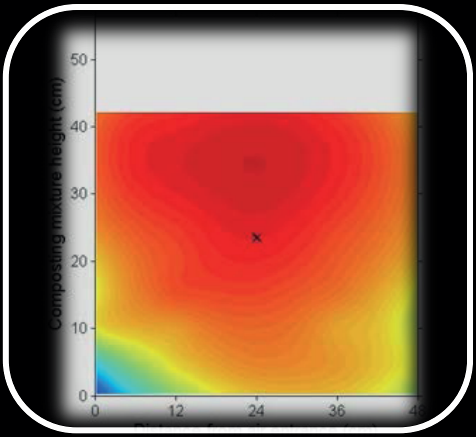

Micaela Alexandra da Rocha Soares

EGGSHELL VALORISATION BY CO-COMPOSTING PROCESS FOR ENVIRONMENTAL APPLICATIONS

PhD thesis in Chemical Engineering supervised by
Prof. Margarida Maria João de Quina and Prof. Rosa Maria de Oliveira Quinta Ferreira
and submitted to the Department of Chemical Engineering,
Faculty of Science and Technology of the University of Coimbra

November 2015

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UNIVERSIDADE DE COIMBRA

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ABSTRACT

Nowadays, the European guidelines boost the concept of “circular economy” where the focus is to reuse, repair, refurbish and recycle existing materials and products. As a result, the waste can be turned into a resource with benefits to the economy and environment. In this scope, composting is a waste management option that can promote the retrieval of waste organic matter and nutrients, which can be tailored to obtain a product for suppressing soil deficiencies.

This work aimed to evaluate the feasibility of co-composting industrial eggshell waste (ES) with other agro wastes (potato peel, grass clippings and rice husks), to obtain an added value product. For attaining this key motivating factor, co-composting was assessed at three levels: i) process performance, ii) end-product quality and iii) added-value of the final product (compost).

In lab-scale self-heating reactors with forced aeration, several mixtures were composted. Firstly, a starting composting mixture of organic rich wastes was selected based upon its biological activity. Then, increasing quantities of industrial eggshell (inorganic material) were added up to 60% (w/w, wet basis). The influence of N-rich sources was also investigated. In these experiments, temperature and oxygen uptake rate were the main process variables monitored and conventional physical, chemical and stability parameters were also evaluated. Principal components analysis was used to unravel the main relationships structuring the variability associated to the composting experiments. Finally, added-value of the composting end-products obtained was appraised, in terms of sorption capacity and immobilisation of metals in soil. In this ambit, the fractionation of metals was determined.

Globally, results showed that co-composting of industrial eggshell waste is feasible to fulfil the pathogen-killing criteria (70ºC, for 1 hour), when an N-rich source was present. At higher level of ES (60% w/w, wet basis) pathogen-killing temperatures were atained, but aerobic biological activity was hindered by some physical properties of the mixture, namely due to high density and low water holding capacity. Eggshell composting product (CES) was stable, non-phytotoxic and can be an effective liming material with intrinsic properties for *in situ* remediation of soil contaminated with lead and zinc.

RESUMO

Hoje em dia, as diretrizes europeias fomentam o conceito de "economia circular", onde o foco é a reutilização, reparação, recuperação e reciclagem de materiais e produtos em fim de vida. Como resultado, os resíduos podem ser transformados em recursos com benefícios para a economia e meio ambiente. Neste âmbito, a compostagem é uma opção de gestão de resíduos que pode promover a recuperação de matéria orgânica e nutrientes através da obtenção de um produto adequado para suprimir determinadas deficiências do solo.

Este trabalho teve como objetivo avaliar a aplicabilidade da co-compostagem de resíduos industriais de casca de ovo (RCO) com outros agro-resíduos (casca de batata, aparas de relva e casca de arroz), de forma a obter um produto de valor acrescentado para remediação ambiental. Para o efeito, o processo de co-compostagem foi avaliado a três níveis: i) desempenho operacional, ii) qualidade do produto final e iii) valor acrescentado do produto final (composto) para aplicação ambiental.

Em reatores laboratoriais de auto-aquecimento e com arejamento forçado foram testadas misturas com várias composições, de forma a selecionar uma mistura de base tendo como critério a atividade biológica. Posteriormente, quantidades crescentes de casca de ovo industrial (material inorgânico) foram adicionadas até um máximo de 60% (p/p, base húmida). Adicionalmente foi também investigada a influência de fontes ricas em azoto no desenvolvimento do processo. Nestas experiências laboratoriais, a temperatura e a taxa de consumo de oxigénio foram os principais variáveis monitorizadas, tendo sido avaliados diversos parâmetros convencionais de natureza física e química, bem como a estabilidade biológica.

A análise de componentes principais (PCA) foi utilizada para mostrar as principais relações que estruturam a variabilidade associada aos testes de compostagem. O valor acrescentado dos produtos finais de compostagem obtidos foi avaliado, em termos de capacidade de sorção e potencial de imobilização de metais no solo. Neste âmbito, foram realizados testes descontínuos de sorção e o fracionamento de metais no solo.

Globalmente, os resultados evidenciaram que a co-compostagem de resíduos industriais de casca de ovo é viável para cumprir os critérios higienização (70ºC, durante 1 h), quando uma fonte de rica em N está presente na mistura de base. No nível mais alto de incorporação de RCO (60% p/p, base húmida), o critério de higienização foi atingido, mas a atividade biológica aeróbia foi dificultada por algumas propriedades físicas da mistura, nomeadamente devido à elevada densidade *bulk* e à baixa capacidade de retenção de água. Os produtos finais de compostagem ricos em RCO são estáveis, não-fitotóxicos e podem ser utilizados como material de calagem

com propriedades intrínsecas para remediação *in situ* de solos contaminados com chumbo e de zinco.

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ACRONYMS

ABP	Animal by product
ANC	Acid neutralisation capacity
B	Biodegradable fraction of volatile solids
BD	Bulk density
BET	Brunauer- Emmett - Teller
BH	Biological heat
C:N	Carbon to nitrogen ratio
CCR	Convective/conductive/radiative heat losses
CEC	Cationic exchange capacity
CES	Compost derived from eggshell co-composting
CFA	Coal fly ashes
CWES	Mature compost without eggshell
D	Diameter
DEFRA	Department for environment food and rural affair
DM	Dry matter
DP	Particle diameter
EC	European comission
EDX	Energy dispensive X ray analysis
ES	Eggshell
EU	European union
FAAS	Flame atomic absorption spectrometry
FAO	Food and Agriculture Organization of the United Nations
FAS	Free air space
FTIR	Fourier transform infrared
GC	Grass clippings
GI	Germination index
L/S	Liquid to solid ratio
MC	Moisture content
MEDA	Multivariate exploratory data analysis
OM	Organic matter
OVR	Oxygen uptake rate
PCA	Principal components analysis
PD	Particle density
PP	Potato peel
PVC	Polyvinyl chloride
PZC	poin of zero charge
RAC	Risk assessment code
RH	Rice husks
RMSE	Relative mean square error
RRG	Relative root growth
RSG	Relative seed growth
SA	Surface area
SD	Sawdust
SEM	Scanning electron microscopy
SHR	Self heating reactor
VS	Volatile solids
WAC	Water absorption capacity
WHC	Water holding capacity

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Part A. Thesis Scope and Outline

The first part of this thesis presents the framework of composting as a valorisation technology for the management of eggshell, which is the main by-product from the egg processing industry.

In the first chapter, eggshell production, properties and applications are revised, along with the legal framework that requires eggshell sanitisation prior to its use. In this scope, composting process is pointed out as a sound solution to promote eggshell sanitisation, thus obtaining an eggshell-rich compost with environmental applications. This issue is the motivation and scope of the doctoral work. The outline of the thesis closes the first chapter.

The second chapter summarises the background work collected along the development of this study enabling to review the main features associated to the composting process, compost quality and utilisation.

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1. Introduction

The first chapter of this thesis addresses the challenges that the egg[1] processing industry faces towards promoting the adequate management of eggshell, which is a product-specific waste from egg breaking operations, from an environmental and economical point of view.

In this ambit, possible valorisation options are addressed, taking into consideration the properties of eggshell waste and the legal constraints associated to its use and/or disposal. Among these valorisation alternatives, composting is highlighted encompassing the motivation and scope of this work.

The information presented in this chapter was partially published upon the following publication:

Soares, M.A.R., Quina M.J., Quinta-Ferreira R., 2014. An overview of eggshell waste potential for sorptive and catalytic processes, presented at CHEMPOR’2014 10-12th September, in Book of Abstracts, Pg 9-49 to 9-51, Porto, Portugal (*oral communication*)**.**

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[1] Eggs from other birds (geese, ducks, plovers, sea- gulls, quails) are of lesser significance in egg market, therefore the term “egg”, without a prefix is related to chicken eggs and is so considered in this thesis.

1.1. The egg processing sector and eggshell production

Hen eggs constitute one of the most important food resources in the framework of world-wide feeding. They are an important source of essential nutrients to human diet providing proteins, fat-soluble vitamins (A, D, E and K) and trace-minerals like iron and zinc (Pirvutoiu and Popescu, 2012; Roberts et al., 2005). According to FAO (2012), in 2009 global consumption of eggs rose to 8.9 kg/capita/year (167 eggs[2] consumed per person on an annual basis) which corresponds to an increase of 41% in comparison to the consumption registered in 1990. As a result of such rise, the egg sector has rapidly expanded, and nowadays the worldwide egg production reaches 6.5 $\times 10^7$ ton/year, representing 185% of the egg produced in 1990 (FAO, 2012).

Currently the egg sector is segmented in three different levels (production, producer market and consumer market) as indicated in Figure 1.1. Most eggs reach the consumer market as shell eggs (flux ❶ in Figure 1.1). However, during the past few years the producer market has grew significantly, as reflect of the increasing demand for liquid, frozen, concentrated or dried powder eggs (flux ❷ in Figure 1.1).

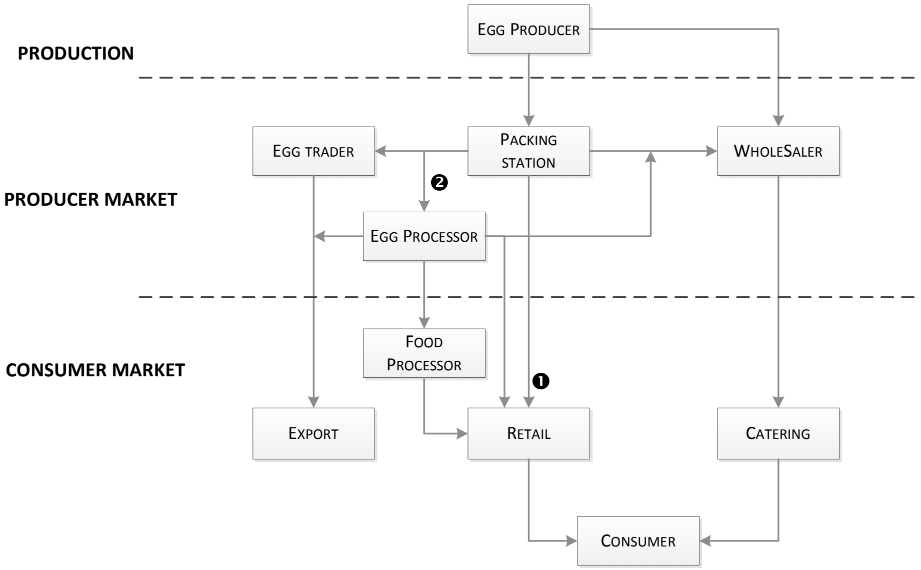

Figure 1.1: General structure of the egg sector.
Adapted from CICEI (n.d.).

These products present some advantages in comparison with shell eggs, like higher product shelf-life, minimal requirements for storage and transportation (Oliveira et al., 2012). They are intended to retailers, catering activities or further used by the food industry, as ingredients, for

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[2] Using a conversion rate of 54 grams per egg (FAO, 2012).

manufacturing processed products with added value, like cooked eggs, bakery products, egg mayonnaise, energetic drinks, etc. (Roberts et al., 2005).

The first step of processing consists in receiving carton packages from the distributors (packing station of Figure 1.1) and storing them in a cool humid atmosphere for preservation until utilisation. After unpacking, visual inspection is performed to detect leaking broken shells and eggs of poor interior quality (e.g. developed embryos, etc.). Washing is performed with detergents and defoaming agents to remove egg solids and adhering foreign material from shell surface and then washed eggs are rinsed with a sanitising agent (Cotterill and McBee, 1995). Egg breaking and separation of shell from liquid content is performed by automated machines and further processing phases of the liquid egg depend on the desired final product, as detailed in Figure 1.2.

In terms of waste generation in egg breaking operations, eggshell (ES) obtained from processing the raw material (shell eggs) is identified as the main process by-product, regardless of the egg product obtained. In the literature, some data are available to quantify the specific waste index of ES. Russ and Pittroff (2004) for example indicate that ES represents 3 to 12% of the egg mass product obtained, depending on the egg shell properties (size and shell thickness). In addition, Jewell et al. (1975) have monitored waste and wastewater production in an American egg breaking industry during three days, and again concluded that ES may represent 13% of edible egg product output.

Nevertheless, nowadays annual industrial production is estimated because data of ES are scarce. Table 1.1 presents information about ES production in Canada, China, United States of America, Portugal and some European Union member states where the largest egg processors are located, namely France, Germany, Italy and Spain (Agra CEAS Consulting Ltd, 2008). In fact, significant amounts of eggshell waste are potentially derived from egg breaking industries. Particularly in Europe, the EU countries considered in Table 1.1 contribute with 34% to the total value estimated for eggshell production. Portugal is the less expressive contributor due to its lower egg production capacity.

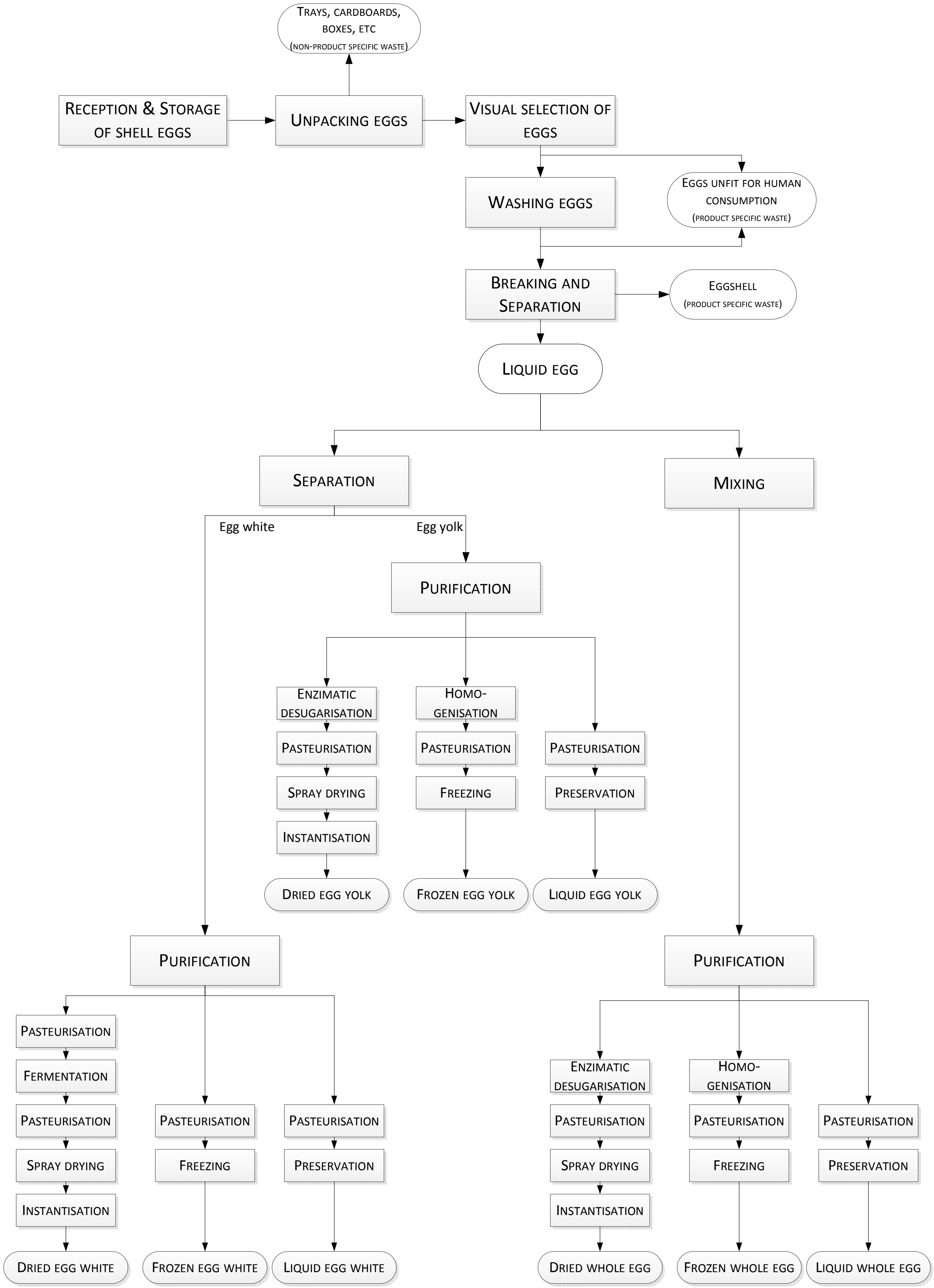

Figure 1.2: Common routes for egg processing.
Adapted from Belitz, Grosch, & Schieberle (2009) and EEPA (2011).

Table 1.1: Estimated annual ES production by the hen egg processing industry, in 2011, for some countries.

Country	Egg production (thousand tonnes)*	Egg for processing (thousand tonnes)**	Estimated eggshell waste (thousand tonnes)***
Canada	436.8	131.1	14.4
China	24231.6	484.6	53.3
United States of America	5419.4	1083.9	119.2
France	839.5	251.9	27.7
Germany	777.1	233.1	25.6
Italy	736.8	221.0	24.3
Portugal	122.8	29.5	3.2
Spain	830.0	249.0	27.4
United Kingdom	662.0	102.1**1)	11.2
All European countries	10638.6	3191.6	351.1

* data according to FAO (2012).

** Egg for processing= Egg production x Processing factor; processing factor equals 0.3 for Canada (Global Poultry Trends, 2011a) and France, Germany, Italy, Spain (Agra CEAS Consulting Ltd, 2008); 0.24 for Portugal (Agra CEAS Consulting Ltd, 2004); 0.2 for USA (Global Poultry Trends, 2011a) and 0.02 for China (Global Poultry Trends, 2011b).

**1) data from UK egg processing values (DEFRA - Department for environment food and rural affairs, 2013).

*** Eggshell waste=Egg for processing x % mass shell in egg; % mass shell in egg=11% (Meski et al., 2011; Oliveira et al., 2013).

