (R:1/1), SA 2: Biochar+urea+SA 2.5% (R:1/5), EC 1: Biochar+urea+EC 10% 10% F (R:1/4), EC 2: Biochar+urea+EC 10% 15%F (R:1/4), AC 1: Biochar+urea+AC 10% 10% F (R:1/2) and AC 2: Biochar+urea+AC 10% 15% F (R:1/6).

Treatments	Shoot dry weight (g)	Roots weight (g)	R/S
C	0.88±0.03 d	0.28±0.01 cd	0.32 ab
U	1.95±0.52 ab	0.74±0.10 b	0.38 ab
ESN	2.46±0.08 a	1.02±0.21 a	0.41 a
BU	1.76±0.08 bc	0.69±0.09 b	0.39 ab
SA 1	1.66±0.09 bc	0.69±0.08 b	0.42 a
SA 2	0.93±0.31 d	0.19±0.01 d	0.20 c
EC 1	1.28±0.22 cd	0.45±0.12 bc	0.32 ab
EC 2	1.41±0.23 bdc	0.54±0.12 bc	0.42 a
AC 1	1.41±0.22 bdc	0.50±0.12 bc	0.36 ab
AC 2	1.23±0.18 cd	0.34±0.09 cd	0.26 bc

*The standard deviation (SD) is the mean of three replicates.

**Different letters indicate significant differences between treatments (Tukey test $P \leq 0.05$).

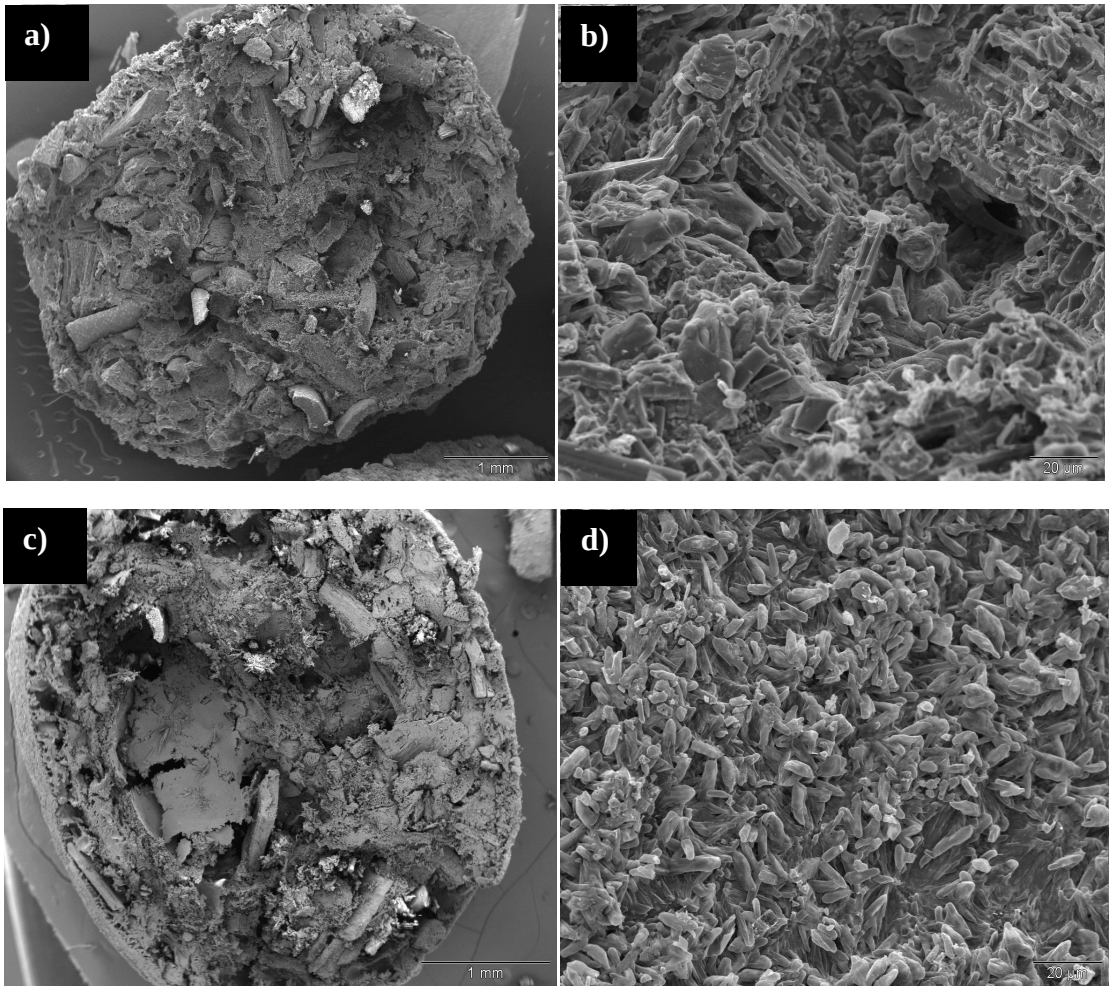

Figure 5.5 Images of scanning electron microscope (SEM) for a) cross section encapsulated SA 1 development using biochar+urea+SA 1% (R: 1/1) overview at 1mm, b) coating image for SA 1 with a resolution of 20µm, c) cross section encapsulated EC 2 developed using biochar+urea+EC 10% 15%F (R: 1/4) overview at 1mm and d) coating image for EC 2 with a resolution of 20µm.

5.4 Conclusions

According to results obtained, it is possible conclude that the polymeric materials can be retard urea hydrolysis occurred during the first days of establishment the essay. The $N\text{-}NH_4^+$ concentration recovered in the leachates in this study were low for all treatments for without and with crop, and the high $N\text{-}NO_3^-$ values, suggest the fast nitrification of NH_4^+ to NO_3^-.

The difference in the amount of N species in leachates fractions collected from soil columns treated with various N formulations could be attributed to differential behavior of the polymeric materials with respect to their ability to release nutrients. In this sense, is recommended using polymeric materials for the CRF formulation in base to biochar, due to biochar impregnated with nitrogen non-encapsulated treatment showed higher values of NO_3^--N, not showing significant difference regarding urea.

In particular, is recommended the use of ethyl cellulose, due to this treatment showing low $N\text{-}NO_3^-$ losses. Besides, this treatment (EC 2) showed higher grain yield compared with other developed encapsulated. Compared with ESN the grain yield was negatively affected in a 83% for C, 81% for SA 2, 70% for AC 2, 62% for AC 1, 52% for EC 1, 38% for SA 1, 28% for EC 2, 23% for BU and 13 % for U.

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Chapter 6

General discussion

General discussion

The present study aimed to evaluate the development of a controlled-release nitrogen fertilizer using biochar as support. In order to achieve this, the subject was divided into three main areas.

In Chapter 3, the production and characterization of biochar using agro-forestry residual biomass as feedstock was studied. In this context, different raw materials (oat hulls and pine bark) and pyrolysis temperature (300 and 500 °C) were the variables studied to obtain the biochar. These working conditions affect the physical, chemical and mineralogical properties as well as the potential applications of these biochars.

Different parameters were taken into account to characterize the biochar such as: chemical composition, surface analysis (surface area and pore size distribution), surface functional groups (by chemical methods and FTIR), optical analysis (scanning electron microscopy (SEM)), among others. As a first application of biochar produced, its potential as support material for the immobilization of *Candida rugosa* lipase was studied.

The results confirmed that the physic-chemical characteristics of the biochar depend on both its raw material and pyrolysis temperature. High pyrolysis temperatures (500 °C) produced a higher surface area (BET), particularly for the biochar synthesized from pine bark. This was attributed to the high content of lignin in this raw material.

The leachability tests of heavy metals (appendix 3) demonstrated that biochar's samples from oat hull and pine bark are considered inert or non-hazardous materials, according to the European Norm 12457-2. However, high enrichment of trace elements such as Ba, Cr, Cu, V and Zn was detected in biochar from pine bark; therefore, it was discarded for lipase immobilization purposes and as support material for the development of a controlled-release fertilizer. In this sense, heavy metals content can be a concern since its regular application involves the gradual accumulation of these compounds in the soil.

Biochar synthesized from oat hull at 300 °C (BO300) showed to be the most suitable support material for the immobilization of *Candida rugosa* lipase. This material presented the highest carboxylic groups content, which can certainly promote enzyme immobilization, through covalent bindings between carboxyl, sulfhydryl, hydroxyl or

phenolic groups found on the biochar surface with the amino groups of the enzyme. Biochar functionality is thus very important for the use of these materials as possible support materials. In particular, for the formulation of controlled-release fertilizer (CRF), these groups can react with ammonia, urea, or other -NH_2 containing materials. Various imide and amide bonds are formed between carboxylic carbons and nitrogen.