The development of an adequate management strategy for this by-product has been considered a challenge for the food industry in what concerns environmental protection, not only due to the large amounts generated but also to its high potential for microbial proliferation or growth of pathogens (Russ and Schnappinger, 2007). In addition, costs related to the growing eggshell disposal are of great concern. Therefore the development of value-added applications for ES would be environmentally and financially beneficial.

1.2. Eggshell properties

Eggs are mainly formed by a central yolk (yellow portion) enclosed by the egg white (albumen) which are surrounded by the shell that is a calcified organic matrix, acting like a natural package of the egg content, Figure 1.3 a). The shell is an essential component of the egg structure and plays a crucial role in the reproduction mechanism of chickens. Its main function is essentially protective (Nys et al., 2004; Roberts et al., 2005): i) to safeguard the egg content from exterior physical and microbial aggressions; ii) to regulate water and gases exchange during the embryo formation; iii) to make available enough calcium for the embryo development. Indeed, eggshell is a biomineralised composite structure of calcite crystals embedded in an organic framework of protein fibres, that represents 11% of the total weight of the egg (Meski et al., 2011; Oliveira et al., 2013). It includes four different layers, as indicated in Figure 1.3 b).

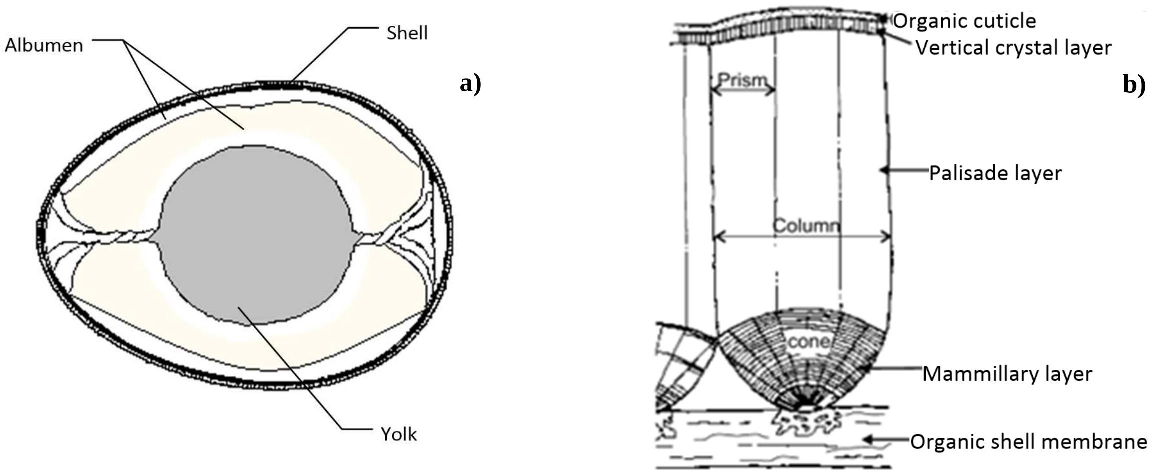

Figure 1.3: Schematic representation of cross section a) egg and b) eggshell.
Figure 1.3 a) adapted from Roberts et al. (2005) and Figure 1.3 b) reprinted from Dauphin et al. (2006) with permission of Springer.

The most internal layer is the organic shell membrane that consists of two parts: the inner membrane that is non-calcified and contacts with the albumen, and a thick outer layer that is attached to the calcified shell. These membranes are constituted by collagen proteins fibres that confer them semi-permeable properties (Lammie et al., 2005; Nys et al., 2004; Rose and Hincke, 2009).

The remaining layers (mammillary, palisade and vertical crystal) form the mineralised shell. The mammillary layer is composed by regular matrix of cones where the fibres of the outer membrane are adhered to and possesses micro crystals of calcite that are readily dissolved whenever needed to mobilise calcium for embryo development (Nys et al., 2004; Rose and Hincke, 2009).

The most extensive layer is the palisade, where calcite crystals grow, in columns and perpendicularly aligned with the surface, from the mammillary zone until the vertical crystal layer (Lammie et al., 2005; Rose and Hincke, 2009). The vertical crystal layer has a strong structure of large crystals and thin inter-crystalline organic layer that absorb external shock impacts and hinder crack propagation to the internal structure (Nys et al., 2004).

Surrounding the elongated calcite crystals of the palisade are narrow pores (7000 to 17000 per egg) with 200 to 400 nm in diameter (Guru and Dash, 2014). Those pores cross the mineralised shell and allow gas exchange (Messens et al., 2005; Solomon, 1997). A thin non calcified protein layer, called cuticle, covers the external surface of the mineralised shell (Lunge et al., 2012). This coat, partly caps the shell narrow pores which remain permeable to gases (Tsai et al., 2006) but inaccessible to microorganisms (Cabeza et al., 2011; Messens et al., 2005). The cuticle is formed by glycoproteins, polysaccharides, lipids and inorganic phosphorous and is important to regulate water exchange mechanism of egg, by repelling or retaining water (Rose and Hincke, 2009).

Some studies have been dedicated to evaluate the physical and chemical properties of eggshell, especially in the powder form. Tsai et al. (2006) studied the physical characteristics of eggshell (ES) and eggshell membranes powders. They concluded that these materials present macropores or open voids with a total volume of 0.006 $cm^3 g^{-1}$. In addition, BET surface area for both materials is in the range of 1.0±0.3 to 1.3±0.4 $m^2 g^{-1}$. Likewise, Gao and Xu (2012) and Ehrampoush et al (2011) reported BET surface area of 0.84 and 1.2 $m^2 g^{-1}$, respectively, for natural eggshell.

In terms of chemical composition, X-ray diffraction patterns of ES show diffraction peaks characteristic of calcite that represents the only crystalline species detected (Freire and Holanda, 2006; Park et al., 2007; Rivera et al., 1999; Witoon, 2011). Actually, mineralised shell presents about 92 to 96% of $CaCO_3$ (Hincke et al., 2010; Lunge et al., 2012; Ok et al., 2011; Tsai et al., 2008). Minor quantities of P_2O_5, Na_2O, SrO, SiO_2, MgO, Cl, Al_2O_3, Fe_2O and NiO have been reported by Freire and Holanda (2006) amounting to 1.5% of eggshell composition. In addition, Fourier transform infrared (FTIR) spectra of ES also confirms the presence of $CaCO_3$, showing significant bands at 1450-1500 cm^{-1}, corresponding to carbonate (C=O) asymmetric stretching vibration, 870 cm^{-1} and 710 cm^{-1} (Ahmad et al., 2012b; Tsai et al., 2006; Witoon, 2011). On the other hand, amides and amines in the eggshell membrane, that contains positively charged functional groups ($-NH_3^+$ and $–CO-N^+H_2-$) are reported by Tsai et al. (2006), due to significant peaks of spectra at 3200-3500 cm^{-1}, 1651 cm^{-1}, 1538 cm^{-1} and 1384 cm^{-1}. In fact, according to (Baláž, 2014) the eggshell membrane contains more than 62 proteins.

Organic matter content of ES is stated to fluctuate between 2% (Hincke et al., 2010) and 4-5% (Freire and Holanda, 2006; Tsai et al., 2006) and its main constituents are proteins, glycoproteins and proteoglycans (Cordeiro and Hincke, 2011).

1.3. Potential valorisation options for eggshell

Aiming to promote eggshell valorisation taking into consideration its characteristics, some technological options have been explored to obtain added-value products from ES that can be grouped in two main categories (Figure 1.4): raw materials for new products manufacture and operating supplies for sorptive and catalytic applications.

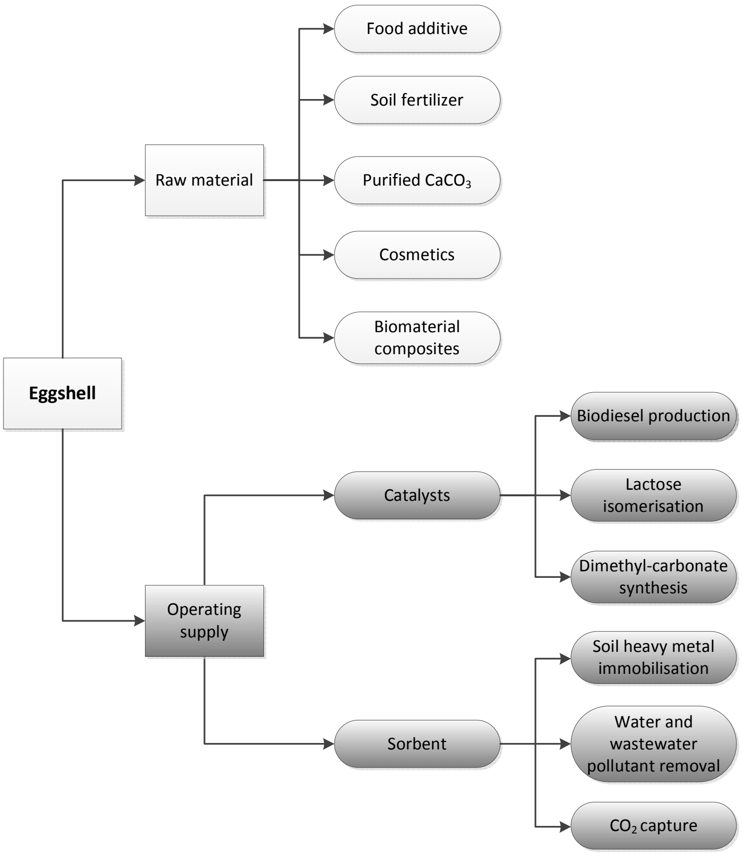

Figure 1.4: Classification of potential uses for ES**.**

1.3.1. Eggshell utilisation as raw material

The utilisation of ES as raw material is a valorisation option, applicable to food product specific wastes, which make use of the bulk properties of the residues (Kosseva, 2009; Laufenberg et al., 2003). For the egg processing industry, options for ES utilisation as raw material include production of food additive, soil fertilizer, purified calcium carbonate, cosmetic products and biomaterial composites as detailed in Figure 1.4.

Food additive for human feed

Eggshell contains high levels of calcium (about 39% w/w) and low levels of toxic elements like Pb, Al, Cd, and Hg and thus can be used in humans, as a dietary supplement. In fact, Schaafsma and co-workers (2000) reported that chicken eggshells present a large store of waste calcium that can be tailored to suppress human needs for this nutrient, especially in the cases of osteoporosis. However, calcium in the carbonate form is less bioavailable than in other forms like citrate (Hanzlik et al., 2005; Nicar and Pak, 1985). Calcium citrate obtained from ES requires a detailed chemical process that involves sequential steps of centrifugation, reaction with citric acid, filtration, washing with ethanol and drying. The overall yield of the process is about 64%, mainly due to the low reaction conversion of $CaCO_3$ to calcium citrate (67%) (Oliveira et al., 2013) .

The eggshell membrane obtained from the calcium citrate production process can be used as dietary ingredient, since it contains glycosaminoglycans and proteins (important to preserve healthy joints and connective tissues). Protein hydrolysates can be also obtained from eggshell membrane to be further used as food supplement (Oliveira et al., 2013). Toxicological tests performed to eggshell membrane suggested that it can be used for human consumption at levels up to 500 mg per day (Ruff et al., 2012).

Food additive for animal feed

ES powder has been employed as a calcium source for laying hens (Cordeiro and Hincke, 2011; Khadka and Subba, 2010). Improved egg production and feed utilisation were obtained in comparison to ground lime (Sim et al., 1983). In fact, ES has been accepted by the Association of American Feed Control Officials as a feed additive for both companion and livestock animals (Ruff et al., 2012).

Eggshell transformation in powder requires drying at 80ºC for moisture removal and microbial inactivation followed by crushing and milling (Rivera, 1999). This is a simple process with minimal losses (about 1%) (Oliveira et al., 2013) and the powder obtained contains the whole eggshell matrix (calcium and proteins from the membrane).

Soil fertilizer

Soil nutrient availability is dependent on soil solution pH. In particular, when soils acidity is high ($pH<4.5$), calcium levels are low and its bioaccessibility is impaired, affecting the normal development of plants (de Varennes, 2003).

Eggshell properties are reported as being potentially acceptable for suppressing soil needs in terms of calcium (Cordeiro and Hincke, 2011; Elwakeel and Yousif, 2010; Tsai et al., 2008; Yoo et al., 2009) due to the high concentrations of $CaCO_3$. Additionally, its carbonate content favours acidic soils pH adjustment (usually called “liming” that consists in applying alkalizing products to raise soil pH). According to Oliveira et al. (2012) ES preparation to soil incorporation follows the procedure previously presented for ES utilisation in animal feed additives. Nevertheless, to the author’s best knowledge, studies available in the literature focusing on understanding the effect of eggshell application on acid soil calcium availability for plants are scarce.

Purified $CaCO_3$

Pure calcium carbonate has many applications in industrial activities. For example, it may be used in the construction sector, as a building material and as an ingredient of cement and mortar[3]. In paper industry, it is also utilised as filler to give brightness and smoothness to paper. Other possibility is as raw material in glass. Application for paints and dyes manufacture has also been reported (Kirboga and Öner, 2013).

To obtain purified $CaCO_3$ (free of organic matrix) from ES, the most efficient method involves a calcination step at 900ºC, to promote organic matter thermal oxidation and carbonate conversion to oxide (Oliveira et al., 2013). Then, water addition in a CO_2 rich environment promotes CaO carbonation and a $CaCO_3$ rich suspension is obtained, which is further centrifuged for water removal and drying. The overall efficiency of this process rounds 80%. This valorisation strategy neglects the potential value of one of the components of eggshell, which is the shell membrane.

A study performed by Yoo et al. (2009) evaluated the utilisation of calcium carbonate from eggshells as a coating pigment for ink-jet printing paper, with additional recovery of shell membrane. A dissolved air flotation (DAF) separation unit was developed. An air/water mixture was introduced at the bottom of the separation vessel and shell membrane was separated from eggshell due to density difference between the two components: membrane floated up and shell particles settled down at bottom. Process efficiency attained 96% recovery of shell membrane and 99% of eggshell particles rich in $CaCO_3$. However, a posterior calcination step at 600ºC for 2 h was required to obtain organic free particles, since the cuticle layer of ES was only successfully removed by thermal oxidation.

In summary, for applications where $CaCO_3$ purity grade is determinant, eggshell should be submitted to thermal processes for removal of the organic matrix, which increases energy cost of eggshell valorisation.

Cosmetics

Collagen is a fibrous protein that plays a relevant role in supporting tissue and structuring proteins and cells, therefore is one of the ingredients of many cosmetics that prevent skin wrinkles and improve its elasticity and thickness (Cordeiro and Hincke, 2011).

Eggshell membrane is rich in collagen protein fibers, so it can be used as a source of collagen to enhance the efficiency of cosmetic products. Furthermore, some studies have reported that collagen obtained from ES is safe to use, since it presents low autoimmune and

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[3] paste used to bind construction blocks together and fill the gaps between them.

allergic reactions in comparison with collagen extracted from other sources (Zhao and Chi, 2009).

Separation methods of membrane from shell may include dissolution of the membrane with consequent solubilisation of collagen in acids, bases, salts, by enzymatic digestion (e.g pepsine digestion) or by fermentation with bacteria, yeast or mold (Vladimir Vlad, 2007; Zhao and Chi, 2009). Additionally systems that apply cavitation (Vlad, 2009) or dissolved air flotation (Yoo et al., 2009) are also referred to thereby separate membrane from shell.

Moreover, shell particles are also indicated as good facial cleansers for skin sebum and dust removal (Cordeiro and Hincke, 2011).

Biomaterials composites

Hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$ is a material with a chemical structure similar to the mineral components found in bones and teeth. It offers an exceptional compatibility with biological systems, especially human soft tissues and therefore can be used in orthopaedic and dental implants. Application of hydroxyapatite may be performed in powder, porous block or composite materials to correct bone or dental deficiencies (Gergely et al., 2010; Rivera et al., 1999).

Synthesis reaction of hydroxyapatite may be described by Eq. (1.1) or (1.2) depending on the form that phosphate is provided:

$$3\ Ca_3(PO_4)_2 + CaO + H_2O \rightarrow Ca_{10}(PO_4)_6(OH)_2 \tag{1.1}$$

$$10\ Ca(OH)_2 + 6\ H_3PO_4 \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 8\ H_2O \tag{1.2}$$

Amongst raw materials used to provide CaO or $Ca(OH)_2$ for the reaction, eggshells have been highlighted (Gergely et al., 2010; Kumar et al., 2012; Prabakaran and Rajeswari, 2009; Rivera et al., 1999; Zhou and Lee, 2011). A prior calcination step at 900ºC for at least 3 h is required to remove the organic matrix of ES and to convert $CaCO_3$ into CaO. When phosphoric acid is used (Eq. 1.2) an additional step is required to endorse CaO hydration (Oliveira et al., 2013).

1.3.2. Eggshell utilisation as operating supplies

Some environmental and chemical applications have been studied for using ES as operating supply. Efforts in utilising ES as catalyst or sorbent are reported in the literature with twofold purposes: to promote valorisation of ES (reducing its environmental impacts) and to decrease operational costs by using low-cost supplies.

As previously presented in Figure 1.4, ES utilisation as operating supply encompasses use as catalyst in biodiesel production, lactose isomerisation or dimethyl carbonate synthesis and as sorbent for pollutant removal or immobilisation in liquid, soil or gaseous emissions.

Eggshell potential for catalysis is mainly allied to base-catalysed reactions. One of the main active phase of this catalyst is the CaO obtained from ES calcination, which has proved activity towards transesterification reactions that occur in biodiesel production (Boey et al., 2011; Boro et al., 2012; Sharma et al., 2011; Wei et al., 2009) or dimethyl carbonate synthesis (Gao and Xu, 2012). In lactose isomerisation, calcium carbonate from ES has also been indicated as a good catalyst in the conversion of lactose in lactulose (Montilla et al., 2005; Paseephol et al., 2008) with the advantage of not requiring significant pre-treatment (only washing and drying) to present potential value as catalytic agent.

Eggshell is mainly composed by calcium carbonate, thus it is expected to behave as a calcite-like sorbent (Guru and Dash, 2014). The presence of proteins in its porous structure endows ES with additional sorptive capacities, reason why some research has been focusing on using ES for organic and inorganic pollutants removal from water, wastewater, soils or gas effluents.

Table 1.2 lists environmental applications of ES as operating supply for sorptive purposes, giving special focus to the pre-treatment required for application as sorbent. Furthermore, sorption capacities are reported for each specific pollutant.

For water treatment, ES usage as sorbent has been mainly related to fluoride and phosphate removal. In this case, ES is pre-treated (calcination and/or additive incorporation) to enhance selectivity and sorption capacity towards the pollutant. Fluoride sorption by ES-based sorbent is well described by the Langmuir isotherm model (Bhaumik et al., 2012; Lunge et al., 2012), while for phosphate uptake, Freundlich (Köse and Kıvanç, 2011) or Langmuir-Freundlich (Mezenner and Bensmaili, 2009) models are more adequate. Chemisorption is indicated as the main sorption mechanism (Bhaumik et al., 2012; Mezenner and Bensmaili, 2009).

The interest upon the capacity of ES for removal of organic pollutants has been exclusively associated to dyes from wastewaters of the textile industry. Podstawczyk et al. (2014) investigated the sorption of malachite green and concluded that physical adsorption and microprecipitation were the main mechanisms involved. Additionally, equilibrium studies regarding the dyes listed in Table 1.2 pointed out that experimental data were in good agreement with Langmuir models. Nevertheless, Freundlich isotherm was also suitable in some cases (Chowdhury and Das, 2011; Ehrampoush et al., 2011; W. Tsai et al., 2008).

Table 1.2: Summary of eggshell application as operating supply for sorption purposes.

		Eggshell pre-treatment							
Application	**Pollutant**	**Water Washing**	**Membrane manual removal**	**Drying 50-110ºC**	**Grounding and sieving**	**Calcination 800-950ºC**	**Others**	**Sorption capacity**	**Reference**
Water	Fluoride						Synthesis of eggshell composite using shell, membrane and aluminum sulphate, by calcination at 450ºC , 6 h	37 mg g^{-1} (30ºC)	Lunge et al. (2012)
Water	Fluoride	X		X	X		-	1.1 mg g^{-1} (30ºC)	Bhaumik et al. (2012)
Water	Phosphate	X		X	X	X	-	23 mg g^{-1} (25ºC)	Köse and Kıvanç (2011)
Water	Phosphate	X		X	X		Sorbent previously submitted to iron adsorption	10.6 mg g^{-1} (25ºC)	Mezenner and Bensmaili (2009)
Wastewater	Malachite green (dye)	X		X	X		-	56.8 mg g^{-1} (30ºC)	Chowdhury and Das (2011)
Wastewater	Reactive red 123 (dye)	X		X	X		-	1.26 mg g^{-1} (25ºC)	Ehrampoush et al. (2011)
Wastewater	Brilliant green (dye)	X		X	X		-	44.7 mg g^{-1} (30ºC)	Kobiraj et al. (2012)
Wastewater	Remazol reactive red 198 (dye)	X	X	X	X		Immobilization with a polymer mixture of alginate and polyvinyl alcohol to obtain biocomposite sorbent	47 mg g^{-1} (22ºC)	Elkady et al. (2011)
Wastewater	Acid orange 51 (Dye)	X	X	X	X		-	114 mg g^{-1} (25ºC)	Tsai et al. (2008)
Wastewater	Reactive yellow 205 (dye)	X		X	X		-	31 mg g^{-1} (35ºC)	Pramanpol and Nitayapat (2006)
Wastewater	Cu (II)	X		X	X		Reaction with $FeSO_4.7H_2O$ and NaOH to obtain iron oxide coated eggshell powder	45 mg g^{-1} (30ºC)	Ahmad et al. (2010)
Wastewater	Radio Co (II)						Convertion to hydroxyapatite by calcination at 900ºC and reaction with PO_4^{3-} compound	3.6x10^{-4} mol g^{-1} (30ºC)	Zhang et al. (2010)
Wastewater	Radio Co (II)						Convertion to magnetite/hydroxyapatite by calcination at 900ºC and *in situ* precipitation with Fe_3O_4	6.7x10^{-4} mol g^{-1} (30ºC)	Hongqin et al. (2011)

Table 1.2: Summary of eggshell application as operating supply for sorption purposes (continued).

		Eggshell pre-treatment							
Application	**Pollutant**	**Water Washing**	**Membrane manual removal**	**Drying 50-110ºC**	**Grounding and sieving**	**Calcination 800-950ºC**	**Others**	**Sorption capacity**	**Reference**
Wastewater	Pb (II), Cu (II)			X	X		Boiling in 1M NaOH to remove impurities and membrane; reaction with $FeCl_2.4H_2O$ to obtain Fe_3O_4 eggshell powder	263 mg g^{-1} (Pb) 250 mg g^{-1} (Cu) (25ºC)	Ren et al. (2012)
Wastewater	Pb, Zn, Cu, Cd, Ni			X	X		Separation of liquid fraction	Total metal uptake:3.8x10^{-2} mEqg^{-1} (25ºC)	De Paula et al. (2008)
Wastewater	Pb (II)	X		X	X		-	154 mg g^{-1} (25ºC)	Vijayaraghavan and Joshi (2013)
Wastewater	Pb (II)						Reaction of eggshell with H_3PO_4 to obtain carbonate hydroxyapatite	500 mg g^{-1} (25ºC)	Meski et al. (2011)
Wastewater	Pb (II)						Reaction with H_3PO_4 at 30-40ºC for 24 h and pH=1-3, to obtain carbonate hydroxyapatite	94 mg g^{-1} (25ºC)	Liao et al. (2010)
Wastewater	Cd (II), Cu (II)						Reaction with H_3PO_4 at 30-40ºC for 24 h and pH=1-3, to obtain carbonate hydroxyapatite	111 mg g^{-1} (Cd); 143 mg g^{-1} (Cu)	Zheng et al. (2007)
Wastewater	Cr(III)	X		X	X		-	160 mg g^{-1} (20ºC)	Chojnacka (2005)
Wastewater	Pb,Cd, Cr	X	X		X	X	-	-	Park et al. (2007)
Soil	Pb	X		X	X	X	-	-	(Ahmad et al., 2012a; Ok et al., 2011)
Gaseous effluents	CO_2	X		X	X	X	-	-	Castilho et al. (2013); Mohammadi et al. (2014); Olivares-Marín et al. (2012); Witoon (2011)

Within the removal of inorganic pollutants from wastewaters, uptake of heavy metals have been exclusively appraised by performing tests in simulated aqueous media (Table 1.2). In that context, ES has been tested as sorbent or precursor of sorbents (carbonate hydroxyapatite or other composites). Interest in ES as a sorbent is mainly associated to (Guru and Dash, 2014): i) carbonate groups which have cation-exchange properties and by increasing pH, negative charge density of the sorbent surface rise, thus prompting the attraction of metallic ions with positive charge; ii) functional groups of proteins (carboxyl, amine and sulfate) can bind metal ions and form ionic bonds, iii) ion exchange between metals and the Ca(II) at ES surface. Besides, aqueous solutions equilibrated with ES become more basic, so that metals can precipitate and deposit on ES surface. Nonetheless, carbonate hydroxyapatite also presents a high sorption capacity for heavy metals, low water solubility, high stability and can be synthesised from ES. These features justify the reason for several studies are addressing the usage of ES as precursor for sorptive purposes.

In soils remediation, eggshell has been indicated as immobilising agent for heavy metals, to reduce its solubility and bioavailability (Guru and Dash, 2014). ES incorporation in soil may enhance soil Pb immobilisation via formation of $Pb(OH)_2$, adsorption on aluminium containing minerals and co-precipitation in carbonate form (Ahmad et al., 2012a).

ES waste has been also highlighted as a competitive sorbent alternative for CO_2 capture, in comparison to commercial materials. In fact, some investigations showed eggshell derived CaO is promising for sorption of anthropogenic CO_2 capture. Experimental results have demonstrated that after commercial CaO, the highest CO_2 uptake was attained with eggshell derived CaO (Olivares-Marín et al., 2012). Carbonation conversion of calcined eggshell was higher than the one observed for calcined commercially available calcium carbonate (Witoon, 2011).

1.4. European legal framework

The European Union (EU) has paid special attention to the disposal and use of animal by-products that correspond to entire bodies or parts of animals, products of animal origin or other products obtained from animals that are not intended for human consumption. These by-products may be a potential source of risks to public and animal health and to avoid any threat of pathogens and disease dispersion, health rules were laid down by Regulation (EC) Nº 1774/2002 of the European Parliament and of the Council of 3 October 2002 that was more recently repealed by Regulation (EC) Nº 1069/2009 of the European Parliament and of the Council of 21 October 2009. Implementation methods defined by this Regulation now in force were formerly concretised by Commission Regulation (EU) Nº142/2011. The level of risk that arises from animal by-products can fall into three categories from 1 (highest risk) to 3 (lowest risk).

ES is a product-specific waste of egg processing industry that is classified as animal by-product and falls into the Category 3, and alternatives for disposal/use are established to reduce spreading risk of pathogens into environment and human health. The different authorised options for disposal or use of eggshells are listed in Table 1.3, where a comparison between legislation repealed and now in force is also presented. Since it is a low-risk material, ES options for use and disposal are much wider in comparison to higher risk animal by-products and enclose incineration, thermal processing, production of petfood, composting, transformation into biogas, fuel combustion (although the calorific value of ES is low) or used/disposed by any other method that prevents biological hazard. Some operating conditions for these legal options are also detailed in Table 1.3.

It should be underlined that EU legal restrictions do not make impractical the full scale implementation of some of the valorisations options detailed in section 1.3. Indeed they became complementary, since legal constrains make valorisation options of ES more safe in terms of environmental and public health, for example:

i) Incineration enables ES conversion to CaO for catalytic application in biodiesel production or dimethyl-carbonate synthesis or for hydroxyapatite manufacture;
ii) Thermal processing allows further application as food additive, soil amendment and/or operating supply for sorption purposes;
iii) Biological treatment by composting yields a final product for soil amending or improvement. However, in this process, one must have into consideration that the level of organic matter available in ES for biodegradation represents a small fraction. Thus, mixing it with other organic wastes with higher self-heating ability, may be necessary to achieve the temperature-time requirements imposed.

Since Regulation (EC) Nº 1069/2009 is in force, ES utilisation without any pre-treatment became possible, under conditions determined by competent authorities, and some EU members have taken advantage of this measure. In fact, United Kingdom has allowed eggshell waste application to land, without prior treatment provided that registration and tracking of microbiological safety is performed (DEFRA - Department for environment food and rural affairs, 2011). Additionally, this EU member has stated to review this measure frequently and in case of suspicion or confirmation of an outbreak of relevant disease or infection this permission may be repealed. In Spain, according to the legal decree 1528/2012, the direct application of ES to soil as fertilizer or amendment is allowed, whenever there is no doubt of disease transmission risk to human, animal and environment. Nevertheless, this measure may have a transitory application if microbiological safety becomes questionable, and in that case other options for use/disposal must be rightly implemented.

From the list of alternatives available, composting presents itself an attractive pre-treatment, because it consists in a biological decomposition and stabilisation of organic subtracts, under aerobic conditions that allow development of thermophilic temperatures as result of biologically produced heat (Haug, 1993). This process fulfils the temperature-time constraint for sanitisation of ES, without requiring an external pasteurisation/sanitation unit. This means that energy costs to sanitise ES could be significantly reduced, by taking advantage of the biological heat produced during the process.

Table 1.3: Alternative methods applicable to eggshell product-specific waste, according to EU regulations.

<table>
<tr><th rowspan="2">Methods to disposal or use</th><th colspan="2">Regulation (EC) Nº 1774/2002 (repealed)</th><th colspan="2">Regulation (EC) Nº 1069/2009 (in force)</th></tr>
<tr><th>Operating Conditions</th><th>Legal framework</th><th>Operating conditions</th><th>Legal framework</th></tr>
<tr><td>Incineration or co-incineration with or without prior processing</td><td>Gas resulting from the process is raised in a controlled and homogeneous way, even under the most unfavourable conditions, to a temperature of 850ºC for 2 s measured by the inner wall or at another representative point of the chamber where the incineration is carried out, as authorized by competent authority</td><td>Article 6 (2) (a)
Article 12
Annex IV</td><td>Gas resulting from the process is raised in a controlled and homogeneous way, even under the most unfavourable conditions, to a temperature of 850ºC for at least 2s or to a temperature of 1100ºC for 0.2 s, as measured by the inner wall or at another representative point of the chamber where the incineration is carried out, as authorized by competent authority</td><td>Article 14 (a), (b)
Regulation (EC) Nº 142/2011: Annex III</td></tr>
<tr><td>Thermal treatment</td><td><table>
<tr><th>Particle size (mm)</th><th>T (ºC)</th><th>Time (min)</th><th>Pressure (bar)</th></tr>
<tr><td><50</td><td>133</td><td>20</td><td>3</td></tr>
<tr><td></td><td>100</td><td>125</td><td>-</td></tr>
<tr><td><150</td><td>110</td><td>120</td><td>-</td></tr>
<tr><td></td><td>120</td><td>50</td><td>-</td></tr>
<tr><td></td><td>100</td><td>95</td><td>-</td></tr>
<tr><td><30</td><td>110</td><td>55</td><td>-</td></tr>
<tr><td></td><td>120</td><td>13</td><td>-</td></tr>
<tr><td></td><td>100</td><td>16</td><td>-</td></tr>
<tr><td><30 (with fat)</td><td>110</td><td>13</td><td>-</td></tr>
<tr><td></td><td>120</td><td>8</td><td>-</td></tr>
<tr><td></td><td>130</td><td>3</td><td>-</td></tr>
<tr><td><20</td><td>80</td><td>120</td><td>-</td></tr>
<tr><td></td><td>100</td><td>60</td><td>-</td></tr>
</table></td><td>Article 6 (2) (b,c)
Article 17
Annex V</td><td>Equal to conditions defined by Regulation (EC) Nº 1774/2002</td><td>Article 24 (e)
Regulation (EC) Nº 142/2011: Annex IV</td></tr>
<tr><td>Petfood and dogchews</td><td>Processed petfood other than canned petfood must be subjected to a heat treatment of at least 90ºC</td><td>Article 6 (2) (e)
Article 18
Annex VIII</td><td>Equal to conditions defined by Regulation (EC) Nº 1774/2002</td><td>Article 14 (d)
Regulation (EC) Nº 142/2011: Annex XIII</td></tr>
<tr><td>Composting</td><td>Closed composting reactor with equipment for monitoring/recording temperature against time; minimum temperature conditions of 70ºC during 1 hour; maximum particle size of 12 mm</td><td>Article 6 (2) (f)
Article 15
Annex VI</td><td>Equal to conditions defined by Regulation (EC) Nº 1774/2002</td><td>Article 14 (f)
Regulation (EC) Nº 142/2011: Annex V</td></tr>
<tr><td>Biogas production</td><td>Biogas plant equipped with a pasteurisation or hygienisation unit where temperature must reach 70ºC during one hour; maximum particle size of 12 mm</td><td>Article 6 (2) (f)
Article 15
Annex VI</td><td>Equal to conditions defined by Regulation (EC) Nº 1774/2002</td><td>Article 14 (f)
Regulation (EC) Nº 142/2011: Annex V</td></tr>
<tr><td>Other methods or uses</td><td>Disposed of by other means in accordance to rules laid down in Article 33(2), after consultation of appropriate scientific committee</td><td>Article 6(2i)
Article 33(2)</td><td>Used under conditions, determined by the competent authority, which prevent risks arising to public and animal health
Other processing method that delivers a product with microbiological hazard level reduced: absence of *Clostridium perfringens* in 1 g
Absence of *Salmonella* in 25 g
Maximum number of bacteria equals 300 for *Enterobacteriaceae*</td><td>Article 14 (h)
Annex IV, chapter III</td></tr>
</table>

1.5. Work motivation and scope

Eggshell is a product-specific waste from the egg processing industry that is classified as an animal by-product, for which specific uses or disposal methods are legally established by European regulations.

Composting has been identified as an environmentally sound option in waste valorisation (Schaub and Leonard, 1996), which can promote sanitation of the materials used and transform organic matter into a humus-like product, to be further used as soil improver or amendment material. However, to successfully achieve pathogen elimination during composting, the starting composting mixture must contain enough available energy (easy biodegradable organic matter) to drive the process into thermophilic temperatures. Since ES is mainly an inorganic material with about 94% (w/w) $CaCO_3$, several questions may arise, namely:

"Is composting a feasibility process for ES pre-treatment, when large amount of this waste is incorporated in the starting mixture?"

"What is the maximum amount of ES that can be introduced in a composting system, to attain sanitation conditions?"

"Is the evolution of composting significantly affected when ES is used as an ingredient?"

"At what level is the final quality of the compost obtained influenced by ES presence?"

"Which is the added value that an ES rich compost may present"?

To the author best knowledge, eggshell waste composting has been scarcely addressed and the answers to these questions have been set aside by the literature. The only study found in the literature addressing eggshell composting, used microbial inoculants to aid in the humus build-up (Kemper and Goodwin, 2009).

In this context, the main goals of this work are to:

i) define/select organic materials with enough biodegradability to attain the desired temperature;
ii) establish the maximum incorporation of ES in the starting mixture, without compromise sanitation conditions and adequate evolution of the microbial degradation/transformation of organic matter;
iii) appraise the quality of final products, in terms of physical, chemical and biological properties, regarding the influence of ES incorporation;
iv) evaluate the added-value of eggshell rich compost, by focusing on its application for environmental purposes, namely immobilisation of heavy metals in contaminated soils and their removal from liquid environmental matrices.

1.6. Thesis structure

This thesis is structured in five Parts (A to E) as indicated in Figure 1.5.