Furthermore, BO300 showed the lowest metal content, avoiding its negative effect on enzyme activity. All these advantages are limited by relatively low specific surface area (0.1 m^2 g^{-1}) and porosity of BO300; however, the functional groups present on the BO300 surface are arguably the key point for assuring an immobilization or adsorption process. In this sense, the binding between carboxylic groups on the BO300 surface and amino groups from the enzyme was confirmed by FTIR analysis.

In the biochar production low molecular-weight polycyclic aromatic hydrocarbons (PAHs) can be generated. These contaminants may be present in biochar matrix and even be bioavailable to organisms. In this study, an aromatic ring was detected in pine bark biomass and in BP300, through FTIR analysis; however, a decrease intensity was observed after a pyrolysis process. Using pyrolysis at the temperature of 300 °C a decrease stretching intensity was measured; while at 500 °C the stretching is completely absent. In biochar from oat hull, pyrolyzed at 300 and 500 °C, the bands of PAHs were not detected. These results correspond with the literature, since in the temperature range between 350-600 °C, very small amounts of PAHs are formed. However, it is necessary quantify total and bioavailable PAHs and perform bio-assays using biochar in soil. Since it is not clear how long these compounds will be biodegradated in the soil. And the most important, the bio-availability of compound in soil is unknown.

The formulation of a controlled-release nitrogen fertilizer using biochar is presented in Chapter 4. A preliminary experiment of nitrogen sorption by biochar using urea as nitrogen source was performed at 25 °C (appendix 1). Under these conditions, the sorption capacity for BO300 and BO500 in the apparent equilibrium was from 17 to 30 mg N-urea g^{-1}, representing 1.7 to 3.0 % of the initial concentration, respectively. In contrast, when the biochar was exposed to urea at elevated temperature (150 °C), BO300 and BO500 showed the highest nitrogen enrichment, from 246 to 252 mg N g^{-1} biochar, representing 49 to 50 %

of the initial concentration, respectively (appendix 1). This increase of nitrogen impregnation was attributed to the presence of urea by-products after thermal decomposition. Nitrogen is likely to be in the form of amides, free NH and NH_2, bonded NH and NH_2, or NH_4^+ species.

At room temperature, the electrostatic interactions between the biochar surface and N-urea seem to be dominant, these interactions are energetically weak; therefore they have a greater chance of desorption. While, at elevated temperatures the interactions between biochar surface and urea are very complex, not only because of the heterogeneity of biochar structure, but also because of the variety of N-reagents that can arise from urea and can react independently with biochar. However, we suggest that the interaction mechanisms between biochar surface and urea, is through covalent bindings between carboxyl, sulfhydryl, hydroxyl or phenolic groups with the amino groups formed during the urea thermal decomposition.

In general terms, nitrogen losses in conventional fertilization of soil (using urea) are between 50 to 60%. It is estimated that the content of active compound of a CRF should be around 15-25% to meet the requirement of crops. Therefore, adsorption of urea onto biochar at room temperature does not meet these requirements for the formulation of a controlled-release fertilizer using biochar as support material. According to this study, the nitrogen impregnation onto biochar is suitable for the production of CRF using biochar as a support or carrier material. However, its disadvantage is nitrogen volatilization in the form of NH_3. This research will serve as a base for future studies focused on exploring variables such as pH variation (preferably acid pH), and the use of biochar as adsorbent for gases generated in the impregnation process (NH_3).

Also in Chapter 4, the use of biodegradable polymeric materials for the encapsulation the biochar/nitrogen mixture was evaluated. Sodium alginate (SA), cellulose acetate (AC) and ethyl cellulose (EC) were evaluated. The polymers selection were based on their degradability (SA > AC ≥ EC) and hydrophobicity (SA > AC > EC). These characteristics influence the controlled release of nutrients to the soil by diffusion through the pores, or by the erosion and the degradation of the encapsulated.

All polymers studied showed a potential use for such encapsulation. The characteristics and properties of encapsulates depend on polymer concentration, method used, use of modifier

agent, as well as the relation polymer/biochar (appendix 2). For example density and viscosity of polymer solutions affect the encapsulation preparation, especially when using phase inversion or precipitation technique in the formulation. These parameters increased with a rises in polymer concentration.

In the case of AC and EC, the addition of formamide (F), as a modifier agent, is essential for the preparation of the encapsulate by the phase inversion technique. Without the addition of this compound, the result is the dissociation of the mixture, forming an asymmetric colloidal membrane. Addition of F to polymeric solutions affected viscosity, density and encapsulated porosity. In this sense, the porosity is a crucial parameter. This property limits the diffusion of water into the fertilizer granule and of nutrients out of the encapsulated. However, the effect of encapsulated porosity on release was not evaluated in this study, therefore in further research it is necessary to evaluate.

Finally, in Chapter 5 the use of different biodegradable polymeric materials as encapsulating agents for the CRF formulation using biochar as support material were tested in order to evaluate the controlled-release characteristics, crop wheat yield and pollution effect.

Using a soil from Manotick series of the Haplorthods Great Soil Group, it was concluded that the polymeric materials can delay urea hydrolysis occurring during the first days of establishment of the assay. Despite the increasing production costs, the use of polymeric materials for CRF formulation is recommended, because biochar impregnated with nitrogen but non-encapsulated showed high losses of NO_3^--N by leaching (35 %). In particular, the use of ethyl cellulose (EC 2) is recommended, because this treatment diminished significantly the N-NO_3^- losses (22 %), thus is more efficient that conventional fertilization that showed a N-NO_3^-losses of 37%. In addition, this treatment showed high grain yield (14 ton ha^{-1}) compared with other polymers tested (e.g., AC 1, 7 ton ha^{-1} and SA 1, 12 ton ha^{-1}).

The difference in the amount of N species in leachates collected from soil columns treated with various N formulations was attributed to differential behavior of the polymeric materials with respect to their ability to release nutrients. For example, the high degradability and hydrophilicity of SA and AC influenced the losses of nitrogen in the

developed formulations, in particular the $N\text{-}NO_3^-$ losses, showed a high amount in the leachates (SA 1, 43.24 mg $N\text{-}NO_3^-$ and AC 1, 41.78 mg $N\text{-}NO_3^-$). In contrast, EC 2 (hydrophobic polymer) showed a low amount of $N\text{-}NO_3^-$ in leachates (15.14 mg).

These studies show that the degradability and hydrophobic/hydrophilic character of the material used in the preparation of the encapsulated has a substantial influence on the nutrient release. However, it is necessary to evaluate the behavior of CRF developed, using different types and different conditions of soil (e.g. organic matter content, pH, temperature). As well as it is necessary to evaluate the effect of the biochar addition on physico-chemical properties of soil. From this study can be inferred that the addition of a CRF, developed base on biochar, does not show an improvement on water holding capacity of the soil (appendix 3).

Chapter 7

General conclusions and outlook

General conclusions

- The structural and chemical properties of biochar are confirmed to depend on the raw material used and pyrolysis temperature. The specific surface area (BET) increased with an increase in pyrolysis temperature. High enrichment of trace elements Ba, Cr, Cu, V and Zn was detected in biochar from pine bark; this made it a bad choice as support material for biological and field applications. To the contrary, biochar produced from oat hull presented low heavy metal content and a significant content of carboxylic surface groups.

- N-urea sorption by biochar at room temperature (25 °C) does not meet the requirements for CRF development. Due to low nitrogen loading onto biochar surface. The interactions mechanisms predominant between the biochar surface and the N-urea are electrostatic interactions, which are interactions energetically weak.

- Nitrogen loading onto biochar impregnation with urea, performed at high temperature (150ºC) produced an enrichment of biochar with nitrogen, phenomena attributed to the presence of urea by-products interacting with the surface after its thermal decomposition. We suggest that the interactions mechanisms between biochar with urea is through covalent bindings between carboxyl, sulfhydryl, hydroxyl or phenolic groups found on the biochar surface with the amino groups formed during the urea thermal decomposition.

- Regarding the use of biodegradable polymeric materials as CRF encapsulation agents, it was concluded that the density and viscosity of polymeric solutions affect the encapsulation process, especially when the technique of phase inversion was used. When cellulose acetate (AC) and ethyl cellulose (EC) were used, addition of formamide (F) as a modifier was essential since, without the addition of this compound, dissociation of the mixture was observed, causing the formation of an asymmetric membrane colloidal.

- The effectiveness of the CRFs developed was tested and compared with the conventional fertilizer (urea) and a commercial CRF (ESN) in soil column experiments. They were less effective than the commercial formulation and conventional fertilizer (U) in terms of grain yield. From the total cumulative nitrogen losses, all developed formulations except EC 2 (Biochar+urea+EC 10% 15%F and relation biochar/polymer 1/4), exhibited the same behavior as the U, whose losses were mainly in $N\text{-}NO_3^-$ form.
-
- The degradability and hydrophobic/hydrophilic characteristic of the material used in the preparation of the encapsulated has a substantial influence on the nutrient release.