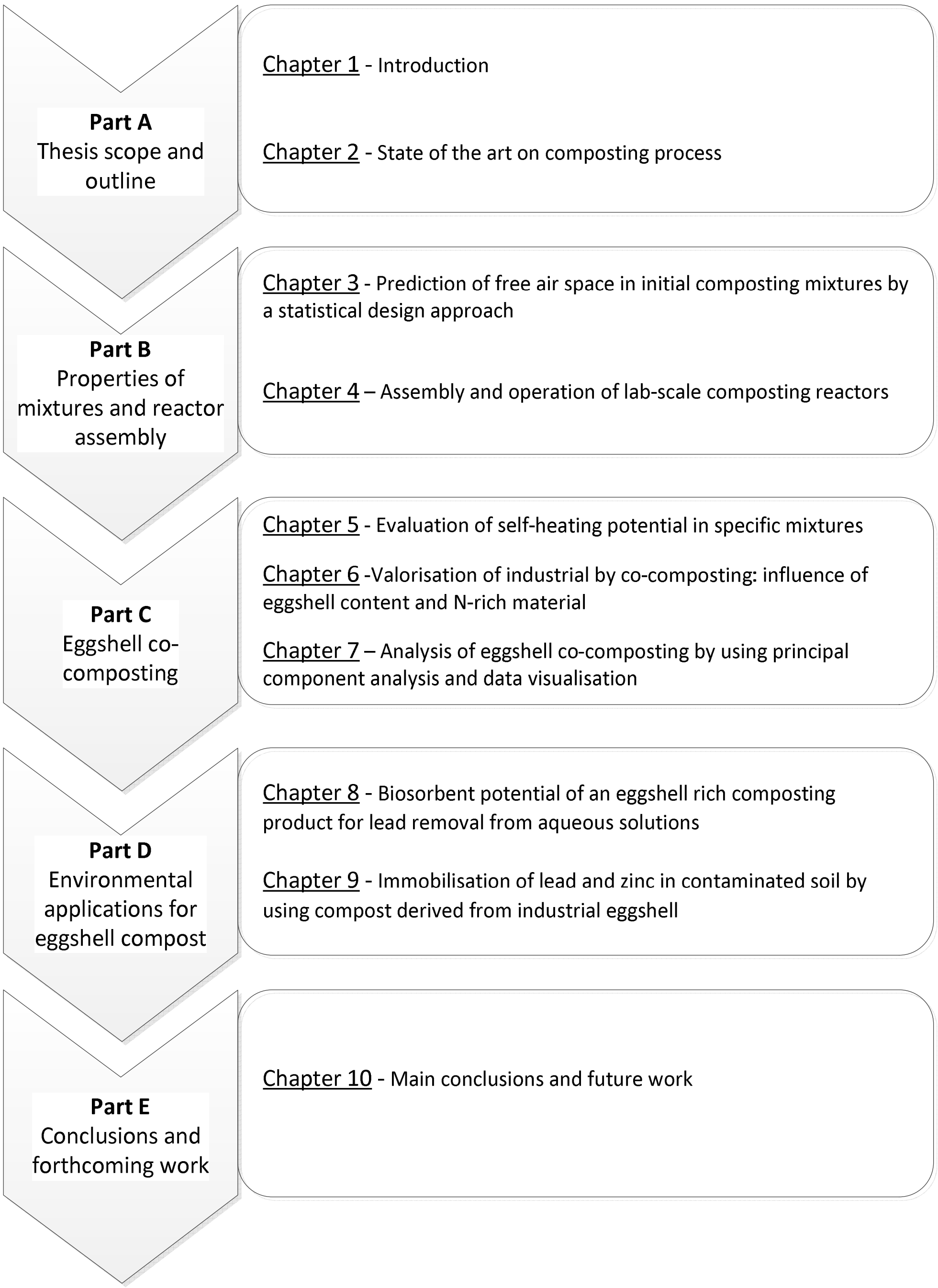

Figure 1.5: Thesis organisation in five parts**.**

The Part A of this document joins together the initial two chapters of the thesis. Chapter 1 comprehends the introduction of the thesis which provides a contextualisation upon the origin, properties, valorisation and legal framework associate to eggshell from the egg processing industry. The role of composting in the valorisation of eggshell is also addressed, covering the scope of this work. In Chapter 2, a review of the abiotic and biotic aspects that influence the composting process is presented. Additionally, the current understanding about organic matter and nitrogen biological transformations that occur during composting are explored and issues related to the quality of composts are also discussed. The potential of composting end-products for environmental applications is also reviewed.

Part B comprises two chapters. In Chapter 3 the properties of the feed stocks primarily selected for this study are addressed, with special focus on the use of experimental mixture design to predict the initial free air space of a composting mixture, which is a physical property that plays an important role in composting. Chapter 4 is devoted to the assembly and experimental validation of the laboratorial composting reactors used for the purpose of this study.

Part C includes all the research on eggshell co-composting. Aiming to evaluate the features of eggshell co-composting, firstly the self-heating ability of some starting composting mixtures is addressed in Chapter 5. Then, the influence of a N-rich material in the physical and chemical changes occurring during co-composting of ES is discussed in Chapter 6. Chapter 7 is focused on evaluating the effect of high levels of ES (60% w/w) on composting evolution by using proper multivariate data analysis techniques and informative visualisation tools.

Part D deals with the application of an eggshell rich-compost for environmental applications, namely removal of lead and zinc from aqueous and solid matrices. Specifically in Chapter 8, compost derived from eggshell waste co-composting was evaluated as a biosorbent for Pb(II) uptake from aqueous medium and for comparison purposes, mature compost without eggshell and natural eggshell were tested as well. Chapter 9 focus on evaluating the feasibility of using the eggshell-rich compost as a soil amendment for immobilisation of Pb and Zn in an acidic contaminated mining soil, by evaluating the environmental availability of metals, toxicity reduction and variation of CO_2 efflux from soil, due to the incorporation of a carbonate rich amendment.

Finally, the most relevant conclusions from this work are resumed in Part E along with further recommendations on future work (Chapter 10).

The experimental methodologies linked to laboratorial tests and analytical techniques are not included as an independent part of the thesis, but are described in each chapter of Part B and C, together with the presentation of the experimental results. This option is believed to be most useful for the reader, since each chapter describes the relevant experimental methodologies used to the issues studied.

The overall information delivered by this thesis has been published or submitted in the following scientific publications (international articles with *peer review* and communications in international conferences):

Soares, M.A.R., Quina, M.J., Quinta-Ferreira, R., 2013. Co-composting of eggshell waste in self-heating reactors: Monitoring and end product quality. Bioresour. Technol. 148, 293–301.

Soares, M.A.R., Quina, M.J., Quinta-Ferreira, R., 2013. Prediction of free air space in initial composting mixtures by a statistical design approach. J. Environ. Manage. 128, 75–82.

Soares, M.A.R., Quina, M.J., Quinta-Ferreira, R., 2015.Immobilisation of lead and zinc in contaminated soil by using compost derived from industrial eggshell. J. Environ. Manage. 164, 137–145.

Soares, M.A.R., Marto, S., Quina, M.J., Gando-Ferreira, L., Quinta-Ferreira, R.. Biosorbent potential of an eggshell rich composting product for lead removal from aqueous solutions. Submitted to Ecological Engineering, in March 2015.

Soares, M.A.R., Quina, M.J., Quinta-Ferreira, R.. Influence of N-rich material in valorisation of industrial eggshell by co-composting. Submitted to Environmental Technology, in October 2015.

Soares, M.A.R., Quina, M.J., Reis, M.S., Quinta-Ferreira, R.. Assessing the impact of using high levels of industrial eggshell in co-composting with agro-wastes, *in preparation*.

Soares, M.A.R., Quina M.J., Quinta-Ferreira R., 2014. An overview of eggshell waste potential for sorptive and catalytic processes, presented at CHEMPOR’2014 10-12th September, in Book of Abstracts, Pg 9-49 to 9-51, Porto, Portugal *(oral communication)*.

Soares, M.A.R., Quina, M.J., Gando-Ferreira, L., Quinta-Ferreira, R., 2014. Removal of Pb (II) from aqueous solutions using eggshell composting products, presented at Athens’2014- 2nd International Conference on Sustainable Solid Waste Management, 12-14th June, In Proceedings, Athens, Greece. *(oral communication)*.

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2. State of the art in composting process

This chapter describes main theoretical concepts associated to the composting process. The temperature dependent phases through which the process evolves, as well as the main biotic aspects and sanitising conditions are firstly tackled. Afterwards, organic matter and nitrogen biological transformations that occur during composting are also explored. Special attention is given to abiotic aspects that influence the reactions attained during aerobic degradation of organic matter. In the last sections of this chapter, issues related to the quality of composting end-products for soil application are discussed too.

2.1. Introduction

Composting may be defined as a process of biological decomposition and stabilisation of organic substrates, under aerobic conditions, that allows development of thermophilic temperatures, as a result of biologically produced heat (Haug, 1993). Due to the metabolic activity of successive microbial populations, the process allows the conversion of organic matter, from animal or vegetal origin, into a final product that should be stable, humus like, free of pathogens and plant seeds (Pare et al., 1998; Shilev and Naydenov, 2007). A generalised equation of composting follows (Peigné and Girardin, 2004):

$$\text{Fresh organic matter} + O_2 \xrightarrow[\text{metabolism}]{\text{microbial}} \text{Stabilised organic material (compost)} + CO_2 + H_2O + \text{Energy (heat)} + \text{other gases} \tag{2.1}$$

Composting is an ancient practice that has been used through centuries by nature and man, especially for farming, as a mean of recycling organic matter to improve soil physical and chemical properties (Rynk et al., 1992). Nowadays, there is a renewed attention concerning composting and its application is not limited to farm context but has been broadened to municipal and industrial frameworks.

Indeed in many nations, legal constrains are reinforcing the waste management hierarchy based on prevention, re-use, recycling and other forms of recovery depreciating disposal in landfills. In addition, mandatory targets have been limiting the amount of biodegradable waste consigned to landfilling, promoting the retrieval of the waste organic matter and nutrients that can be tailored to energy recovery or supress soil deficiencies. In this scope, composting has been identified as an environmentally sound alternative (Chroni et al., 2012; Farrell and Jones, 2009; Gajalakshmi and Abbasi, 2008; Sharma et al., 1997; Tuomela et al., 2000).

Composting has been used to recycle wastes such as municipal solid wastes (Castaldi et al., 2005; Gestel et al., 2003; Kumar and Goel, 2009), sewage sludges (Kang et al., 2011; Manios, 2004; Paredes et al., 2005; Wei and Liu, 2005), agro-food residues (Grigatti et al., 2011; Pepe et al., 2013; Sellami et al., 2008) and many others. In addition, aerobic conversion is reported to be useful in the remediation of soils contaminated with some chemical pollutants like aromatic hydrocarbons, pesticides or chlorophenols (Houot et al., 2012; Semple et al., 2001).

2.2. Temperature-dependent phases and general biotic aspects

Temperature plays a determinant role in the evolution of composting since it dictates the phases through which the process evolves. Under adequate abiotic conditions for microbial development (namely moisture, pH, oxygen, nutrients and porosity) composting process can be divided into four phases (Fogarty and Tuovinen, 1991; Ryckeboer et al., 2003), as detailed in Figure 2.1.

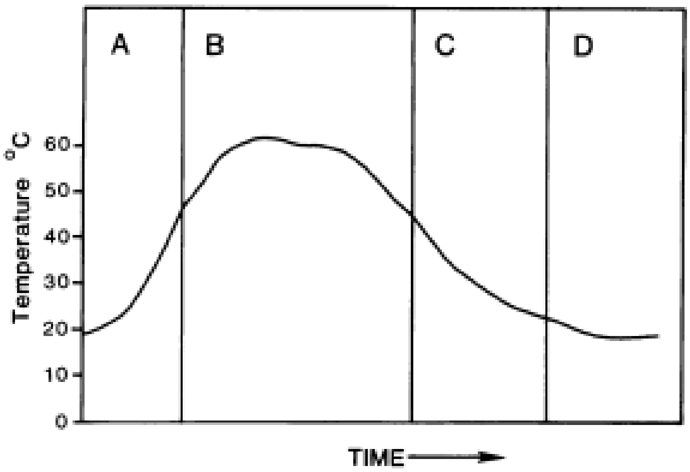

Figure 2.1: Phases of composting: A-initial mesophilic phase; B-thermophilic phase; C-cooling phase; D-maturation phase.
Reprinted from Fogarty and Tuovinen (1991) with permission of American Society for Microbiology.

The early stage of composting is characterised by a mesophilic stage ruled by the activity and growth of mesophilic organisms and rapid uptake of easily biodegradable organic matter. *Bacillus* spp and *Azotobacter* spp are some of the main bacteria responsible for degradation (Nakasaki et al., 1985) and fungal populations seem to be only modestly present during this initial phase (Hansgate et al., 2005). As temperature increases, non-thermo-tolerants communities become less competitive and are replaced by thermophilies. During this stage, organic matter degradation rates and oxygen consumption levels are high and most of the biomass formation takes place. Predominance of thermophilic microbiota mainly associated to the genus *Bacillus* is reported (Strom, 1985), but if temperature is in between 40-50ºC, some thermo-tolerant micro-fungi and actinomycetes have favourable growth conditions, colonise the mixture and initiate cellulose degradation and lignin solubilisation and or degradation. In fact, during this stage, actinomycetes are important promoters of lignocellulose break down, because they are more thermo-tolerant than fungi, although fungi lignin degradation ability is higher (Tuomela et al., 2000).

After the peak of organic matter degradation, thermophilic activity decreases, mesophilic biological activity arises and once again dictate the temperature profile that attains the cooling phase. Benefiting from the temperature decline, during this stage active degradation of hemicellulose by actinomycetes may occur (Gajalakshmi and Abbasi, 2008).

Once the temperature reaches ambient levels, the maturation phase is started, occurring recolonisation. Diffusion of actinomycetes and fungal recolonisation benefit from temperature decrease and degradation of complex organic substances into humus-like compounds becomes more expressive. Although in this phase, reaction rates are typically low (Bertoldi et al., 1983). Lignin degradation is mainly promoted by fungi species belonging to the subdivision Basidiomycotina (Bertoldi et al., 1983; Grinhut et al., 2007; Tuomela et al., 2000) but actinomycetes are also able to degrade and transform resistant polymers.

Generally, composting is driven by the indigenous microorganisms found in feedstocks. However, inoculation with specific organisms to favour a particular biochemical transformation of organic matter or enhance global biotransformation has been studied for several feedstock like olive mill wastes (Agnolucci et al., 2013; Echeverria et al., 2012), cattle/chicken wastes (Wang et al., 2011), waste paint sludges (Tian et al., 2012), municipal solid wastes (Xi et al., 2012), spent coffee ground (Hachicha et al., 2012) and cow manure (Liu et al., 2011).

Nevertheless, positive effects of inoculation may be sometimes limited since most organic wastes already contain diverse communities of organisms that multiply rapidly when favorable conditions are established, though inhibiting the growth of the inoculants added (Hubbe et al., 2010).

2.3. Pathogen destruction

Many organic wastes contain some indigenous microorganisms that are considered pathogenic to humans, animals and plants.

Table 2.1 summarises some of the pathogens that can be found in sewage sludge and solid urban wastes, which are often treated by composting. Since end-products are intended to be suitable for soil incorporation, the process must be conducted in such a way that the final composts do not contain viable pathogens in levels that can trigger disease spread to humans, animals and plants (Wichuk et al., 2011).

The mechanisms identified to promote pathogen eradication during composting are diverse (Noble and Roberts, 2004; Ryckeboer et al., 2002; Wichuk et al., 2011; Wichuk and McCartney, 2007):

1) thermal denaturation of enzymes and consequent cell death;
2) production of toxic substances, like organic acids, ammonia and phenolic compounds;
3) nutrient deplection, specially during maturation stage;
4) lytic activity of enzymes produced in the compost;
5) antagonistic relations between microorganisms associated to the presence of antibiotic substances produced by certain fungi and actinomycetes.

Table 2.1: Pathogenic microorganisms isolated from soils urban wastes and sewage sludge and diseases induced to humans and animals. Adapted from Bertoldi et al. (1983); Haug (1993).

	Pathogenic organism	Induced diseases
Viruses	Polioviruses	Poliomyelitis
	Coxsackie	Mild infections
	Echoviruses	Mild infections
	Hepatitis A vírus	Hepatitis A
	Adenoviruses	Respiratory infections
	Reoviruses	Diarrhea; gastroenteritis
Bacteria	*Salmonella*	Salmonellosis
	Escherichia coli and other coliform species	Diarrhea; gastroenteritis
	Yersinia	Gastroenteritis
	Bacillus anthracis	Anthrax
	Listeria	Listerosis
	Vibrio cholerae	Cholera
	Mycobacterium	Tuberculosis
	Leptospira	Leptospirosis
	Campylobacter	Gastroenteritis
Fungi	*Aspergillus fumigatus*	Aspergillosis
	Candida albicans	Candidiasis
	Cryprococcus neoformans	Cryptococosis
Parasites	*Taenia saginata*	Tapeworm infections
	Ascaris lumbrocoides	Ascariasis
	Toxocara	Visceral larva migrans
	Echinococcus	Hydatid disease

Thermal inactivation is considered the main responsible mechanism for pathogen eradication (Farrell and Jones, 2009; Haug, 1993; Noble and Roberts, 2004; Vinnerås et al., 2003; Wichuk et al., 2011) and depends on two factors: temperature level and exposure time. Thus, low temperature during a long period can be equally effective as high temperature for a short period, as detailed in Table 2.2.

Table 2.2: Pathogen destruction by thermal inactivation in sewage sludge: Temperature and time relations. Adapted from Haug (1993).

	Destruction Temperature-time			
Microorganism	Temp. (ºC)	Time (min)	Temp. (ºC)	Time (min)
Salmonella typhosa	55-60	30	60	20
Salmonella sp.	55	60	60	15-20
Shigella sp.	55	60	-	-
Entamoeba histolytica cysts	45	few	55	few seconds
Taenia	55	few	-	-
Trichinella spiralis larvae	55	quickly	60	few seconds
Brucella abortis	55	30	62.5	3
Micrococcus pyogenes	50	10	-	-
Streptococcus pyogenes	54	10		
Mycobacterium tuberculosis	66	15-20	67	few
Corynebacterium diphtheriae	55	45	-	-
Necator americanus	45	50	-	-
Ascaris lumbricoides eggs	50	60	-	-
Escherichia coli	55	60	60	15-20

During composting, high temperatures might be maintained for several days if abiotic conditions are favourable. Therefore, this is a well-established pathogen reduction technology that can lessen the presence of most pathogenic bacteria, viruses, fungi, protozoa and helminth ova to safe low levels. Guidelines to assure effective inactivation during composting have been established in many countries, based on temperature monitoring and control, Table 2.3.

Table 2.3: An overview of regulatory temperature-time relations during composting. Adapted from British Standard Institute (2011); CCME Guidelines for Compost Quality (2005); JRC - European Commission (2012); U.S. Environmental Protection Agency (2003).

	Composting technology			
Country	Windrow	Aerated static pile	In vessel (reactor)	Scope of application
Canada	T≥55ºC for at least 15 days, with 5 turnings	T≥55ºC for 3 days	T≥55ºC for 3 days	All feedstock
United States of America	T≥55ºC for at least 15 days, with 5 turnings	T≥55ºC for 3 days	T≥55ºC for 3 days	Biosolids
United Kingdom	T≥65ºC for 7 days (not necessarily consecutive) Moisture=51%	T≥65ºC for 7 days (not necessarily consecutive) Moisture=51%	T≥65ºC for 7 days (not necessarily consecutive) Moisture=51%%	Segregated biowastes, biodegradable polymers and materials; anaerobic digestate
Belgium	T≥60ºC for 4 days or T≥55ºC for 12 days			-
Czech Republic	T≥65ºC for 5 days or T≥55ºC for 21 days			-
German	T≥65ºC for 5 days or T≥55ºC for 14 days	T≥60ºC for 5 days or T≥55ºC for 14 days	-	-
Denmark	T≥55ºC for 14 days			-
France	T≥60ºC for 4 days			-
Italy	T≥55ºC for 3 days			-
Netherlands	T≥65ºC for 4 days			-
Slovenia	T≥65ºC for 4 days or T≥60ºC for 5 days or T≥55ºC for 14 days			-
EU members (proposal)	T≥65ºC for 5 days, T≥60ºC for 5 days or T≥55ºC for 14 days			Biowaste
EU members	T≥70ºC for 1 hour			Animal by-products

In general, regulations from European countries are much more conservative in terms of inactivation temperature in comparison to American and Canadian guidelines. In fact, in a review about effectiveness of time-temperature regulations on pathogen inactivation, Wichuk and McCartney (2007) reported that pathogens have been detected in end-products of processes that fulfilled the requirements of 55ºC for 3 days. Thus, they underlined that this regulation would not be sufficient to ensure a complete inactivation. Although the applicability of the guidelines is rarely questionable, some studies reported by Wichuk and McCartney (2007) have emphasised that: i) 55ºC during 15 days in windrows is not enough to eliminate protozoan parasites; ii) some virus inactivation was not possible at 55ºC during 3 days; *Salmonella* may survive at 60ºC for 5 days in biosolids composting.