- Under the particular conditions experiments performed in this study, grain yield in soil columns treated with EC 2 was 23 and 16% less than for ESN and U, respectively. However, this formulation, had similar behavior to the commercial formulation, thus avoiding nitrogen losses by leaching. From this result it can be concluded that controlled-release fertilizers using biochar as support and polymers like ethyl cellulose could reduce environmental pollution produced by leaching and have similar productivity, as compared to conventional fertilizers.

Outlook

The information generated in this doctoral thesis suggests the potential use of biochar in several applications particularly as support/matrix material for the development of control release fertilizers and bio-molecules immobilization.

In this sense, results obtained evidence that the use of controlled-release fertilizers based on biochar as support material could improve nutrients uptake by plants, while reducing environmental pollution produced by nutrients leaching, as compared to conventional fertilization. However, new research should focus on the nitrogen impregnation process optimization in order to reduce NH_3 losses during the process. On the other hand, is necessary elucidating the mechanisms involved in the interaction between biochar and urea or its derivatives in the thermal decomposition. This information can be helpful when explaining the release of active compound once it is applied to the soil.

New researches are need for to elucidate the release rate of active components from the encapsulated. In this sense, it is necessary studies on the effect of the solution polymer concentration, inasmuch as influences the porosity of the prepared encapsulated. This property limits the diffusion of water into the fertilizer granule and of nutrients out of the granule.

Also it is necessary to evaluate the behavior of CRF developed under different types of soil and different conditions, such as: organic matter content, different soil pH, temperatures, and rainfall regimes; among others parameters.

In summary, technology such as biochar is presented as a promising material for the development of controlled-release fertilizers, not only acting as a soil conditioner but also promoting global warming mitigation.

Chapter 8

Appendix

Appendix 1

8.1 Methodology

8.1.1 Effect of washing and particle size of biochar on the N-urea sorption

Biochar was produced by pyrolysis of oat hulls at 300°C (BO300). A factorial design with 3 experimental blocks (B1: hexane; B2: hexane-methanol; B3: methanol washing) was used to study the effect of the variables: particle size (Xt) and washing time (XL) on the N-urea sorption onto biochar (Y: mg N-urea g^{-1} biochar). Both variables were studied in 3 levels (Table 8.1).

Table 8.1 Levels of the tested variables in experimental design.

Level	**Code Value**	**X_L (h)**	**X_t**
Low	-1	0	53-150 μm
Medium	0	2.5	150-500 μm
High	+1	5	>500 μm

The N-urea sorption was carried out in 50 mL centrifuge tubes at 25 ± 0.2 °C for 24 h in mechanical shaker (150 rpm). After incubation period, the samples were filtered through 0.45 μm pore size membrane and N-urea content at the liquid phase was determined by Kjeldahl method (APHA, 1995). The N-urea adsorbed was calculated by difference between the initial and final aqueous concentrations. All experiments were performed in duplicate and the average values are reported. For all experiment of sorption the amount of urea adsorbed was determined using the following mass balance equation:

$$qe = \frac{(C_0 - Ce) \times V}{W}$$

where qe is the amount (mg N-urea g^{-1}) of N-urea adsorbed, C_0 and Ce are the initial and equilibrium N-urea concentrations (mg N-urea L^{-1}) in solution, V is the adsorbate volume (L), and W is the adsorbent weight (g).

8.1.2 N-urea sorption kinetic

Urea solutions were prepared by dissolving urea with a 46% N (LOBA CHEMIE, PA) in DI water. The experiments were carried out in 50 mL centrifuge tubes at 25 ± 0.2 °C. The N-urea sorption capacity on biochar was evaluated using 0.25g of BO300, BO500, BP300, BP500 (previously washed with hexane -methanol and selected the 53-150 μm particle size) and activated carbon (AC) (Merk). The tubes were placed on an orbital shaker at 150 rpm at 25 ºC for 30 min, 1, 2, 4, 8, 24, 48 and 72 hours. After incubation period, the samples were filtered through 0.45 μm pore size membrane and the nitrogen content at the liquid phase was determined Kjeldahl method (APHA, 1995) . N-urea adsorbed was calculated by difference between the initial and final aqueous concentrations. For all experiment of sorption the amount of N-urea adsorbed was determined using the following mass balance equation:

$$qe = \frac{(C_0 - Ce) \times V}{W}$$

Where, qe is the amount (mg g^{-1}) of N-urea sorbed, C_0 and C_e are the initial and equilibrium N-urea concentrations (mg L^{-1}) in solution, V is the adsorbate volume (L), and W is the adsorbent weight (g).

The kinetic model used to describe N-urea sorption onto the biochar was the Elovich equation:

$$q_t = q_0 + (1/\beta)\ln(\alpha\beta) + (1/\beta)\ln t$$

where, α is the sorption rate (mg $g^{-1}h^{-1}$) and β is the desorption rate (mg $g^{-1}h^{-1}$), q_0 is the compound adsorbed at time zero (mg g^{-1}) and q_t is the maximum sorption of compound (mg g^{-1}) at time t (h) (Cea et al., 2010).

8.1.3 Effects of temperature and particle size in the nitrogen impregnation onto biochar

After determine the time of nitrogen impregnation (10 min), a factorial design with three experimental blocks was evaluated for BO300 and BO500 (B1: 1:0.5:5; B2: 1:1:5 and B3: 1:2:5 proportions of reactants, biochar: nitrogen: deionized water, respectively). The experimental design was used to study the effect of the variables: temperature (Xt) and particle size (Xp) on the nitrogen impregnation onto biochar (Y: mg N g^{-1}biochar). Both variables were studied at three levels (Table 8.2).

Table 8.2 Levels of the tested variables in experimental design.

Level	Code value	Xt (°C)	Xp
Low	-1	50	≤500 μm
Medium	0	75	Ground unsieved
High	+1	150	≥500 μm

Then, the analysis of nitrogen content at the solid phase was determined by Kjeldahl method (Sadzawka et al., 2004).

8.2 Results and discussion

8.2.1 Effect of washing and particle size of biochar on the N-urea sorption

It was found that urea sorption was not replicable, even when using biochar coming from one same batch of production. This is probably due to a contamination of the biochar surface by bio-oil condensed during the pyrolysis process. Therefore, a washing stage was introduced in order to obtain a uniform biochar. Moreover, it is believed that particle size also can affect the urea sorption. Because of before mentioned, it was decided to study the effect of biochar washing and particle size on urea sorption. For this study a factorial design methodology was used and it is presented in Table 8.3. The design consists of three experimental blocks: Block 1 (B1), Block 2 (B2) and Blok 3 (B3). In this Blocks hexane,

hexane followed by methanol and methanol were employed for washing the biochar (BO300), respectively. Additionally, for each Block the influence of washing time biochar (X_L) and biochar particle size (X_t) on the urea sorption was studied. The response to each factor of the experimental blocks are shown in Table 8.4.

Table 8.3 Urea sorption on BO300 depending of the studied variables.

B1			**B2**			**B3**		
X_L	***X_t***	***Y1***	***X_L***	***X_t***	***Y2***	***X_L***	***X_t***	***Y3***
1	1	91	-1	-1	78	-1	1	34
-1	-1	50	0	0	63	-1	-1	38
1	-1	61	1	-1	106	-1	1	41
1	1	52	1	-1	116	-1	-1	45
0	0	46	-1	-1	84	1	1	57
-1	1	68	1	1	42	1	-1	125
-1	1	294	-1	1	46	1	-1	108
-1	-1	62	1	1	44	0	0	56
1	-1	44	-1	1	60	1	1	53

Table 8.4 Effect of factors on the response to the experimental blocks B1, B2 and B3.

Effect	***B1***	***B2***	***B3***
X_L	-56	10	46
X_t	72	-48	-33
$X_L X_t$	53	-19	-29

Data analysis for B1 (hexane) suggests that in the case of not washing and using the largest particle size the urea sorption on BO300 is variable. Also by means of ANOVA it was determined that in B1 the effect of the studied variables is not significant in the response (Figure 8.1a), indicating that the material still has a high content of impurities.

Additionally, in B1 it was observed that using smaller particles the urea sorption increased, which can be due to an a reduction of particle size increase the specific surface area.

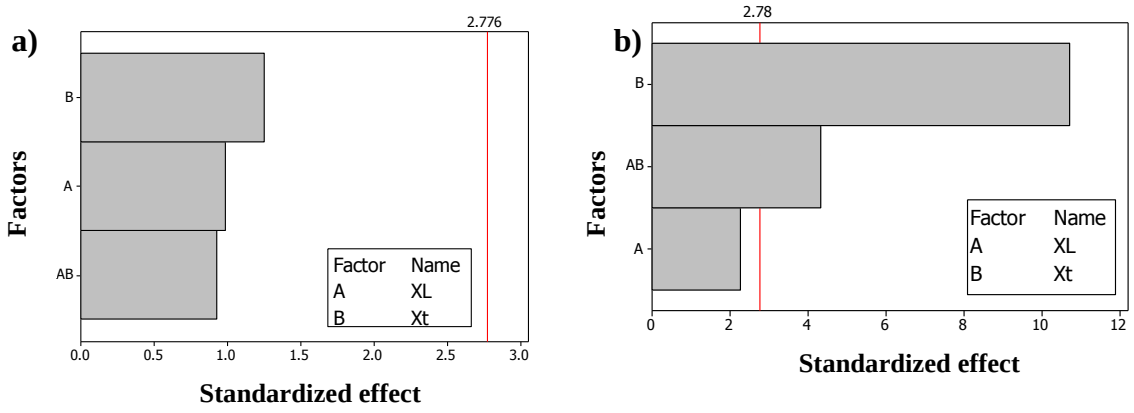

Figure 8.1 Effect of variable X_L (washing) and X_t (particle size) on the N-urea sorption a) for B1 (hexane) and b) for B2 (hexane-methanol), for BO300.

On the other hand, the results for B2 (hexane-methanol) indicated that the particle size (X_t) and the interaction with the washing time (X_LX_t) have a significant influence over urea sorption (Figure 8.1b) for a significance level $\alpha = 0.05$, with a greater influence of particle size. Furthermore, as is shown in Table 8.3, the effect of the variable Xt is negative indicating that the smaller the particle of BO300 higher the value of the response. For X_LX_t the coefficient is also negative, so smaller particle size and longer washing time, greater is the urea sorption.

Consequently B2 shows that the hexane-methanol system is suitable for cleaning of biochar, being a wash time of 5 hours and a particle size between 50-153μm the condition that gave more reproducible results.

Finally, in B3 (methanol) the results indicated that the two studied variables plus their interaction had a significant influence for the response (Figure 8.2)

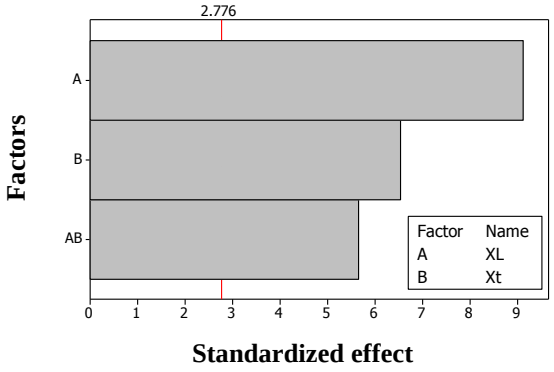

Figure 8.2 Effect of variable X_L (washing) and X_t (size particle) for B3 on the N-urea sorption for BO300.

Note that in B3 the effect of particle size (X_t) and the interaction with the washing time (X_LX_t) have a significant influence over the N-urea sorption, as was obserbed for B2. However, the effect of washing (X_L) becomes more relevant than for B2. This is caused by the bio-oil that is condensed at the pores of biochar surface. Consequently, when the sample is more contaminated with bio-oil, washing becomes more relevant, as well as the effect of the washing variable.

Washing is a recurrent technique to remove impurities deposited on the biochar, which are typical of the pyrolysis process. Some studies suggest biochar washing before use, especially for enzymes and microorganisms immobilization it is a good practice (Liu et al. 2011; Lin et al. 2010; Dehkhoda et al. 2010). Washing of biochar, is also an issue of concern to for their suitability as a soil amendment. In previous research, Brown et al. (1951), Turner (1955) and Gibson and Nutman (1960) used extensive washing procedures of charcoal-type to remove both organic and inorganic substances before application to soil. From the various washing methodologies, it is concluded that increasing the polarity of the solvent (from hexane to methanol) increases the N-urea sorption on the BO300. Moreover, despite the fact that the hexane and methanol washing showed no significant improvement compared to methanol washing. However, it is recommended washing with hexane and methanol, since it achieves the highest observed bio-oil removal percentages of around 0.4 % (w w^{-1}).

Regarding the particle size is recommended to work in a range between 53 and 150 μm and that in all cases showed a higher N-urea sorption. Studies realized by Zheng et al. (2010) show that a smaller biochar particle size (> 75m) ensures a greater adsorption of atrazine and simazine. Therefore, small particle sizes allow the sorbate to reach the microporous region of the biochar.

8.2.2 N-urea sorption kinetic

Figure 8.3 shows the amount of N-urea sorbed withing the time by the different biochars and activated carbon described with the Elovich equation. For both oat hull and pine bark biochar and for activated carbon was observed an apparent equilibrium after 48 h of incubation; however, there are differences between the maximum amounts adsorbed.

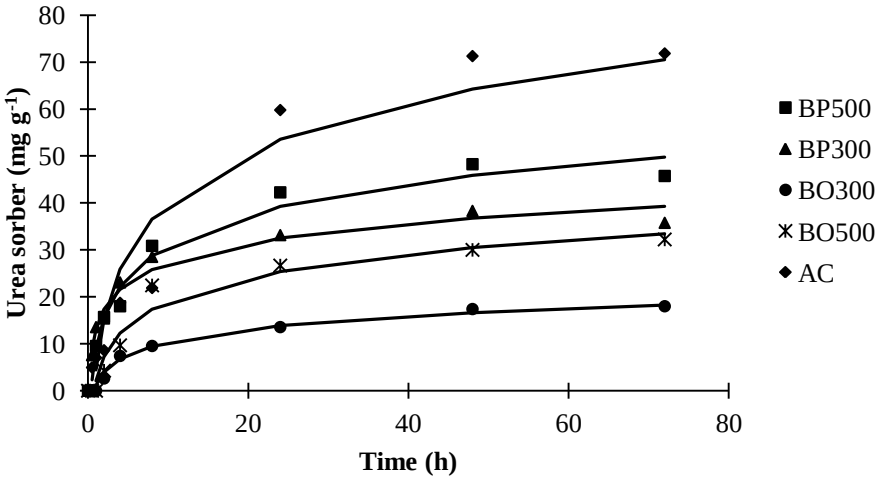

Figure 8.3 N-urea sorption on biochar's samples and activated carbon modeled by Elovich equation by 72 h at 25 °C, 150 rpm and initial concentrations of 1000 mg N-urea L^{-1}.

The quantity of N-Urea sorbed by BO300 and BO500 in the apparent equilibrium was of 17 and 30 mg N-urea g^{-1}, representing the 1.7 and 3 % of the initial concentration, respectively. For BP300 and BP500 the amount sorbed at 48 h was 38 and 42 mg N-urea g^{-1}, representing the 3.8 and 4.2 % of initial concentration, respectively. Also, we observed that AC sorbed the 7.2 % of the initial concentration after 48 h.

The Elovich equation was originally developed to describe the kinetics of heterogeneous chemical adsorption of gases on solid surfaces. The equation can be used to describe a variety of rate-controlling mechanisms, such as surface diffusion, activation and inactivation of catalytic surfaces (Sparks, 1999).Previously, the Elovich equation has been used to describe adsorption and desorption kinetics for a wide variety of organic and inorganic substances by activated carbon (Nemr et al., 2008; Halim et al., 2010).

Using the Elovich rate parameters, α and β, it is possible to obtain the equilibrium constant (K_{eq}) for the sorption process, where:

$$K_{eq}= \alpha/\beta$$

Figure 8.3 and Table 8.5 indicate that the sorption kinetics of N-urea on biochar samples and activated carbon were well described by the Elovich equation, with R^2 values ranging from 0.91 to 0.98.

Table 8.5 Elovich parameters for N-urea sorption (C_0=1000 mg N-urea L^{-1}) onto biochar's samples and activated carbon.

Samples	α (mg $g^{-1}h^{-1}$)	β (mg $g^{-1}h^{-1}$)	R^2	Log K_{eq}
BP300	52.05	0.163	0.96	2.50
BP500	24.30	0.104	0.96	2.37
BO300	5.37	0.250	0.98	1.33
BO500	9.78	0.136	0.95	1.85
AC	20.61	0.064	0.91	2.50

According to the Elovich rate parameters, the initial rate for BP300 sorption (α) was much greater for than the rates observed for the other biochar's evaluated and activated carbon. The sorption rates decreased as follows BP300> BP500> AC> BO500> BO300. In relation with the desorption rate (β), AC showed the minimum desorption rate, indicating a slow N-urea desorption. Regarding to the biochar samples BO300 showed the maximum desorption rate value. The fast desorption can be explain due to the electrostatic interactions between the biochar surface and urea, which are interactions energetically weak, therefore a greater chance of desorption (Wang et al., 1975; Cea et al., 2010).

The maximum value of log K_{eq} was for BP300 and AC these results demonstrated the affinity between these material and urea. In contrast, biochar from oat hull showed low log K_{eq}, thus the affinity between these materials and urea is very low, especially for BO300.

8.2.3 Effects of temperature and particle size in the nitrogen impregnation onto biochar

The effect of temperature and particle size onto the nitrogen impregnation capacity was evaluated. In this study, a factorial design methodology was used. Table 8.6 and Table 8.7 show the factorial design and the responses for BO300 and BO500, respectively. The obtained results showed that temperature is a key factor in nitrogen impregnation, for both BO300 and BO500. Besides, the designs, which consider greater amounts of nitrogen exhibit, greater impregnation capacity on biochar.