It should be pointed out that the criteria reported in Table 2.3 presume that all particles reach these conditions. So, cooler zones inside the matrix where pathogen may survive and the use of discrete temperature measurements at fixed locations of the mixture are the main reasons to fail pathogen inactivation (Wichuk and McCartney, 2007). Therefore, the establishment of time-temperature regulations have tended to be more conservative.

2.4. Organic matter and nitrogen transformations

Organic matter constituted by lignin, carbohydrates (including cellulose, hemicellulose, starch, monosaccharides and oligosaccharides), proteins and lipids suffers mineralisation, transformation and stabilisation along several biochemical pathways that are catalysed by enzymes, throughout composting (Tuomela et al., 2000). As result, changes in carbon and nitrogen content occur as composting evolves. Carbon is used mainly as energy source for the microorganisms and may also be incorporated into their cells (at lower extents). Nitrogen is a constituent of enzymes, co-enzymes, proteins and nucleic acids that have a determinant role in cell growth and maintenance. In the following sections, an overview of the transformation of organic matter and nitrogen during the main stages of composting is presented.

2.4.1. Organic matter dynamics

During the active phase of composting, organic matter mineralisation prevails with quick degradation of more labile compounds like simple carbohydrates, fats and amino acids forming carbon dioxide, water and ammonia (Amir et al., 2010; Bernal et al., 2009). As available organic substances become scarce, degradation rates decrease and resistant organic substrate such as cellulose, hemicellulose and lignin start to be partially degraded. Then, aliphatic compounds and phenols of low structural complexity are released and further degraded by microbial activity or transformed into new complex and polymerized compounds with similar properties to humic substances[1] found in soil (Sánchez–Monedero and Roig, 1999).

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[1] Humic substances are considered to be a series of relatively high-molecular-weight substances, yellow to black coloured organic substances formed by secondary synthesis reactions in soils (Harrison, 2008)

On the latter stages of composting (maturation), the transformations processes prevail over mineralisation and organic compounds with high stabilisation degree remain (Bernal et al., 2009). These organic substances are generally characterised by small particle size, dark colour, with a complex chemical structure rich in phenolic, carboxylic and amine functional groups (Harrison, 2008).

Several studies in the literature have been dedicated to track the fate of organic matter substances during composting for better understanding biochemical mechanisms that lead to composts with high level of stability. Some works focus on evaluating water soluble organic substances (Castaldi et al., 2005; Charest, 2004; Said-Pullicino and Gigliotti, 2007; Said-Pullicino et al., 2007a, 2007b; Sánchez–Monedero and Roig, 1999) since organic matter degradation and transformation occurs at the liquid biofilm surrounding the solid particles, when organic compounds are depolymerised by enzymatic activities, into smaller molecules water soluble and consequently assimilated by the microorganisms (Gajalakshmi and Abbasi, 2008). For this reason, water-soluble substances have been considered to reflect the biochemical alterations of organic matter during composting, allowing the assessment of compost stabilisation (Bernal et al., 2009; Castaldi et al., 2005).

On the other hand, monitoring humic substances is also of interest to understand the mechanisms of polymerisation and transformation of humic compounds during composting. Humic substances are heterogeneous mixture of organic molecules that can be fractioned according to its solubility at specific pH into fulvic acids, humic acids and humin (as detailed in Figure 2.2 a)). Although these fractions present chemical and physical differences, it does not indicate the existence of three distinct types of organic molecules (Sutton and Sposito, 2005).

The chemical structure for humic substances is not well defined, since a number of precursors and possible combinations between them is extremely wide. However, the final mature compost is believed to contain large organic molecules with aromatic COOH, aliphatic COOH, phenolic OH, H-bonded phenolic OH, saccharide, quinone, peptide and cyclic N units (Harrison, 2008; Stevenson, 1994). Therefore, most of the studies focusing on humic substances evolution throughout composting are based on identifying the presence of these characteristic chemical groups or by fractioning and purifying humic substances according to its pH solubility (Figure 2.2 a)) (Amir et al., 2010; Barje et al., 2012; Droussi et al., 2009; Lhadi et al., 2006; Zbytniewski and Buszewski, 2005).

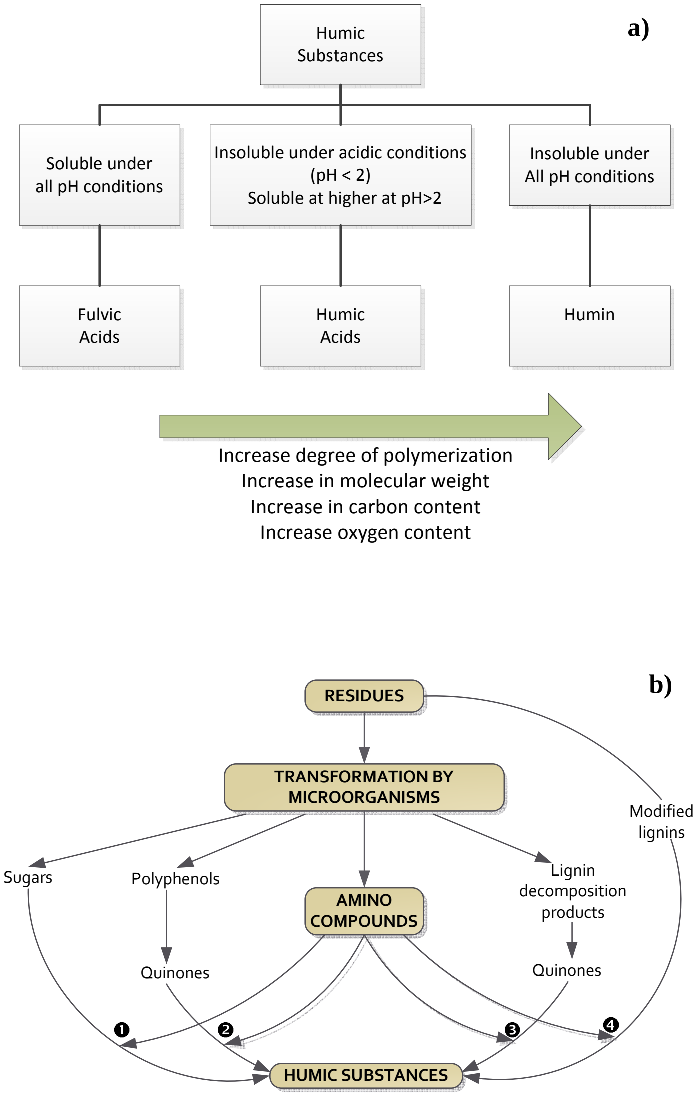


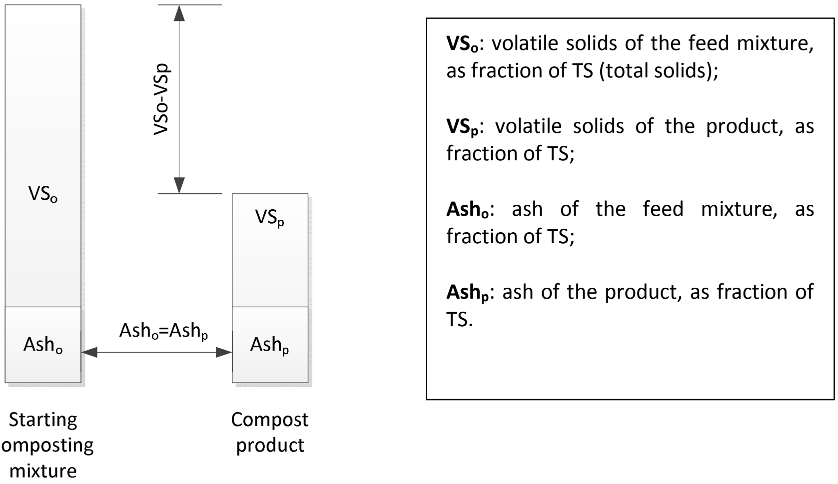
Figure 2.2: Humic substances: a) classification and chemical properties, b) proposed formation mechanisms. Adapted from Stevenson (1994).

Despite significant interest in the biochemical features of humic substances formation, this issue is still considered one of the less understood aspects of humus chemistry. According to Stevenson (1994) four mechanisms may be considered (Figure 2.2 b)) where amino compounds synthesised by microorganisms are perceived to react with modified lignins (pathway ❹), quinones (pathways ❷ and ❸) and reducing sugars (pathway ❶) to form complex dark-coloured polymers by polymerisation and condensation reactions (Harrison, 2008). Indeed, the exact mechanism of breakdown and formation of humic substances during composting is not consensus (Barje et al., 2012; Harrison, 2008) and it is likely that the prevalence of one or more of the mechanisms here indicated (or other yet not understood) is dependent on the nature of the feedstocks' organic matter and on the composting conditions.

The fate of organic matter substances during aerobic conversion may also be tracked by following the evolution of polysaccharides and lignin concentration (Alburquerque et al., 2009; Charest, 2004; Francou et al., 2008; Lashermes et al., 2012; Lhadi et al., 2006; Paradelo et al., 2013). In fact, since these substances are considered to undergo biodegradation and not synthesis in the course of composting, variations in their concentration are regarded to be better indicators of organic matter evolution in comparison to other organic matter fractions (Paradelo et al., 2013).

Furthermore, mineralisation of organic matter can be quantified based upon the ash conservation principle, which assumes that ash of a composting matrix remains constant throughout the process (Figure 2.3).

Despite nitrification (that will be presented in section 2.4.2) can slightly increase the ash fraction due to nitrite and nitrate formation, this contribution is considered negligible in comparison to the overall content (Himanen and Hänninen, 2011).



VS_o: volatile solids of the feed mixture, as fraction of TS (total solids);

VS_p: volatile solids of the product, as fraction of TS;

Ash_o: ash of the feed mixture, as fraction of TS;

Ash_p: ash of the product, as fraction of TS.

Figure 2.3: Generalised bar diagram showing VS and ash components for starting composting mixture and compost product. Ash fraction is conservative. Adapted from Haug (1993).

Thus, the mineralisation during composting can be defined as (Haug, 1993):

$$k_m = \frac{VS_0 - VS_p}{VS_0} \tag{2.2}$$

The percentage of ash in feed (Ash_0%) can be determined as:

$$Ash_0\% = \frac{Ash_0 \times 100}{Ash_0 + VS_0} \tag{2.3}$$

Then,

$$VS_0 = \frac{Ash_0 \times 100}{Ash_0\%} - Ash_0 \tag{2.4}$$

Similary, Ash_p% and VS_p can be determined as:

$$Ash_p\% = \frac{Ash_p \times 100}{Ash_p + VS_p} \tag{2.5}$$

$$VS_p = \frac{Ash_p \times 100}{Ash_p\%} - Ash_p \tag{2.6}$$

Thus substituting Eqs (2.4) and (2.6) into (2.2) and rearranging:

$$k_m = \frac{(Ash_p\% - Ash_0\%) \times 100}{Ash_p\% \times (100 - Ash_0\%)} \tag{2.7}$$

Additionally, decomposition of organic materials can also be appraised by monitoring CO_2 production (Cayuela et al., 2012; Chang and Hsu, 2008; Contreras-Ramos et al., 2004; de Guardia et al., 2010b; Gomes and Pereira, 2008; De Guardia et al., 2008; Komilis and Ham, 2006; Komilis, 2006; Kulcu and Yaldiz, 2004; Saidi et al., 2008; Tosun et al., 2008) or organic matter losses (Gomes and Pereira, 2008; Paredes et al., 2002, 2001, 2000; Tortosa et al., 2012) due to aerobic biological degradation. The main objective of these studies is essentially to understand the mineralisation phase of organic matter transformations by characterising the kinetic or stoichiometric behaviour of the biochemical reactions involved. Some models have been used for that purpose, as indicated in Table 2.4.

Table 2.4: Mineralisation of organic matter during composting: an overview of kinetic models. Adapted from Mason (2006), Sleutel et al. (2005), Tosun et al. (2008).

Model type	Description	Model equation	Application
Single-first order	Rate of mineralisation is proportional to the amount of one pool of available organic matter, following a first order kinetic.	$C(t) = C_A(1 - e^{-kt})$ (2.8)	Organic carbon and organic matter
Parallel first-order	Existence of two pools of available organic matter which are both mineralised according to first order kinetics.	$C(t) = C_{Af}(1 - e^{-k_f t}) + C_{As}(1 - e^{-k_s t})$ (2.9)	Organic carbon
Combined first and zero-order	Organic matter presents an easily mineralisable pool assuming a first order kinetics and a more resistant pool that follows a zero-order mineralisation kinetic.	$C(t) = C_{Af}(1 - e^{-k_f t}) + C_{As}k_s t$ (2.10)	Organic carbon
Second order	Rate of decomposition is proportional to the product of organic matter and microorganisms concentration.	$C(t) = C_A - \frac{C_A}{1 + k_2 a(1 - a)C_A t}$ (2.11)	Organic carbon
Levi -Minzi	Net mineralisation follows an exponential kinetic	$C(t) = k_3 t^m$ (2.12)	Organic carbon
Chen-Hosshimoto	Considers the organic matter as dependent on a refractory coefficient (R), a kinetic constant (K) and maximum specific growth rate of microorganisms	$\frac{C(t)}{C_o} = R + \frac{(1 - R)K}{\mu_m t - 1 + K}$ (2.13)	Organic carbon

Legend: C(t) is the cumulative amount of C mineralised at time t; C_A is the amount of mineralisable C.
$C_{A,f}$ and $C_{A,s}$ are the C pools with fast and slow turn over time, respectively; k_f and k_s are the mineralisation rate constants of these pools; a is the fraction of microbial biomass; k_2 is the second-order mineralisation rate constant; R is the refractory coefficient, K is Chen and Hashimoto dimensionless kinetic constant, and μ_m is the maximum specific growth rate of microorganisms.

In summary, different approaches may be used to track organic matter transformation during composting. The main routes are by analysing water soluble organic matter, humic substances or carbon/organic matter mineralisation. However, it is noteworthy that organic matter dynamics is highly dependent on factors like nature of organic feedstocks and operating conditions, which determine the microorganisms' development and succession with further implications on organic matter transformation and stabilisation.

2.4.2. Nitrogen dynamics

Nitrogen dynamics during composting are complex and follow a set of successive processes that can be summarised in ammonification, nitrification, immobilisation and denitrification, as detailed in Figure 2.4.

Ammonification (also called mineralisation) corresponds to the conversion of organic nitrogen (proteins, aminoacids, urea, etc.) into free ammonium (NH_4^+/NH_3) and this transformation occurs concurrently with organic matter biodegradation throughout composting. Thus, NH_4^+/NH_3 formed during ammonification is added to initial content present in feedstock and constitutes a single pool. Then, nitrogen can be transferred to leachates, emitted as ammonia by stripping, immobilised in turn to organic form by microbial synthesis, directly accumulated in composting end-product or oxidised to NO_3^- by a two-step biological process called nitrification (de Guardia et al., 2010b; Zeng et al., 2012a).

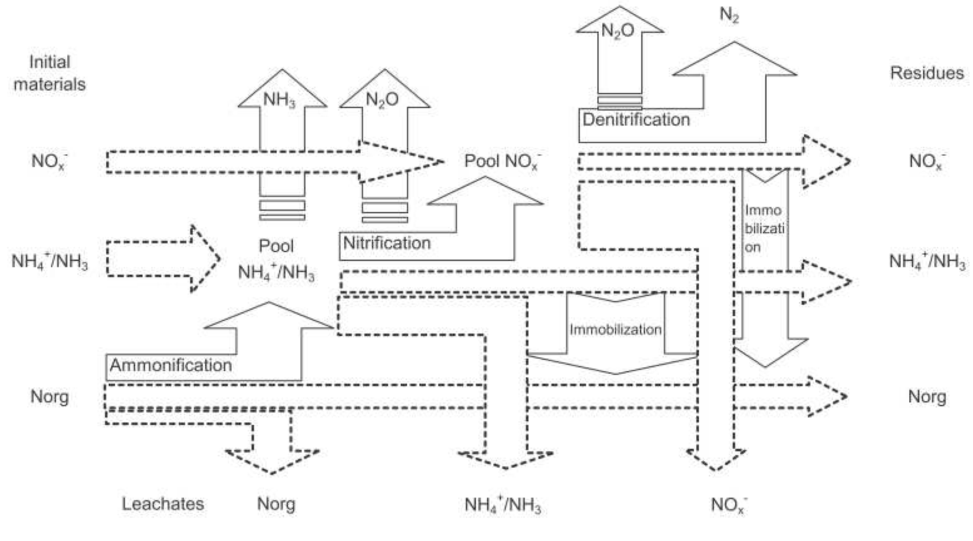


Figure 2.4: Schematic representation of nitrogen transformations and transfer throughout composting. Reprinted from Zeng et al., (2012a) with permission of Elsevier.

Nitrification occurs during the maturation stage of composting. Firstly, ammonia is biological oxidised to nitrite (NO_2^-) (nitritation) by ammonia-oxidising bacteria and then converted to nitrate NO_{3-} (nitratation) by nitrite-oxidising bacteria. Nitritation is considered the rate-limiting step of nitrification and N_2O is a by-product of NH_4^+/NH_3 oxidation (Sánchez-Monedero et al., 2010). Then, the pool of NO_3^-/NO_2^- (NO_x^-) can be transformed by microbial activity to N_2 via denitrification, with N_2O gas emission, or suffer immobilisation. Main transfer routes for NO_x^- are leaching and accumulation in composting end-product (De Guardia et al., 2010b; Zeng et al., 2012b).

Therefore during aerobic degradation of organic solids, N can be exported in the form of leachates or gaseous emissions characterised by ammonia, nitrous oxide and N_2. These gas emissions are of environmental concern. Ammonia (NH_3) contributes to eutrophication and acid rain (Colón et al., 2010) and nitrous oxide is a greenhouse gas molecule whose contribution to global warming is even more significant than CH_4 or CO_2 (Shen et al., 2011). Accordingly, one of the major challenges regarding N transfer and transformation processes is how to minimise the loss of nitrogen during composting, in order to preserve the agronomic value of the composting end-product and to avoid environmental problems associated to N losses (Hubbe et al., 2010). Several studies in literature have addressed this issue in order to minimise N export from compost.