Table 8.6 Nitrogen impregnation capacity onto BO300 depending of the studied variables and the experimental blocks.

B1 (1:0.5:5)			**B2 (1:1:5)**			**B3 (1:2:5)**		
Xt	*Xp*	*Y1* *(mg N g^{-1} BO300)*	*Xt*	*Xp*	*Y2* *(mg N g^{-1} BO300)*	*Xt*	*Xp*	*Y3* *(mg N g^{-1} BO300)*
1	1	203	0	0	180	1	-1	338
-1	1	47	1	-1	320	1	-1	337
0	0	92	0	0	173	1	1	331
-1	-1	82	-1	1	108.	-1	1	109
-1	-1	83	-1	-1	97	0	0	374
1	-1	245	-1	1	105	-1	-1	142
1	1	211	1	1	289	-1	-1	121
1	-1	246	1	1	286	1	1	337
-1	1	48	1	-1	315	0	0	374
0	0	89	-1	-1	93	-1	1	107

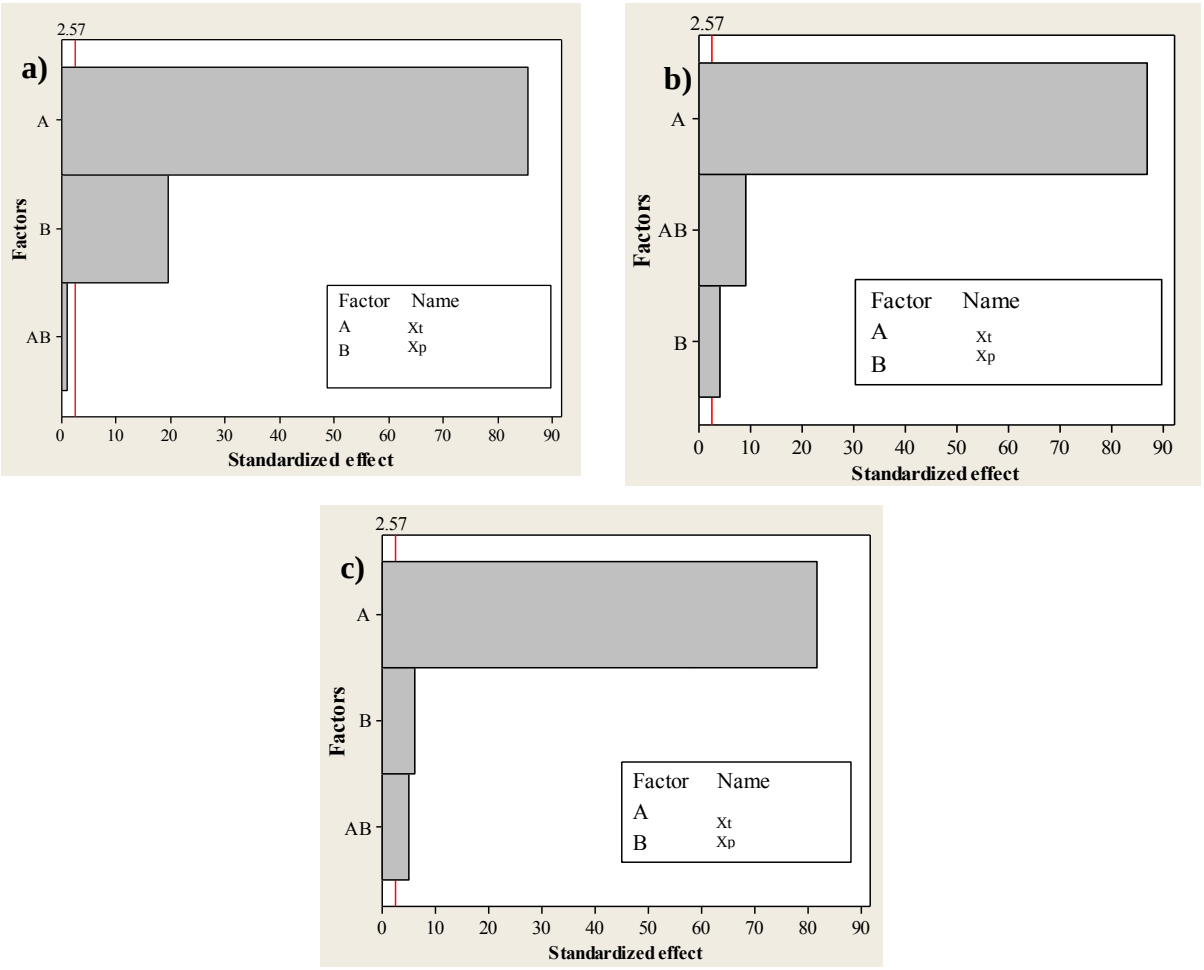

Figure 8.4 Effect of variable Xt (temperature) and Xp (particle size) on the nitrogen impregnation a) B1 (1:0.5:5), b) B2 (1:1:5) and c) B3 (1:2:5) for BO300.

Data analysis for B1 (1:0.5:5) suggests that in case of high temperatures (150 °C) and small particle size (<500 μm) the nitrogen impregnation capacity of BO300 is high (246 mg N g^{-1} BO300). Furthermore, as the nitrogen proportion increases the nitrogen impregnation capacity increase, being 320 and 337 mg N g^{-1} BO300, for B2 and B3, respectively.

Besides, it was determined by means of ANOVA analysis, that in B1 the effect of the studied variables is significant in the response (Figure 8.4a) for both temperature (Xt) and particle size (Xp), being temperature more significant. Additionally, it was observed in B1 that using smaller ranges of particle size (<500 μm) the nitrogen impregnation was increased. However, the interaction between factors (XtXp) is not significant in the response. On the other hand, the results of B2 (1:1:5) showed the same tendency of B1.

However, the interaction of the factors (XtXp) have a relevant influence on the response (Figure 8.4b) for a significance level $\alpha = 0.05$, with a greater influence of temperature. Finally, the experimental results indicated in B3 (1:2:5) that the two studied variables plus their interaction had a significant influence for the response (Figure 8.4c). It should be noted that the interaction (XtXp) in B3 has a significant influence on nitrogen impregnation, as in B2.

Table 8.7 Nitrogen impregnation capacity onto BO500 depending of the studied variables and the experimental blocks.

B1 (1:0.5:5)			**B2 (1:1:5)**			**B3 (1:2:5)**		
Xt	*Xp*	*Y1* *[mg N g^{-1}BO500]*	*Xt*	*Xp*	*Y2* *[mg N g^{-1}BO500]*	*Xt*	*Xp*	*Y3* *[mg N g^{-1}BO500]*
1	1	205	-1	1	97	1	-1	343
0	0	91	1	-1	319	-1	-1	112
-1	-1	88	-1	1	96	-1	-1	118
1	1	208	1	1	289	0	0	287
-1	1	52	0	0	183	-1	1	112
1	-1	249	0	0	184	0	0	282
-1	-1	83	-1	-1	93	1	1	356
1	-1	254	-1	-1	84	-1	1	116
-1	1	51	1	1	288	1	1	359
0	0	90	1	-1	319	1	-1	347

The tendency of the factorial design for BO500 showed similar results to those obtained for BO300, where temperature is the most important factor affecting the nitrogen impregnation, followed by the particle size.

Data analysis of B1 (1:0.5:5) for BO500 as well as for BO300, suggests that in the case of high temperatures (150 °C) and small particle size (<500 μm) the nitrogen impregnation capacity of BO500 is high (253 mg N g^{-1}BO500). Moreover, increasing the nitrogen proportion the nitrogen impregnation capacity increases significantly, being 319 and 347 mg N g^{-1} BO500 for B2 and B3, respectively.