Pagans et al. (2006) studied the influence of temperature in ammonia emissions during composting of several wastes (organic fraction of municipal solid wastes, dewatered raw sludge and anaerobically digested sludge, animal by-products from slaughterhouses and hydrolysed hair from the leather production industry). They concluded that ammonia emissions exhibited a similar pattern to temperature profile that means an exponential correlation during thermophilic stage and a linear relationship during the final mesophilic stage.

De Guardia et al. (2008) evaluated the influence of aeration on N dynamics during composting of wastewater sludge with wood chips and concluded that ammonification, ammonia emissions and nitrification are controlled by this parameter. In fact, higher aeration rate induced ammonia emissions, decreased nitrogen losses by leaching and increased the concentrations of total ammoniacal nitrogen in the composting mixture. Furthermore, a proportional relationship was found between nitrification rate and the mean content of NH_4^+/NH_3 in the mixture.

Additionally, De Guardia et al. (2010) developed studies on ammonification, accumulation, stripping and conversion of NH_4^+/NH_3 and highlighted the significance of organic mineralisation in N losses due to an increase of ammonia emissions when organic nitrogen (Nbio) content in the mixture was higher. Latter, Zeng et al. (2012a) proposed a correlation to estimate N losses from the initial NH_4^+/NH_3 content of the composting mixture and the Nbio content:

$$\mathrm{N_{losses}} = \alpha_3 + \beta_3 \times \left[N_{bio} + \left(NH_4^+ / NH_3 \right)_{initial} \right] \tag{2.14}$$

where α_3=0.213, β_3=0.913, N_{bio} is the organic nitrogen decomposed (g N kg^{-1} $VS_{initial}$) and $(NH_4^+/NH_3)_{initial}$ is the ammonia content in the initial composting matrix (g N kg^{-1} $VS_{initial}$).

Nitrogen losses are also related to the level of carbon present in the initial mixtures and a negative relationship between C:N and N losses has been found (Liang et al., 2006; Meunchang et al., 2005; Sanchez-Monedero et al., 2001). Indeed, a carbon source may promote immobilisation, thus reducing the effects of ammonification (Barrington et al., 2002). However, according to Li et al. (2013) not all carbon sources are effective in reducing ammonia emissions and even using readily available carbon compounds, emissions reduction is dependent on the characteristics of the source.

In what concerns nitrification, it has been a challenge to understand why this process occurs in the later stages of composting and how to minimise N_2O emissions. It has been noted that high temperatures or low pH due to intense biodegradation inhibit nitrification (Sanchez-Monedero et al., 2001). However some recent studies revealed the existence of thermophilic ammonia oxidisers (Jarvis et al., 2009; Yamamoto et al., 2011, 2010) capable of promote ammonia oxidation during the early days of composting. Hence, it has been suggested that biodegradation of organic matter might also be responsible for the latter occurrence of nitrification (Zeng et al., 2013, 2012b), probably because the decomposition of easily biodegradable organic matter leads to a sharp rise in O_2 demand in situ, that limits oxygen available for ammonia oxidisers.

2.5 Abiotic factors

Aerobic degradation of organic material results from the interaction of processes that occur in solid, liquid and gaseous phases (Figure 2.5).

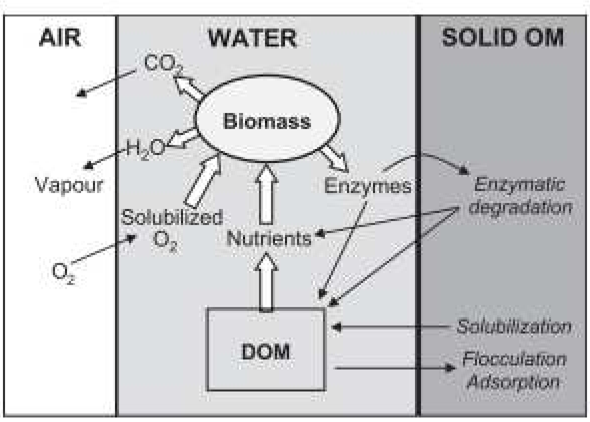


Figure 2.5: Schematic interactions involved in solid-liquid-gas phases during composting. Reprinted from Said-Pullicino et al. (2007a) with permission of Elsevier.

The solid phase, constituted by particles that contain organic and inorganic substances, interacts with aqueous phase. This liquid phase adheres to particles forming a biofilm which hosts biomass (microorganisms) and the biological processes of organic matter transformation. The biofilm contains soluble substrate that is oxidised by biomass in the presence of O_2 provided by gaseous phase (Petric and Selimbasic, 2008; Said-Pullicino et al., 2007a). The equilibrium between the reactions in each phase depends upon a set of abiotic factors, namely substrate biodegradability, moisture, free air space, nutrients, oxygen and pH.

2.5.1. Substrate biodegradability

Organic matter present in composting mixture is the main source of substrate to the microorganisms. Its properties determines the biological reactions rate (Berthe et al., 2007), and thus the length of the composting temperature-dependent phases.

In particular, the biodegradable organic matter content is of most importance, once it influences the self-heating capacity of the mixture to attain thermophilic conditions necessary for sanitising (Barrena et al., 2011; Ponsá et al., 2010). To categorise the potential biodegradability of organic wastes (to compare potential self-heating capacity), Barrena et al., (2011) proposed three main categories based on the measurement of the respiration activity expressed as the rate of oxygen consumption:

i) high biodegradable (≥5 mg O_2 gVS^{-1} h^{-1});
ii) moderate biodegradable (within 2 to 5 mg O_2 gVS^{-1} h^{-1});
iii)low biodegradable (≤2 mg O_2 gVS^{-1} h^{-1}).

where VS represents the volatile solids in the mixture.

In addition, biodegradability of a mixture has been related to its lignin content (Komilis and Ham, 2003; López et al., 2010; Vikman et al., 2002). Lignin is known to hinder the biodegradation of cellulose, by preventing ready access of microorganisms to this substrate. This aspect has implications in terms of composting process evolution namely thermophilic temperatures, reactions rates and time of composting. A correlation for biodegradable fraction of volatile solids (B) of a composting mixture has been formulated by Komilis and Ham (2003) as follows:

$$B = 0.850\,(\pm 0.084) - 0.010\,(\pm 0.003) \times L \qquad (2.15)$$

where B represents the biodegradable fraction of VS (in %) and L is the initial lignin content (as % VS) ranging from 10 to 55%.

In other studies, the cellulose to lignin ratio (C/L) has been used to assess the biological stability of organic mixtures (Paradelo et al. 2013). In case of C/L is up to 4, this indicates the organic matter is still potentially biodegradable, but values of 0.5 or less are indicative of biologically stable substrates (Komilis and Ham, 2003).

2.5.2. Moisture

Moisture content is considered as a key factor for successful composting. The main role of this parameter in the process is to host the biomass activity, by providing a medium for transport of organic substrate and oxygen diffusion required for metabolic reactions of microorganisms (Haug, 1993; Hubbe et al., 2010).

High moisture contents may fill the pores between particles, inducing a higher biofilm thickness that constrains oxygen permeability and diffusion, therefore enhancing anaerobic clumps (Liang et al., 2003). Contrarily, low moisture conditions can restrict the activities of microorganisms due to dehydration and provide a physically stable but biological unstable compost (Bertoldi et al., 1983).

In the literature, there is a wide range of reported moisture values that covers from 25% to 80% on a wet basis (Ahn et al., 2008a). But the most recommended values are between 50-70% (Haug, 1993; Liang et al., 2003; Richard and Hamelers, 2002).

Nevertheless, Ahn et al. (2008a) have pointed out that each material has singular physical, chemical and biological properties that can affect the relationship between moisture content and the water availability, particle size, porosity, and permeability of the overall composting mixture. In fact, Richard et al. (2004) verified that in manure-straw composting mixtures, gas permeability was augmented by increasing the moisture content from 50% to 79%, apparently due to the aggregations of fines resulting in an increasing proportion of large pores. In addition, an optimum moisture content was suggested to be 60-80% of the material water holding capacity (Ahn et al., 2008a).

Moisture regulation of a starting composting mixture is considered to be a simple task, requiring water addition, when overall moisture is low, or amendment with drying agents when excessive water is present.

2.5.3. Free air space and particle size

Free air space (FAS) is defined as the ratio of gas filled pore volume (Vg) to total compost mixture volume (Vt), as indicated in Figure 2.6. However, the total air void volume can be fractioned into inter-particle and intra-particle volumes. Usually, the voids inside the particle are considered inaccessible to microorganisms (Alburquerque et al., 2008; Ruggieri et al., 2009). Nevertheless, in practice the distinction between these two fractions is difficult to establish and thus the total air void fraction (here assumed as FAS) is the parameter considered (Alburquerque et al., 2008).

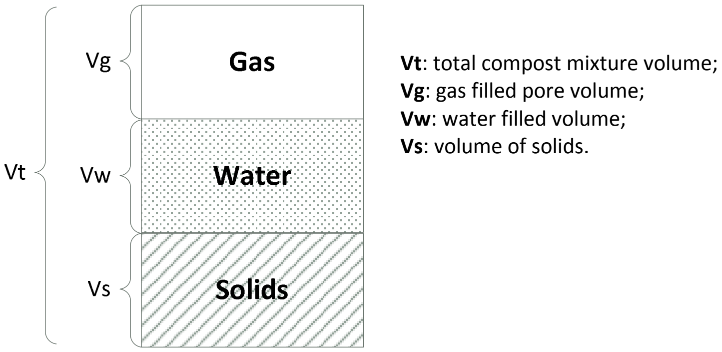


Figure 2.6: Schematic representation of volume fractions in a composting matrix. Adapted from Haug, (1993).

FAS is a physical property that may play an important role during composting, because it determines not only the air quantity throughout the mixture, but also the mass and heat transport mechanisms that intrinsically regulate microbial kinetics (Agnew and Leonard, 2003; Richard et al., 2004).

Suitable FAS is necessary to maintain aerobic conditions and compensate oxygen depletion that results from substrate degradation. Optimum values for FAS may be in the range of 30-60% (Ruggieri et al., 2009). Lack of air voids can increase the length of the composting time and lead to anaerobic conditions. However, excessive FAS allows significant heat and moisture losses which can also hinder microbial reaction rates. Nevertheless, references to FAS as high as 85-90% without significant negative impact are reported by Ahn et al. (2008b).

FAS depends not only on the moisture content, as previously stated in section 2.5.2, but also on the structural characteristics of the materials used such as bulk density (BD) and particle size. BD represents the mass of material within a given volume and a decrease of air voids volume is in general associated to high BD. Particle size determines the availability of substrate in biofilms that host microbial activity and a maximum accessibility is attained with smaller particle sizes. However, smaller particles reduce FAS and therefore a balance between these two properties must be found for successful composting (Rynk et al., 1992).

Air picnometry is considered to be the most adequate methodology to quantify FAS. The air volume of a mixture is determined by applying the Boyle’s Law to an air picnometer constituted by two chambers (Figure 2.7).

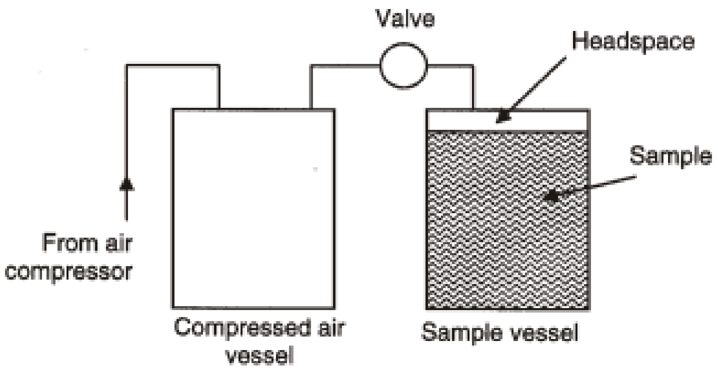


Figure 2.7: Simple representation of air picnometer. Adapted from Agnew et al. (2003).

Those chambers are connected with a valve, so the compressed air vessel with known volume (V_{gc}) can be isolated. The sample is placed in the sample vessel and the air vessel is pressurised to a set pressure (P_1). When both chambers are connected in closed system, the air pressure is allowed to equilibrate (P_2). By assuming that in a closed system with moderate pressures the temperature remains constant, then the term nRT of the ideal gas law remains constant (Agnew et al., 2003). Boyle's law can be used to derive the general equation for the pressure–volume relationships under these two pressure regimes, by assuming that relative pressure in the reservoir chamber is initially zero:

$$P_1V_{gc} = P_2V_t \tag{2.16}$$

where V_t, volume of overall system (compressed air chamber, air volume in sample chamber, pipes and fittings). By ignoring the air volume of fittings and pipes, Eq (2.16) results in:

$$V_g = \frac{(P_1-P_2)}{P_2}V_{gc} \tag{2.17}$$

where Vg is the volume of gas air voids in the sample.

FAS can be expressed as the ratio of gas filled pore volume of the sample (Vg) to total sample volume (Vs),

$$FAS = \frac{(P_1-P_2)V_{gc}}{P_2V_S} \tag{2.18}$$

The unavailability of commercial picnometers with adequate volume to analyse this type of solid matrices can be a drawback in quantifying FAS. Thus many researchers have been using self-made picnometers with 1 to 25 L of capacity (Agnew et al., 2003; Ahn et al., 2008b; Berthe et al., 2007; Richard et al., 2004). Additionally, some theoretical and empirical correlations have been proposed in the literature to quantify FAS, as detailed in Table 2.5.

Table 2.5: Overview of theoretical and empirical relationships used for FAS determination.
Adapted from Alburquerque et al., (2008); Ruggieri et al., (2009).

	Equation		Reference	Observations
Theoretical	$FAS = 1 - BD_w\left(\frac{1-DM}{D_w} + \frac{DM \times OM}{PD_{OM}} + \frac{DM \times (1-OM)}{PD_{ash}}\right)$	(2.19)	Richard et al. (2004)	More adequate when bulk densities are higher than 400 kg m^{-3}.
	$FAS = 1 - BD_w\left(\frac{MC}{D_w} + \frac{(1-MC)}{PD}\right)$	(2.20)	Agnew and Leonard (2003)	-
	$FAS = 1 - \left(\frac{BD_w \times DM}{G_S \times SG_w}\right) - \left[\frac{BD_w \times (1-DM)}{SG_w}\right]$ With $\frac{1}{G_S} = \frac{OM}{SG_{OM}} + \frac{(1-OM)}{SG_{ash}}$	(2.21)	Haug (1993)	-
	$FAS = \left(1 - \frac{BD_w}{PD_w}\right)$	(2.22)	Adhikari et al. (2009)	-
Empirical	$FAS\ (\%) = 100 - 0.0889 \times BD_w$	(2.23)	Agnew and Leonard (2003)	Applicable for high-moisture and/or low density mixtures
	$FAS = 0.91 - 2.46 \times MC^{8.69}$	(2.24)	Uao et al. (1993)	-
	$FAS\ (\%) = 101.84 - 0.0922BD_w$	(2.25)	Mohee and Mudhoo (2005)	Applicable for high-moisture and/or low density mixtures
	$FAS\ (\%) = 91.558 - 0.1713BD_d$	(2.26)	Mohee and Mudhoo (2005)	Applicable for high-moisture and/or low density mixtures

BD_d: dry bulk density; BD_w: wet bulk density; DM: dry matter fraction; D_w: water density (1000 kg m^{-3}); MC: moisture fraction; OM: organic matter fraction; PD: particle density; PD_w: wet particle density; PD_{OM}: organic matter particle density (1600 kgm^{-3}); PD_{ash}: ash particle density (2500 kgm^{-3}); SG_w: water specific gravity; SG_{OM}: organic matter specific gravity (1.0); SG_{ash}: ash specific gravity (2.5).

According to Ruggieri et al., (2009) when air picnometry to FAS quantification is not possible, equations (2.19), (2.23) and (2.25) are considered to be adequate alternatives, taking into account the observations indicated in Table 2.5.

2.5.4. C:N ratio

Carbon and nitrogen are the most important nutrients for microbial decomposition. Carbon is the main energy source and the basic building block for growth, while nitrogen is a key element of proteins, amino acids and enzymes necessary for cell synthesis and function (Haug, 1993; Rynk et al., 1992).

Therefore, composting is mainly dependent on the relative proportion of carbon and nitrogen (C:N ratio) in a starting mixture. If nitrogen is limiting (high C:N ratio), development of microbial populations will be impaired and the available carbon will be slowly decompose, thus extending the needed composting period. On the other hand, excess nitrogen (low C:N ratio) will allow that available carbon to be fully used, without complete nitrogen stabilisation. In these conditions, nitrogen losses by volatilisation or leaching may be enhanced (Gajalakshmi and Abbasi, 2008).

An initial C:N ratio ranging from 25-30 is often considered optimum for composting evolution (Abdullah and Chin, 2010; Hamoda et al., 1998; Huang et al., 2006). Although C:N ratios from 20 to 40 are also considered adequate (Rynk et al., 1992).

These ratios are established assuming that both carbon and nitrogen are relatively biodegradable. However in practice, C and N content are quantified by chemical analysis, which does not necessarily reflect the bioavailable content of these elements. According to Haug (1993) most of nitrogen is relatively biodegradable, since it is mainly present in the form of proteins. On the contrary, carbon bioavailability is dependent on the nature of bounds in the different compounds, which in certain cases can be highly resistant to biological degradation.

For the formulation of balanced mixtures that can potentiate nitrogen preservation during composting, C:N ratio must be based on aerobic biodegradable carbon (Sánchez, 2007). In fact, De Guardia et al. (2010a) concluded that chemical C:N ratio may not allow to predict the amount of organic matter biodegradable during composting.

A methodology to determine aerobic biodegradable organic carbon (BOC) has been proposed by Puyuelo et al. (2011) by studying sixteen organic solid wastes from different origins (manures, municipal solid wastes and their organic fraction, sludge and pruning wastes). BOC determination consisted in a small scale respirometric test (150 g of sample) where CO_2 liberated during aerobic degradation of organic matter was monitored and assumed to be a direct measure of the aerobic biodegradable carbon. Results indicated that all samples presented BOC:N ratios significantly lower that C:N values (representing 6 to 60% of the C:N ratio based on total organic carbon (TOC) measure) which supported the recommendations to use BOC:N, instead of C:N. In practice, differences between BOC:N and C:N can justify the fact that a higher C:N ratio does not always indicate prevention of N loss during composting of organic wastes (Liang et al., 2006). Nevertheless, the time of essay necessary to quantify BOC can be long (25 days or more) which represents a drawback in comparison to TOC determination. Therefore, an alternative method to estimate BOC in a short period of time is still necessary.

2.5.5. Aeration

In aerobic processes, oxygen concentration is a critical factor that influences microbial growth, maintenance and synthesis (Garcia-Ochoa et al., 2010). During composting, air must be provided not only to satisfy the oxygen demand for organic matter oxidation, but also to ensure oxygen does not become limiting leading to anaerobic conditions (Kulcu and Yaldiz, 2004).