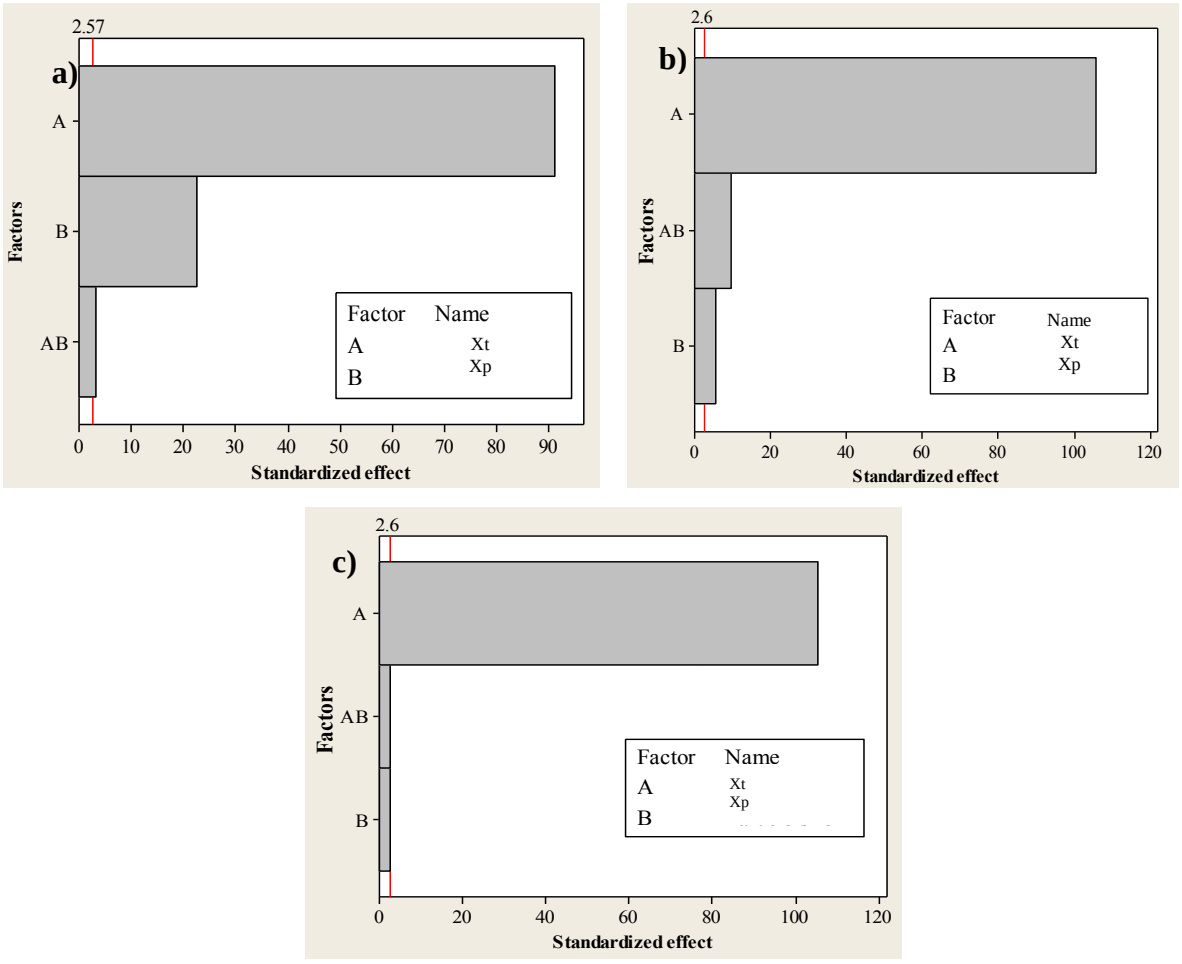

Figure 8.5 Effect of variable Xt (temperature) and Xp (size particle) on the nitrogen impregnation a) B1 (1:0.5:5), b) B2 (1:1:5) and c) B3 (1:2:5), for BO500.

Besides it was determined by means of ANOVA analysis that in B1 the effect of the studied variables is significant in the response (Figure 8.5a) for both temperature (Xt) and particle size (Xp), being more significant the effect of temperature. Additionally, it was observed in D1 that by using smaller particle (<500 μm), the nitrogen impregnation increased. In addition, the interaction between factors (XtXp) is significant in the response.

On the other hand, the results of B2 (1:1:5) showed the same tendency as B1 (Figure 8.5b) for a significance level $\alpha = 0.05$, with a greater influence of temperature.

In the case of B3 (1:2:5) the experimental results indicate that only the temperature is relevant in the nitrogen impregnation (Figure 8.5c).

Finally, the effect of each factor on the response of the experimental blocks is shown in Table 8.8 for BO300 and BO500.

Table 8.8 Effect of factors on the response to the experimental blocks B1, B2 and B3 for BO300 and BO500.

Biochar	**Effect**	**B1 (1:0.5:5)**	**B2 (1:1:5)**	**B3 (1:2:5)**
BO300	*Xt*	161.17	201.81	213.176
	Xp	-37.01	-9.24	-15.733
	XtXp	-2.06	-20.96	12.981
BO500	*Xt*	160.27	211.05	236.763
	Xp	-39.51	-11.21	5.628
	XtXp	-5.58	-19.16	6.235

The obtained results showed that temperature is a key factor in nitrogen impregnation for both BO300 and BO500. However, studies carried out by Drage et al. (2007) showed that the nitrogen content in charcoals decreases significantly with increase of reaction temperature.

This practice of enriching a carbonaceous material with nitrogen compounds has been described in the formulation of charcoal-based slow release nitrogen fertilizer using chemical reaction between a nitrogen source and lignocellulosic matrices (Coca et al., 1984; Kim et al., 1981; Ramírez-Cano et al., 2001). However, this method has been used nowadays for obtain the activated carbons through nitrogen group introduction (Mangun et al. 2001; Zhuravsky et al. 2012)

In this sense, Bimer et al. (1998) using the same nitrogen source and similar condition, but at a higher temperature (300 °C), reported values between 13 and 16.7% in the total nitrogen content in the charcoal. Adib et al. (2000) also obtained nitrogen contents of 7.5 and 2.4% in the charcoal using urea with as source of nitrogen at 450 °C and 950 °C, respectively.

The studies suggest that the chemistry of the reaction of biochar with urea is very complex not only because of the heterogeneity of biochar structure but also because of the variety of

N-reagents that can arise from urea and can react independently with biochar (Bimer et al. 1998). On the other hand, theses interaction between biochar and N-reagents will depend of temperature of impregnation (Pietrzak, 2009).

On the other hand, the designs which consider greater amounts of nitrogen exhibit greater nitrogen impregnation capacity of biochar. Despite a twofold increasing of the amount of nitrogen, the ability of impregnation was not significantly increased.

Considering the nitrogen content in the biochar and the amounts of nitrogen evaluated in B1, B2 and B3, it is advisable to work with the proportion 1:0.5:5 (biochar: nitrogen: deionized water). This proportion ensures lower losses of nitrogen during the impregnation process. As for the particle size, it is recommended to use sizes <500 μm, since they presented a higher impregnation capacity.

The BO300 and BO500 did not present significant differences in the nitrogen impregnation capacity in B1: 246 and 248 mg N g^{-1}, respectively. Therefore, we recommend using BO300, because pyrolysis at low temperature guarantees a better performance in the biochar.

Appendix 2

8.2 Methodology

8.2.1 Evaluation of polymers for CRF formulation

The polymers used in the preparation of encapsulates were cellulose acetate (AC), ethyl cellulose (EC) and sodium alginate (SA).

The polymer solutions were prepared by dissolution of the solid polymer in an adequate solvent. Acetone was used for CA and EC, whereas distilled water was used for SA.

Formamide (F) was used as modifying agent in the preparation of CA and EC solutions (Jarosiewicz and Tomaszewska, 2003). The densities (gravimetric) and viscosities (digital viscometer VIS-79) of the resulting polymer solutions were measured at room temperature in the case of density and at 20 °C for the viscosities. Table 8.9 shows the compositions of polymer solutions applied for the formation of each formulation.

Encapsulates were formed from the mixture of polymeric solution/biochar by phase inversion technique, for the case of AC and EC. The technique used for the formulation of encapsulates of cross linking technique was used for SA.

Biochar in different proportions was gradually added to polymer solutions. Then for AC and EC, the mixture between polymer and biochar was dropped into the precipitation bath (distilled water), where the solvent-nonsolvent exchange proceeded, resulting in the formation of encapsulates (gelation process). The temperature of the precipitation bath was 25°C. The beads were left in the distilled water for 5 min to ensure complete gelling, then separated and dried.

Afterwards, the mix between polymer and biochar in different proportion was then dropped into the precipitation bath of $CaCl_2$ for SA. On the other hand, the effect of the different concentrations of calcium chloride in SA gelation was studied. The evaluated concentrations of $CaCl_2$ were 0.1, 0.25 and 0.5 M. The spherical beads were left in the $CaCl_2$ solution for 5 min to ensure complete gelling and then separated from the solution and dried.

Table 8.9 Composition of the polymer solution for encapsulates formation.

Polymer	***Polymer concentration (wt%)***	***Solvent concentration (wt%)***	***Modifying agent concentration (wt%)***
Cellulose acetate	10	90	0
	15	85	0
	20	80	0
	10	85	5
	15	80	5
	20	75	5
	10	80	10
	15	75	10
	20	70	10
	10	75	15
	15	70	15
	20	65	15
Ethyl cellulose	10	90	0
	15	85	0
	20	80	0
	10	85	5
	15	80	5
	20	75	5
	10	80	10
	15	75	10
	20	70	10
	10	75	15
	15	70	15
	20	65	15
	Polymer concentration (%)		
Sodium alginate	1	2.5	5

8. 3 Results and discussion

8.3.1 *Evaluation of polymers for CRF formulation*

Density and viscosity are important factors to consider in the encapsulation of biochar impregnated with nitrogen. These parameters affect the preparation of encapsulates and the properties of CRF. Figure 8.6 shows the density of different polymeric solutions. Of the polymers used in this study, SA has the highest density, followed by AC and EC. However, this parameter is proportional to the concentration of polymers. For AC and EC, the

densities are affected by the addition of formamide (F); the higher the concentration of F, the higher the density shown by these polymers. The same trend is reflected in the viscosity for CA and EC (Figure 8.7a). It is worth pointing out that SA presents high viscosity (Figure 8.7b), this can be a drawback for the preparation of encapsulate.