Stoichiometric oxygen demand may vary between 1.2 to 4.0 g of O_2 per gram of biodegradable volatile solids and a pore space minimum oxygen concentration of 5% (v/v) is necessary to assure aerobic conditions (Haug, 1993).

In addition, aeration removes heat, water vapour and other gases from the composting matrix. Heat removal can be necessary to prevent high temperatures that hamper biological activity. Moisture removal, by means of evaporative cooling, is also required to avoid water accumulation between pore space, which constrains oxygen permeability and diffusion. Nevertheless, the balance between the aeration rate and water/heat removal is not an easy task and depends on the stage of composting. The initial stages are characterised by high oxygen consumption (high aeration rates), while during maturation phase air flow rate can be much smaller.

Main aerations methods are based on natural convection or forced aeration (Haug, 1993) and different control strategies have been used/tested to regulate the aeration rate throughout the composting process, as indicated in Table 2.6.

According to Papadimitriou et al. (2010), aeration rate is the most significant manipulated parameter in composting, since it directly affects a wide number of parameters such as temperature gradients, water availability, O_2 concentration, reaction rate and moisture gradient.

Table 2.6: Brief description of control systems to regulate aeration rate in composting.

Aeration rate controlling strategies	Description	References
Feedback control based on O_2 content	Control signal is provided by oxygen sensors to adjust inlet airflow rate and maintain a set point for O_2. Several set point ranges have been recommended: 10-18%, 15-20%. Also, outlet oxygen gas levels between 5-15% are suggested.	de Bertoldi et al. (1988); Ekinci et al. (2006); Magalhães et al. (1993); Puyuelo et al. (2010)
Feedback control based on temperature	If temperature exceeds the set point, aeration rates are increased to maintain a desired temperature. On the other hand, when temperature is lower than set point, air flow rates are reduced or air can be provided in an on/off sequence. Usual threshold lays between 50-60ºC.	Bari and Koenig (2001); Bari et al. (2000); Ekinci et al. (2006)
Feedback control based on oxygen uptake rate	Biological activity is evaluated through the continuous measure of the oxygen uptake rate (OUR). The control loop determines variations between consecutive OUR measures and evaluates if those variations are associated with an increase, decrease or constant airflow.	Puyuelo et al. (2010)

2.5.6. pH

pH of the substrate matrix may change as result of the several biochemical transformations to which organic matter is exposed. These alterations may affect the type and activity of the microorganisms involved (Gajalakshmi and Abbasi, 2008). Bacteria prefer pH near neutrality (6-7.5) and fungi develop better in slightly acidic environments although, they are more tolerable to a wider range of pH (5.5-8.0) than bacteria (Bertoldi et al., 1983; Zorpas et al., 2003).

Starting from a neutral mixture (pH 7), normal evolution of pH during composting is reported to present an initial drop to acidic values, as consequence of volatile fatty acids (VFA) formation and poor buffer properties of the mixture. However, the presence of VFA is transitory, since as the process evolves they are then consumed and ammonification increases. So, usually pH rises towards 8 or even 9 (Gajalakshmi and Abbasi, 2008; Rynk et al., 1992). While pH remains acidic, a reduction on the degradations rates and delay of the thermophilic phase are observed. On the other hand, when pH reaches alkaline values, conditions for ammonia volatilisation are favoured if organic matrix temperatures are thermophilic (Pagans et al., 2006).

In some studies lime was added to starting mixtures for preventing initial drop of pH to acidic values (Fang and Wong, 1999; Gabhane et al., 2012; Singh and Kalamdhad, 2013; Wong et al., 2009) which enhanced degradation rates. Nevertheless, this strategy must be cautiously used, since it can directly affect the acid-base equilibrium that regulate nitrogen conservation by increasing ammonia losses. Therefore, in practice pH control is not an easy task.

2.6. Compost quality criteria

Final composting products may offer many potential benefits, including soil amendment and other environmental applications. Indeed, compost is commonly used to improve physical, biological and chemical soil properties, because it can increase soil organic matter, reduce erosion, enhance water retention capacity and pH buffer capacity. In addition, compost may also enhance soil physical structure (aggregate stability, density, pore size) and biological activity (JRC - European Commission, 2012).

In this scope, the main question is “what defines the quality of composting end-product for soil amendment and which are the parameters that reflect that quality?”

According to Bernal et al. (2009) composting end-products must present “…a high degree of stability or maturity, which implies a stable OM content and the absence of phytotoxic compounds and plant or animal pathogens”. Nevertheless stability and maturity are two distinct concepts:

1) Stability refers to “…the extent to which readily biodegradable organic matter has decomposed…” (Adani et al., 2006; Komilis, 2015; Wichuk and McCartney, 2010) and is determined by indices of microbial activity. However, microbial decomposition can be inhibited by other factors than low amount of biodegradable organic material, like lack of nutrients, low moisture content or even inhibitory substances. Therefore, Komilis (2015)

proposes that stability would be better defined as "the resistance against further microbial decomposition as long as there is no inhibition by other factors not relevant to organic matter".

2) Maturity is defined as the "…degree or level of completeness of composting …" without " … a negative effect on seed germination or plant growth" (Bernal et al., 2009). This means that maturity is an agronomic parameter, meaning that a mature compost should be non-phytotoxic to plants, but ideally should be beneficial to their growth (Komilis, 2015).

A set of parameters has been indicated in the literature to assess compost quality, though it is acceptable that "quality" cannot be established by a single property (Bernal et al., 2009; Komilis, 2015). Figure 2.8 shows an overview about parameters to assess compost quality.

Typically, the biological-microbiological parameters are allied with the term ''stability'', while the seed/plant growth indexes are related with ''maturity''. However, the association of the physico-chemical properties cannot be clearly associated to these terms. (Komilis, 2015).

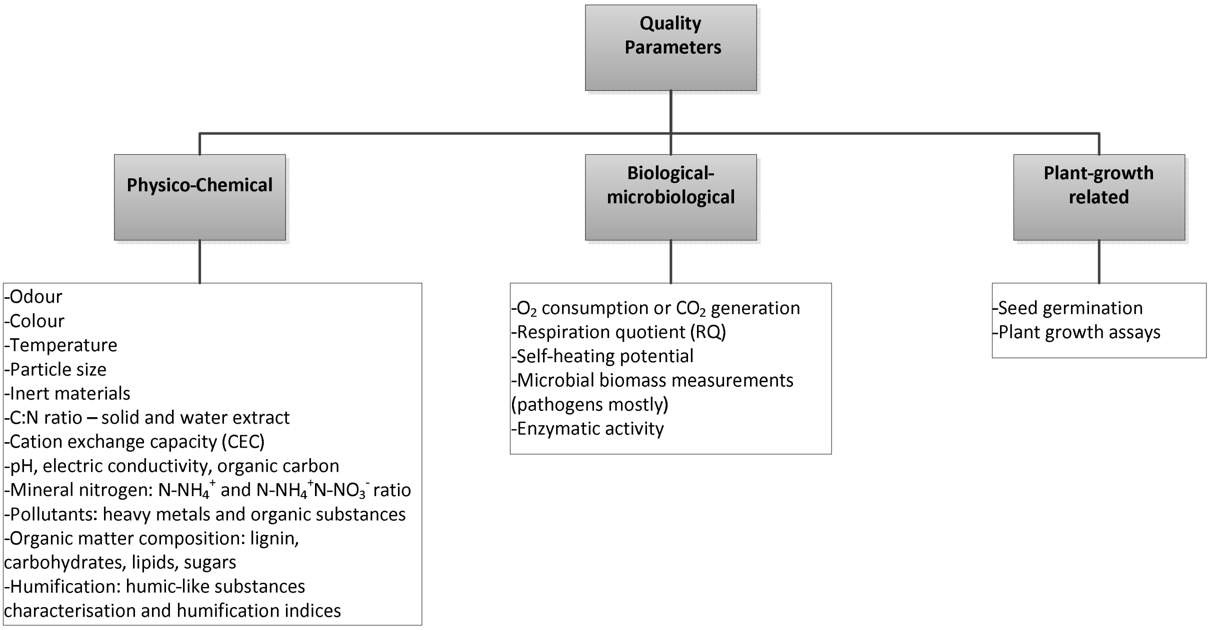


Figure 2.8: Overview of parameters proposed to assess compost quality.
Adapted from Bernal et al. (2009) and Komilis (2015).

Compost physical appearance may provide a general idea of the extent of decomposition and maturity of the material, by presenting a black dark colour with an earthy odour, soil-like texture and no recognisable substrate particle (Adhikari et al., 2009; Iqbal et al., 2010; Mohee and Mudhoo, 2005; Neklyudov et al., 2006). Nevertheless, these properties do not deliver a quantitative information about the maturity level achieved by the compost (Bernal et al., 2009).

Chemical properties are widely taken into consideration for establishing compost maturity degree, namely C:N ratio, cation exchange capacity, mineral nitrogen and humic substances (Bernal et al., 2009).

C: N ratio is frequently stated as a maturity parameter, because it is used to evaluate the progress of composting, and it should decline as process evolves. In that scope, starting from a C:N balanced mixture, the final composting end-product should exhibit a C:N ratio less than 20 or even between 10 to15, which is the value for stable soil organic matter (de Varennes, 2003; Gajalakshmi and Abbasi, 2008). In fact, when an immature compost with a higher C:N ratio is added to soil, the microorganisms will appropriate some of the soil available nitrogen to transform the organic carbon of the compost, which may cause a plant nitrogen deficit. Nevertheless, some studies refer that C:N is not an adequate indicator of maturity (Goyal et al., 2005; Komilis, 2015), since C:N ratio of the mature compost is greatly affected by characteristics of the mixture, namely in terms of carbon biodegradability, as stated in section 2.5.4 and no limit value can be defined for general application. Goyal et al. (2005) emphasised that the decrease of C:N ratio along with other chemical properties of the composts, can be taken as a reliable parameter for compost maturity assessment.

Cation exchange capacity (CEC) represents the maximum quantity of total cations that a matrix can adsorb or exchange at a given pH. As composting progresses and organic matter is humified, more carboxyl and phenolic functional groups are formed and CEC is increased. Minimum values of CEC for mature composts are described to be 60 to 67 cmol kg^{-1} on a dry ash free basis (Harada and Inoko, 1980; Jiménez and García, 1992).

The ratio between the mineral forms of nitrogen has been also used as a maturity criteria. In fact, during the early stages of composting, ammonium is released by ammonification and then through the maturation phase it can be nitrified to nitrate. Therefore, immature composting products can present high $N\text{-}NH_4^+$:$N\text{-}NO_3^-$ ratio, while as maturation proceeds there is a sink of $N\text{-}NH_4^+$ with a parallel increase of nitrate and consequent depletion of $N\text{-}NH_4^+$:$N\text{-}NO_3^-$. A value of 0.16 has been established by Bernal et al. (1998) to describe a mature compost. However, this ratio is only useful when the amount of available nitrogen is high and thus a limit of concentration for nitrate of 50 mg NO_3^- kg^{-1} fresh weight has been suggested (Wichuk and McCartney, 2010). Additionally, the individual concentration of NH_4^+ has been indicated as a maturity index and a limit value of 400 mg $N\text{-}NH_4^+$ was proposed by Zuconni and de Bertoldi (1987).

The degree of organic matter humification is considered to be an important criterion of maturity and stability. For that purpose, humification indexes associated to humic substances quantities and fractioning have been established and are summarised in Table 2.7. Nevertheless, these indexes are not useful for all composts, since their final values depend on the properties of the materials used in the starting composting mixture (Bernal et al., 2009). In particular, some wastes like manure (Bernal et al., 1998; Bustamante et al., 2008b; Paredes et al., 2000) or anaerobic digestates (Bustamante et al., 2012) present humification indexes, at the beginning of the composting, higher than the limits indicated in Table 2.7 and therefore maturity can be established using these guidelines. As alternative, evolution of humification parameters during composting (that are expected to increase) is considered a good indicator of organic matter stabilisation and maturation, but a single value cannot be defined (Bernal et al., 2009; Wichuk and McCartney, 2010).

Table 2.7: Humification indexes for evaluation of compost maturity degree.
Adapted from Jiménez and García (1992); Liu and Hue (1995).

Humification index	**Calculation expression**		**Optimal value for acceptable degree of maturity**
Humification ratio – HR	$HR = \frac{C_{ext}}{TOC} \times 100$	(2.27)	
Humification index – HI	$HI = \frac{C_{HA}}{TOC} \times 100$	(2.28)	>13%
Percentage of humic acids – PHA	$PHA = \frac{C_{HA}}{C_{ext}} \times 100$	(2.29)	>62%
Polimerisation degree – DP	$DP = \frac{C_{HA}}{C_{FA}}$	(2.30)	>1.6
Fulvic acid concentration - C_{FA}	-		≤12.5 g kg^{-1}
Extractable carbon - C_{ext}	-		≤60 g kg^{-1} ≥6 g g^{-1} water soluble organic carbon

C_{ext} – NaOH extractable carbon; C_{HA} – carbon associated to extractable humic acids; C_{FA} – carbon associated to extactable fulvic acids; TOC – total organic carbon in solid sample.

Biological tests are also considered important for estimating quality of composting end-products. Aerobic respiration rates have been widely used for assessing compost stability and biological activity. They are based on assessing the heat released (self-heating test) or on determining either the O_2 uptake or the CO_2 production from the biological and chemical activity of a compost sample (Gómez et al., 2006). An equivalency between CO_2 production and self-heating test is presented in Table 2.8.

Table 2.8: Relationship between respirometric tests.
Adapted from Gómez et al. (2006).

CO_2 production ($mg\ CO_2\ g^{-1}\ C\ d^{-1}$)	**Respiration rate**	**Self-heating grade equivalent**	**Material status**
0-2	Very slow	V	Stable
2-8	Moderately slow	IV-III	Stable
8-15	Medium	II-I	Fresh
15-25	Medium-high	I	Fresh
>25	High	I	Raw

Concerning O_2 consumption tests, values less than 0.5 mg O_2 g^{-1} VS h^{-1} are referred to stable materials (Gómez et al., 2006). These tests are indicative of the biodegradability level of organic matter still present in the final compost and which is inversely associated to the stability level (Bernal et al., 2009; Wichuk and McCartney, 2010).

Biochemical reactions during composting are catalysed by enzymes and their activity can be related with the level of organic matter stability (Gajalakshmi and Abbasi, 2008; Wichuk and McCartney, 2010). In this scope, dehydrogenases (DH) are involved in the oxidation of glucose and the measurement of its activity has been used to assess the microbial activity, due to DH role in the microbial respiratory chain (Tiquia et al., 2002). The dehydrogenase activity is considered to be a general index of biological activity (Vargas-García et al., 2010) which is expected to decrease as organic matter is transformed into more stable forms. Although enzymatic activity is a relatively simple and fast determination, Wichuk and McCartney (2010) pointed out that enzymatic activities in stable composts are dependent of the feedstock’s origin, thus making difficult to set a limiting value.

Phytotoxicity tests are also used to appraise compost maturity degree. Phytotoxicity is associated with the phenomenon of accumulation in plant tissues of potentially harmful substances (e.g. volatile fatty acids, ammonia, phenolic substances, soluble salts, etc) at levels that might impair normal growth and development (Tiquia et al., 1996).

Phytotoxicity assays are classified into germination and growth tests (Bernal et al., 2009). Germination tests are capable of providing information about the presence or not of significant quantities of phytotoxins in a compost, are rapid to perform (48-76 hours) and widely used (Gajalakshmi and Abbasi, 2008). On the other hand, plant growth essays provide information about compost effect after the development of plant (roots and elongation). But these tests are more time consuming (10-12 days). In general, through phytotoxicity tests is not possible to identify the specific substance that is present in final compost, and only information about the overall acceptability of the compost is obtained. Germination index (GI) is a lumped parameter often used to evaluate phytotoxicity and values greater than 50% are indicative of a phytotoxin-free compost (Bernal et al., 2009). But other values are reported for safe soil application, namely 60% (Gómez-Brandón et al., 2008) and 80% (Tiquia and Tam, 1998).

Biological tests may also encompass sanitation degree of final compost by evaluating the presence of pathogens. *Salmonella* spp and *E. coli* are the indirect indicator species mostly determined. *E.coli* is usually investigated when faecal materials are used in the starting mixture. *Salmonella* may be present in food wastes and in raw materials with an animal origin (Bustamante et al., 2008a). It is generally accepted that in mature composts, *Salmonella* spp.

must be absent in 25 g of compost and *E. coli* must not exceed 1000 colony forming unit (cfu) per g of compost (Noble et al., 2009).

In summary, the establishment of the compost quality is of utmost importance to potentiate composting end-products value for soil application. In practice, despite the recognised importance, there is still lacking an adequate integration of the more reliable parameters for quality classification (Komilis, 2015). Nevertheless, quality standards are being defined. According to technical proposal of the end-of-waste criteria, in Europe, on biodegradable waste subject to biological treatment (JRC - European Commission, 2014) there are several national compost quality standards, but a harmonisation of the criteria established is still required. In fact, there are some European countries where the compost quality assurance is required or promoted by legislation or regulatory authorities (Luxemburg, Spain and Belgium), whilst in others countries quality declaration is purely voluntary or even inexistent. In general, compost quality regulations or schemes include minimum quality requirements for achieving desired levels of health and environmental protection, like:

1) minimum organic matter content, to ensure basic usefulness and to prevent dilution with inorganic materials;

2) sufficient stability/maturity;

3) absence of certain pathogens that pose health risks;

4) limited amount of macroscopic impurities (as a basic requirement for usefulness and to limit the risks of injuries);

5) concentrations of pollutants (mainly heavy metals and specific organic pollutants).

Nevertheless, the limits set for each requirement differ from country to country, as well as the guidelines for compliance testing (namely number of tests, protocols for sampling, analysis, etc). In this scope, efforts to establish an European harmonised standard for composting end-product quality are ongoing and to the author’s best knowledge the lasted proposed criteria for compost quality are the ones presented in Table 2.9.

Table 2.9: European proposed quality criteria for composting end-products.
Adapted from JRC - European Commission (2014).