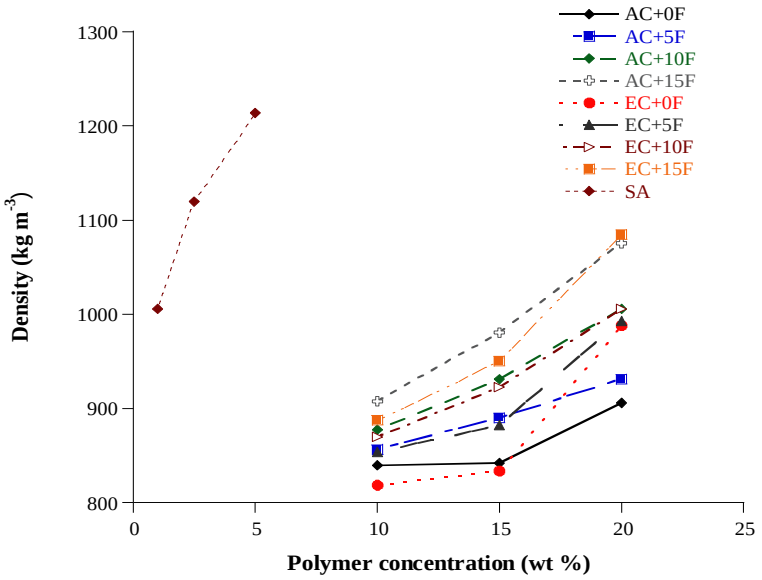

Figure 8.6 Densities of polymer solutions measured at room temperature.

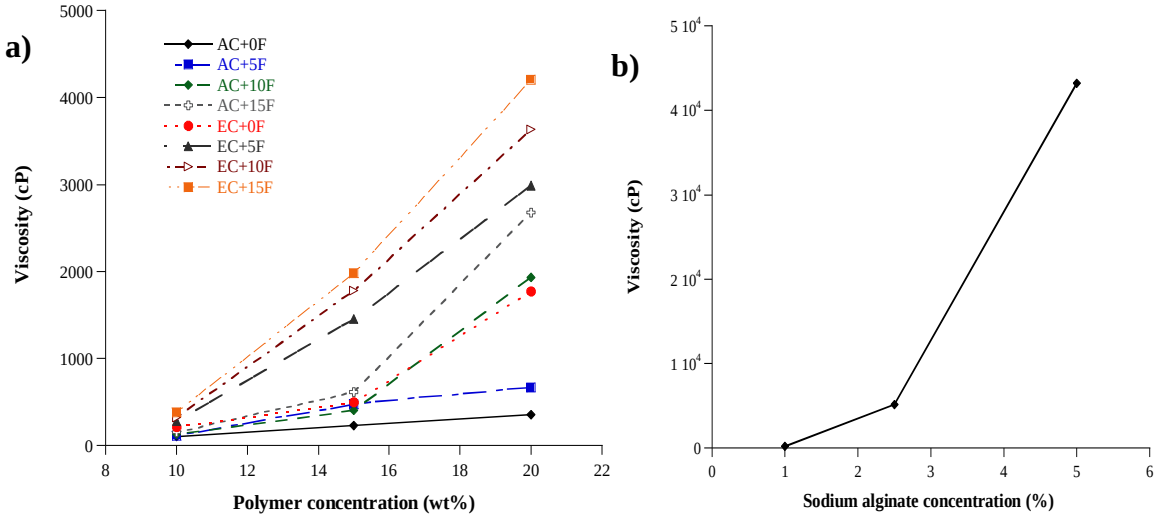

Figure 8.7 Viscosities of polymer solutions measured at 20°C, a) cellulose acetate and ethyl cellulose at different concentration of formamide and b) sodium alginate.

Once these parameters obtained, mixing of biochar and the polymeric solutions was carried out and evaluated for different proportions (Table 8.10).

The resulting mixture was introduced in a syringe with openings of approximately 5 mm diameter and, by means of dropping, the sample was precipitated in different solutions, depending on the studied polymer.

For CA and EC distilled water was used as precipitation bath. In the case of SA $CaCla_2$ was used as precipitation bath.

In order to evaluate the effect of the $CaCl_2$ concentrations used as precipitation bath for gelation of SA, three different concentrations: 0.1, 0.25 and 0.5 M were tested.

Table 8.10 Composition of biochar and polymer solution for encapsulation formation.

Polymer	Polymer concentration (wt%)	Solvent concentration (wt%)	Modifying agent concentration (wt%)	Proportion biochar/polymer (ww^{-1})			Capacity to form spheres					
Cellulose acetate (AC)	10	90	0	1/2	1/3		No		No			
	15	85	0	1/2	1/3		No		No			
	20	80	0	1/3	1/5		No		No			
	10	85	5	1/2	1/3	1/6	No		No		No	
	15	80	5	1/3	1/5		No		No			
	20	75	5	1/5			No					
	10	80	10	1/2	1/3		Yes		Yes			
	15	75	10	1/3	1/6		No		Yes			
	20	70	10	1/3	1/6		No		No			
	10	75	15	1/3	1/6		No		Yes			
	15	70	15	1/3	1/6		No		No			
	20	65	15	1/8			No					
Ethyl cellulose (EC)	10	90	0	1/2	1/3		No		No			
	15	85	0	1/3	1/5		No		No			
	20	80	0	1/5			No					
	10	85	5	1/3	1/5		No		No			
	15	80	5	1/5	1/6		No		No			
	20	75	5	1/6	1/8		No		No			
	10	80	10	1/2	1/4		No		Yes			
	15	75	10	1/3	1/6		No		No			
	20	70	10	1/5			No					
	10	75	15	1/4	1/6		Yes		Yes			
	15	70	15	1/3	1/6		No		No			
	20	65	15	1/5			No					
							$CaCl_2$ 0.1 M		$CaCl_2$ 0.25 M		$CaCl_2$ 0.5 M	
Sodium alginate (SA)	1	-	-	1/1	1/3		Yes	Yes	Yes	Yes	Yes	Yes
	2.5	-	-	1/2	1/6		No	Yes	No	Yes	No	Yes
	5	-	-	1/6	1/8		No	No	No	No	No	No

In Table 8.10 it can be observed that the proportion of biochar/polymer and polymer concentration affect the formation of encapsulate. On the other hand, the addition of formamide (F) in AC and EC is relevant to achieve spherical shape of encapsulates. As the concentration of the modifying agent increases, the spheres show a more regular shape. However, due to the low density of AC and EC in comparison with of SA, the form of encapsulate is a flattened ellipsoid.

The polymers that showed a higher viscosity for example AC 20%+5F, AC 20%+10F, AC 20%+15F, EC 20%+5F, EC 20%+10F, EC 20%+15F and SA5% are not suitable for the formulation of encapsulate, because they have a greater resistance to dripping.

The best results were obtained for AC 10%+10F, AC 15%+10F, AC 10%+15F, EC 10%+10F, EC 10%+15F and SA 1 and 2.5% (Figure 8.8). However, the shape that shows encapsulate, especially when using AC and EC, is flattened ellipsoid.

In addition, it was shown that the concentration of $CaCl_2$ is not relevant in SA gelation, for the formulation of encapsulate.

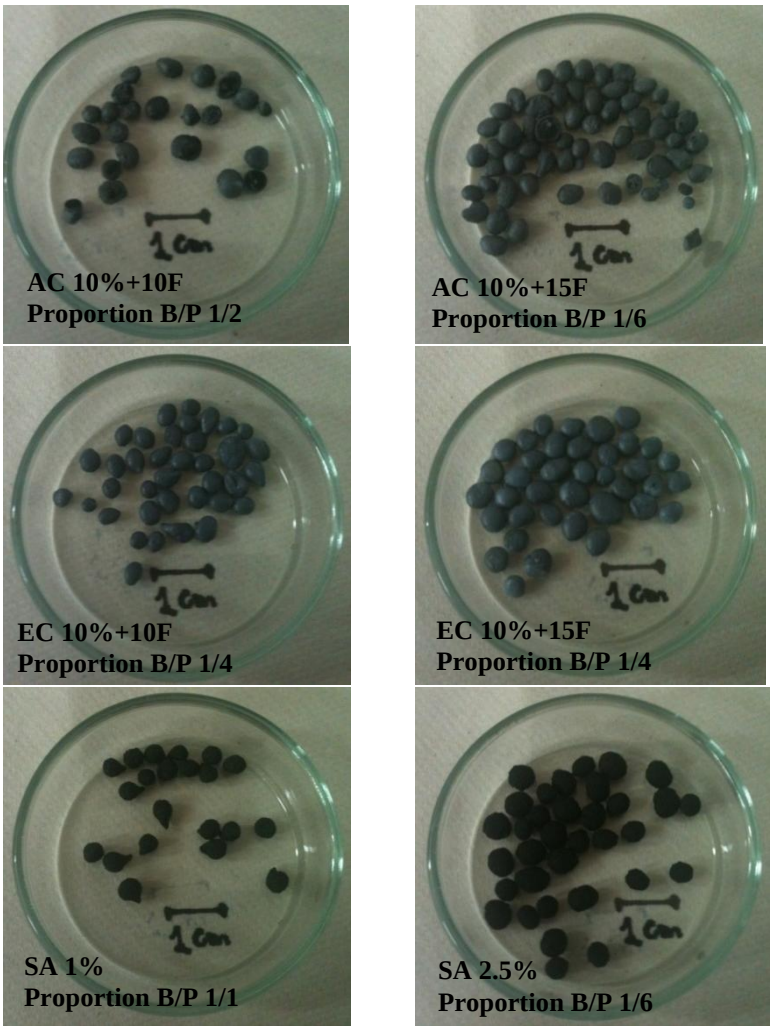

Figure 8.8 Encapsulate developed using different polymers.

In recent years, much attention has been paid to the use of polymeric matrices in the development of controlled-release technology in the areas of fertilizer herbicides and pesticides (Desai et al., 2006).

In the area of controlled release fertilizer (CRF), the effectiveness of a specific controlled-release polymeric system is determined in part by its specific chemical and physical properties, its biodegradation rate, and the used fertilizer source (Mikkelsen, 1994).

Within these physical characteristics, density and viscosity of polymer solutions affect the encapsulation preparation, especially when using phase inversion technique in the formulation.

Solution viscosities that are too low or too high cause granules of encapsulate to be incomplete or damaged. These defects affect the properties of the fertilizer, and the active components were dissolved very quickly and released (Jarosiewicz and Tomaszewska, 2003).

As expected in all the evaluated polymeric solutions, the densities and viscosities increased with a rises in polymer concentration. However, the viscosities of SA solutions were much higher than those of the other polymers.

In the case of AC and EC, the addition of formamide (F) as a modifier agent is essential for the preparation of encapsulate by the phase inversion technique. Without the addition of this compound, the result is the dissociation of the mixture, forming an asymmetric colloidal membrane. This tendency to form spherical encapsulates has been described by Kesting and Maneffe (1968). The presence of formamide increases the solvent loss (acetone) until the miscibility of the polymeric solution in water is no longer possible. As a result encapsulate is formed. On the other hand, Jarosiewicz and Tomaszewska (2003) report that the use of formamide added to the polymer solution also influences the coating porosity. Higher amounts of formamide increased the coating porosity.

It was already mentioned that F increases the viscosity of polymer solutions, in this sense, the viscosity is a critical factor for the formulation of encapsulates. In this study, the polymers that showed a higher viscosity for example AC 20%+5F, AC 20%+10F, AC 20%+15F, EC 20%+5F, EC 20%+10F, EC 20%+15F and SA5%, are not suitable for the formulation of encapsulate, because they have a greater resistance to dripping.

The use of polymers as coating material for conventional fertilizers has been reported recently. However, the use polymers for the formulation of encapsulate using adsorbent materials such as biochar has not been described. For this reason, the densities of the polymer solutions are important since the mixture between them generates a greater resistance to dripping.

The best results were obtained for the encapsulation using AC 10%+10F, AC 15%+10F, AC 10%+15F, EC 10%+10F, EC 10%+15F and SA 1 and 2.5%. However, the shapes of the resulting encapsulate, especially when using AC and EC are flattened ellipsoids. Probably, this shape is due to the low density that presents the polymeric solution of AC and EC in comparison with SA.

Due to their lower density, the drops of polymeric solutions form encapsulate that float in the precipitation bath. This causes that the resulting encapsulates present a flattened ellipsoid shape, rather than spherical.

It was also shown that the concentration of $CaCl_2$ is not relevant in SA gelation, for the formulation of encapsulate. However, a disadvantage to use $CaCl_2$ as precipitation bath is the need to change the $CaCl_2$ solution at certain intervals. The literature does not describe any optimal concentration of $CaCl_2$ for gelation. It has been generally reported that for concentrations of SA between 1-4%, concentrations of $CaCl_2$ ranging from 1 to 3% (w/v) are used (Almeida and Almeida, 2004).

The beads produced by dropping a SA/biochar into a $CaCl_2$ solution showed a more regular spherical form, unlike the case of AC and EC. On the other hand, the use of AC and EC presents the disadvantage of using acetone as a solvent, and also formamide, which is a toxic compound.

Appendix 3

Table 10. Leaching test values of European Norm EN 1245-2 for biochar samples (mg kg^{-1}).

Compound	Inert	Non-hazardous	Hazardous	BO300	BO500	BP300	BP500
	(mg kg^{-1})						
As	0.5	2	25	<0.1	<0.1	<0.1	<0.1
Ba	20	100	300	0.1	<0.1	0.20	0.54
Cd	0.04	1	5	<0.1	<0.1	<0.1	<0.1
Cr	0.5	10	70	<0.1	<0.1	0.89	1.48
Cu	2	50	100	<0.2	<0.1	<0.1	0.52
Hg	0.01	0.2	2	<0.01	<0.01	<0.01	<0.01
Mo	0.5	10	30	<0.1	<0.1	<0.1	0.36
Ni	0.4	10	40	<0.1	<0.1	<0.1	0.11
Pb	0.5	10	50	<0.1	<0.1	<0.1	<0.1
Sb	0.06	0.7	5	<0.1	<0.1	<0.1	0.2
Se	0.1	0.5	7	<0.1	<0.1	<0.1	<0.1
Zn	4	50	200	<0.1	0.33	0.44	4.38

Table 8.11 Leachates collected during assays where, U: urea, ESN: commercial N-CRF, BU: Biochar impregnated with non-encapsulated, SA 1: Biochar+urea+SA 1% (R:1/1), SA 2: Biochar+urea+SA 2.5% (R:1/5), EC 1: Biochar+urea+EC 10% 10% F (R:1/4), EC 2: Biochar+urea+EC 10% 15%F (R:1/4), AC 1: Biochar+urea+AC 10% 10% F (R:1/2) and AC 2: Biochar+urea+AC 10% 15% F (R:1/6).

	Leachates	**Formulations**									
	(mL)	**C**	**U**	**ESN**	**BU**	**SA 1**	**SA 2**	**EC 1**	**EC 2**	**AC 1**	**AC 2**
With plant	*L1*	65±4	89±10	65±11	67±9	69±3	47±9	88±10	89±8	89±10	93±5
	L2	108±7	115±11	100±6	101±9	114±12	105±4	103±12	106±12	121±9	122±16
	L3	116±15	128±13	113±9	122±9	99±10	107±4	118±12	117±11	119±13	119±9
	L4	130±14	103±13	127±8	55±6	90±10	76±6	94±11	85±16	87±14	79±9
	L5	141±12	120±8	112±11	136±13	111±9	155±7	127±16	133±8	147±12	107±17
	L6	168±15	130±0	105±10	137±14	132±15	143±15	137±15	140±7	137±14	147±14
	L7	135±6	129±11	163±11	163±15	136±11	130±10	153±10	142±14	144±11	135±6
	L8	120±15	130±17	150±10	110±13	143±12	150±0	147±16	123±12	160±10	147±16
	L9	140±10	100±8	82±3	113±5	113±10	133±15	133±18	118±15	112±12	122±11
	L10	153±11	140±10	142±16	133±15	160±18	133±17	170±10	137±17	137±13	143±12
Without plant	*L1*	94±7	109±5	103±4	108±7	102±3	95±6	90±4	99±3	80±4	103±10
	L2	143±8	121±10	142±13	137±14	132±9	129±13	117±13	114±11	123±17	120±12
	L3	121±12	133±9	120±6	112±11	124±11	113±16	115±15	130±12	116±9	118±8
	L4	125±8	119±6	135±12	118±13	127±4	118±10	117±11	123±15	137±13	134±4
	L5	91±6	89±8	74±9	82±7	93±5	83±4	71±2	87±11	104±12	112±9
	L6	152±4	160±9	161±13	160±0	152±7	162±11	159±4	161±14	159±15	167±3
	L7	166±12	100±10	142±9	157±15	137±8	149±16	120±7	140±17	153±6	141±12
	L8	118±3	110±0	101±9	99±10	100±8	100±2	126±12	113±7	120±0	130±8
	L9	110±10	128±5	133±14	116±6	109±10	113±17	119±18	123±12	120±0	126±15
	L10	136±5	140±5	126±7	129±10	135±16	136±5	130±10	140±10	141±13	145±12

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