Parameter		Value	Comment
Minimum organic content		15% (dry weight basis)	Minimum content after composting phase and prior to any mixing with other materials; limit established to prevent dilution with mineral components like sand or soil
Stability		-Respirometirc index of maximum 25 mmol O_2 kg^{-1} $_{\text{organic matter}}$ h^{-1}, measured according to EN 16087-1; -Minimum Rottegrad III, IV or V (self-heating test temperature rise of maximum 30ºC above ambient temperature) measured according to EN 16087-2	A minimum stability should avoid unwanted emissions during transport and storage and prevent materials from entering the market without proper treatment.
Pathogens content		No *Salmonella* spp in 25g sample Maximum 1000 CFU of *E. Coli* in 1g of fresh sample	Requirement to be complemented with process temperature-time criteria (as detailed in Table 2.3).
Viable weeds and plant propagules		2 viable weed seeds L^{-1} of compost	Requirement to be complemented with process temperature-time criteria (as detailed in Table 2.3).
Macroscopic impurities > 2 mm		0.5 % (dry weight basis)	There is a need to distinguish natural impurities from man-made impurities (glass, metal and plastics).
Heavy metal content		(mg kg^{-1} dry weight basis)	Maximum content after composting phase and prior to any mixing with other materials
	Zn	600	
	Cu	200	
	Ni	50	
	Cd	1.5	
	Pb	120	
	Hg	1	
	Cr	100	
Organic pollutants			Maximum content after composting phase and prior to any mixing with other materials
	PAH 16	6 mg kg^{-1} dry weight basis	

PAH16: sum of 16 US EPA priority list polycyclic aromatic hydrocarbons

2.7. Compost utilisation

Composting of organic wastes is an important beneficial recycling method and the soil application of compost end-product can assume different suitable uses. Figure 2.9 provides an overview of compost use in Europe.

By far, agriculture and landscaping are the main application areas for compost. The potential of final compost to enhance soil quality in terms of physical, chemical and biological characteristics makes composting end-products attractive for these uses.

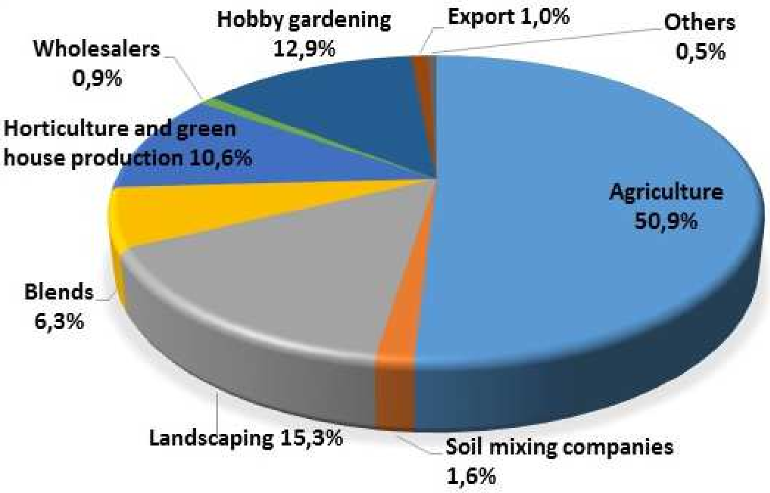


Figure 2.9: Composting end-products use for soil application. Adapted from JRC - European Commission (2014).

Nevertheless, several authors highlight that compost concentration in some nutrients, especially nitrogen, cannot be sufficient to provide a complete nutritional support for crops (Gajalakshmi and Abbasi, 2008; Hargreaves et al., 2008; Iglesias-Jimenez and Alvarez, 1993) because the N fraction that is available for plants is relative small in comparison with mineral fertilisers. Therefore, the use of compost with a suitable and controller supplement of N fertilisers are recommend with beneficial effects for soil-plant system (Iglesias-Jimenez and Alvarez, 1993).

Application of composting end-products in the landscaping represents also an important use. In particular for the remediation of brownfield areas that exhibit very degraded top soils, compost incorporation is considered to provide finely texture particles capable of retaining water and nutrients (Jones et al., 2009).

In addition to heavy metals contaminated sites for which remediation strategies are generally based on the extraction or stabilisation of the pollutants (Cunha-Queda et al., 2010; Farrell and Jones, 2009), special attention has been focused on the approaches that promote the stabilisation of the contaminants, since they are less onerous and do not affect soil structure as extraction methods do (Mench et al., 2003).

In this context, composts obtained from several organic wastes have been studied and considered for heavy-metal immobilisation in soils, by inducing metal precipitation at alkaline pH level or by promoting metals sorption into the organic matter matrix (Cunha-Queda et al., 2010; Farrell and Jones, 2009; Paradelo et al., 2011; Park et al., 2011; Tandy et al., 2009; Theodoratos et al., 2000). For example, soil application of poultry compost decreased Cd uptake of plants by 56-62.5% and green waste compost reduced Cd and Zn leaching from soil by 48% (Park et al., 2011).

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Part B. Properties of mixtures and reactor assembly

This second part of the thesis includes two studies that precede the composting experimental tests.

Firstly in Chapter 3, statistical mixture design tools are applied to predict and understand the influence of a mixture proportion on free air space. In Chapter 4, special attention is given to the definition and operational testing of an experimental apparatus for monitoring the composting process.

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3. Prediction of free air space in initial composting mixtures by a statistical design approach*

Free air space (FAS) is a physical parameter that can play an important role in composting processes to maintain favorable aerobic conditions. Aiming to predict the FAS of initial composting mixtures, specific materials proportions ranged from 0 to 1 were tested for a case study comprising industrial potato peel waste characterized by low air void volume, thus requiring additional components for its composting.

The characterization and prediction of FAS for initial mixtures involving potato peel, grass clippings and rice husks (set A) or sawdust (set B) was accomplished by means of an augmented simplex-centroid mixture design approach. The experimental data were fitted to second order Scheffé polynomials. Synergistic or antagonistic effects of mixture proportions in the FAS response were identified from the surface and response trace plots in the FAS response. Moreover, a good agreement was achieved between the model predictions and supplementary experimental data. In addition, theoretical and empirical approaches for estimating FAS available in literature were compared with the predictions generated by the mixture design approach.

This study demonstrated that the mixture design methodology can be a valuable tool to predict the initial FAS of composting mixtures, specifically in making adjustments to improve composting processes containing primarily potato peel.

Keywords
Industrial potato peel; free air space; mixture design; composting

*The information presented in this chapter was based upon the publications:

Soares, M.A.R., Quina, M.J., Quinta-Ferreira, R., 2013. Prediction of free air space in initial composting mixtures by a statistical design approach. J. Environ. Manage. 128, 75–82.

Soares, M.A.R.; Quina, M.J.; Quinta-Ferreira, R, 2012. Industrial potato peel composting: blend formulation using mixture design analysis, presented at ORBIT’2012 – Global assessment for organic resources and waste management, 12-15th June, In Proceedings, pg 5a-190 to 5a-197, Rennes, France *(oral communication)*.

3.1. Introduction

Potato peel (PP) waste is a by-product from the processed potato products industries such as French fries, chips and puree. Although PP is a zero value waste for those plants, disposal, sanitation, and environmental problems must be overcome (Arapoglou et al., 2010). During the processing of potatoes at industrial level, depending on the technology used (steam, abrasion or lye peeling) losses caused by potato peeling can reach 15 to 40% of the total raw materials (Schieber et al., 2001).

Though the food industry manages PP waste as a non-valuable by-product, its composition may be suitable for several applications such as dietary fibre for baking products and animal feeding (Djomo et al., 2008), biohydrogen and ethanol production (Arapoglou et al., 2010; Djomo et al., 2008; Mars et al., 2010), source of natural antioxidants (Al-Weshahy et al., 2010; Schieber et al., 2001; Wijngaard et al., 2012) and bio-methane production (Kaparaju and Rintala, 2005; Kryvoruchko et al., 2009; Parawira et al., 2004). Our study addressed the PP valorisation through composting. This process may be defined as the biological decomposition and stabilization of organic subtracts, under aerobic conditions that allow development of thermophilic temperatures as result of biologically generated heat, to obtain a final product that is stable, free of pathogens and plant seeds that can be beneficially applied to land (Haug, 1993). It should be noted that PP valorisation by composting can be quite interesting for the food industry, given that a volume reduction of by-products up to 40% can be achieved (Schaub and Leonard, 1996). Nevertheless, to the best of our knowledge, PP composting has been scarcely addressed in literature. PP waste is usually characterised by high interparticle water content which might hinder composting evolution, due to higher oxygen diffusion resistance in pores between particles. Therefore, its valorisation may require previous mixture with other materials, aiming to reach an adequate formulation for composting.

Mixture formulations for composting are often based on physical and chemical properties of the wastes (Barrena et al., 2011) in order to adjust moisture content and C:N ratio to optimal values and favourable to the growth and activity of microbial populations. Indeed, it is well known that one of the most important factors for thermophilic composting is the carbon to nitrogen ratio (C:N) with optimum values around 25-30:1 (Abdullah and Chin, 2010; Hamoda et al., 1998; Huang et al., 2006) and moisture content in the range of 50-65% (Abdullah and Chin, 2010; Liang et al., 2003).

However, more recently the free air space (FAS) of a mixture has also been indicated as a physical property that may play an important role during composting (Agnew et al., 2003;

Barrena et al., 2011; Eftoda and McCartney, 2004; Richard et al., 2004; Ruggieri et al., 2009). This parameter is defined as the ratio of gas filled pore volume to total compost mixture volume and it determines the air quantity and movement inside the process, as well as the intrinsic air content, carbon dioxide, moisture and heat removal from the system (Richard et al., 2004; Ruggieri et al., 2009). FAS depends on the structural characteristics of the materials used such as bulk density, particle density and water content. A minimum value of 30% is usually required to ensure aerobic condition in the composting mixture (Haug, 1993), and optimum values for FAS may be in the range of 60%. In literature there are references to FAS as high as 85 to 90% without significant negative impact (Ahn et al., 2008; Ruggieri et al., 2009). To quantify FAS for a given composting mixture, some theoretical empirical correlations have been developed (Agnew et al., 2003; Haug, 1993; Oppenheimer et al., 1997; Richard et al., 2004). Moreover, experimental procedures by using air pycnometry have been studied and compared (Ruggieri et al., 2009). But so far, the study of an approach able to predict FAS for a given mixture based on its individual components proportions has been seldom addressed (Soares et al., 2012).

Mixture design is a statistical technique associated to the concept of planning and execution of informative experiments concerning a mixture of different components, and it has been widely used to establish formulations in chemical, pharmaceutical and food industries (Eriksson et al., 1998). The method consists in varying the proportions of two or more ingredients of the mixture and studying the influence of the independent variables (proportions of different components) into the measured response, which is dependent on the ingredient composition (Akalin et al., 2010).

In this scope, the present work aims to use the mixture design approach to understand and predict the influence of each component, on the FAS of an initial mixture containing primarily potato peel waste (PP) for further composting. Rice husk (RH) or sawdust (SD) were selected as bulking agents, and grass clippings (GC) as nitrogen source.

3.2. Materials and methods

3.2.1. Composting materials

The materials used were collected from different sources: potato peel (PP) is from a national industry of potato chips, rice husk (RH) was provided by a rice husking factory, sawdust (SD) is from a local pine sawmill, and grass clippings (GC) were obtained from a national football stadium. Grass clippings were sieved through a 5 cm mesh to obtain a homogeneous material in size and shape. The other materials did not require any specific treatment. The characterisation of the each material was carried out using a composite sample of 25 L obtained from five individual samples of 5 L taken from the initial laboratory samples of about 120 L. Then, each composite sample of 25 L was homogenised and divided into four parts, with one being eliminated. This procedure was repeated until samples of about 1-2 L were obtained for further analysis.

3.2.2. Mixture design establishment and validation

The mixtures tested in this study were grouped in two sets (Set A and Set B) according to the type of bulking agent used. Each set comprised three mixture factors or ingredients (set A: PP + GC + RH; set B: PP + GC + SD) and their mixing proportions were individually allowed to range from 0 to 1.

Therefore, a regular and triangular experimental design region is expected for each set, with the constraint that the sum of all feedstock's proportions must be 1. Vertices of the design region correspond to the formulations that are pure blends.

The main objective of this design was to predict the free air space (desired response) for any mixture tested by modelling the mixing surface with mathematical equations. It was assumed that the measured response was only dependent on the relative proportions of the ingredients (considered as independent variables) but not on the amount of the mixture.

The models considered in this study were Scheffé canonical polynomials (Smith, 2005):

Linear:

$$E(Y)=\sum_{i=1}^{q}\beta_i X_i \tag{3.1}$$

Quadratic:

$$E(Y)=\sum_{i=1}^{q}\beta_i X_i+\sum_{i=1}^{q-1}\sum_{j=i+1}^{q}\beta_{ij}X_iX_j \tag{3.2}$$

Special cubic:

$$E(Y)=\sum_{i=1}^{q}\beta_i X_i+\sum_{i=1}^{q-1}\sum_{j=i+1}^{q}\beta_{ij}X_iX_j+\sum_{i=1}^{q-2}\sum_{j=i+1}^{q-1}\sum_{k=j+1}^{q}\beta_{ijk}X_iX_jX_k \tag{3.3}$$

Full cubic:

$$E(Y)=\sum_{i=1}^{q}\beta_i X_i+\sum_{i=1}^{q-1}\sum_{j=i+1}^{q}\beta_{ij}X_iX_j$$
$$+\sum_{i=1}^{q-1}\sum_{j=i+1}^{q}\gamma_{ij}X_iX_j\left(X_i-X_j\right)+\sum_{i=1}^{q-2}\sum_{j=i+1}^{q-1}\sum_{k=j+1}^{q}\beta_{ijk}X_iX_jX_k \tag{3.4}$$

Special Quartic:

$$
\begin{aligned}
E(Y) = {} & \sum_{i=1}^{q}\beta_i X_i + \sum_{i=1}^{q-1}\sum_{j=i+1}^{q}\beta_{ij} X_i X_j \\
& + \sum_{i=1}^{q-1}\sum_{j=i+1}^{q}\gamma_{ij} X_i X_j \left(X_i - X_j\right) + \sum_{i=1}^{q-2}\sum_{j=i+1}^{q-1}\sum_{k=j+1}^{q}\beta_{ijk} X_i^2 X_j X_k + \\
& \sum_{i=1}^{q-2}\sum_{j=i+1}^{q-1}\sum_{k=j+1}^{q}\beta_{ijk} X_i X_j^2 X_k + \sum_{i=1}^{q-2}\sum_{j=i+1}^{q-1}\sum_{k=j+1}^{q}\beta_{ijk} X_i X_j X_k^2
\end{aligned}
\tag{3.5}
$$

where *E(Y)* is the expected value of the output variable *Y*, *X* are the independent variables, β corresponds to the polynomial coefficients, *q* is the number of components (in this case *q*=3).

Since the experimental mixture region is regular and constitutes a simplex shaped region, an augmented simplex-centroid mixture design approach was used to define the number of mixtures necessary to attain the desired response (FAS value). This approach allows to detect the model curvature in the interior of the design region and it includes the points (mixtures) indicated in Figure 3.1, namely pure mixtures (vertices of the triangle), binary mixtures (midpoints at each side of the triangle), and mixtures involving three components.

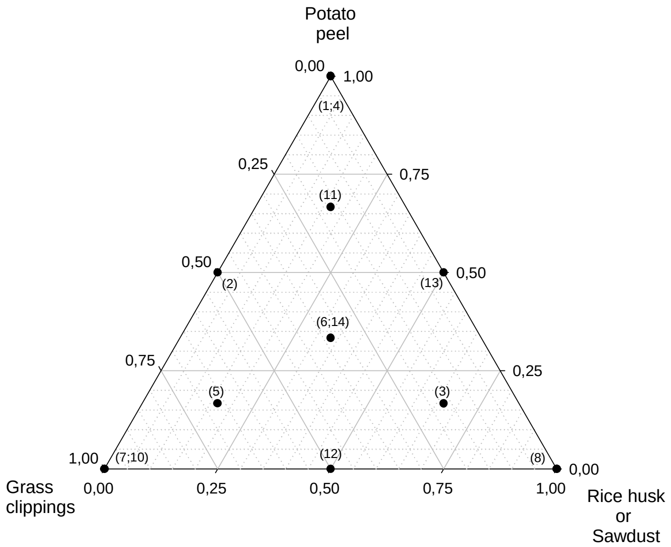

Figure 3.1: Mixtures tested according to a simplex-centroid mixture design (values in brackets represent blend order preparation and analysis).

In the interior area of the triangle there is a mixture of one third each of all ingredients (called centroid point) and three mixtures located midway between the centroid and each pure material. The pure and centroid mixtures were analysed twice to detect possible lack of fit and pure experimental error. According to this methodology, each set comprised 14 experiments.

Each mix was prepared, for a total load of 2 kg, by weighing the different components and mixing them by hand during 10 min, according to the pre-defined proportions and random sequential order indicated in Figure 3.1. Before mixture preparation, every single individual ingredient was manually mixed to promote homogenisation prior to weighing. Design-Expert ® version 8.0.4 was used to analyse experimental data and to evaluate of the model that best fits the composition effects in the response variables.

Some extra mixtures were prepared and analysed to validate the response of the model. This assessment was accomplished by comparing the model predictions and experimental FAS values for these specific mixtures.

3.2.3. Analytical methods

The main response variable under analysis is FAS and was calculated according to Eq. (3.6) (Adhikari et al., 2009):

$$FAS = \left(1 - \frac{BD}{PD}\right) \times 100\% \qquad (3.6)$$

where *BD* is the wet bulk density (kg m^{-3}) and *PD* is the wet particle density of the mixture (kg m^{-3}).

The parameter *BD* was determined according to a standard procedure described elsewhere (US Department of Agriculture and US Composting Council, 2001). Briefly the procedure consisted in the following steps:

a) transfer a 600 cm^3 aliquot of as-received compost into a 2000-mL graduated beaker through the wide neck funnel. Promote uniform material packing by allowing the beaker+material to fall freely onto a rubber mat once from height of 15 cm;
b) repeat the filling with 600 cm^3 and free falling operation, two more times (three times total). After the third free-fall drop, fill the graduated beaker to volume with sample material, 1800 mL. Do not repeat free-fall drop after topping off. Topping off should be limited to 2-3 cm;
c) weigh and record gross weight of 2000-mL graduated beaker containing 1800 cm^3 of as-received material;
d) calculate BD from the mass of material recorded in step c) and the volume of material used (1800 mL).

PD was estimated based on the procedure defined by Adhikari et al. (2009):

i) measuring the density of kerosene by adding a specific volume and weighing a graduate cylinder of known mass;

ii) adding a known mass of fresh composting mixture to another dry graduate cylinder, previously weighed, and then adding kerosene until all the particles are covered and free of air bubbles;

iii) recording the total volume of the cylinder content, as well as the weight of the its content.

The volume of the particles is calculated by subtracting the total volume recorded in step iii) from the volume of added kerosene (calculated from the density in step i) and the mass of kerosene obtained in step ii). By knowing the mass of particles (from step ii)), one can calculate the particle density.

Water absorption capacity (WAC) of the bulking agents tested was performed based on the procedure described by Adhikari et al. (2008). A known mass of dry material (at 105ºC) was soaked in distilled water for 24 h. Then water was drained off by gravity for 24 h under cover to limit evaporation, and then weighed. The WAC (%) was computed as:

$$WAC(\%) = \frac{m_{soaked\ and\ drained\ sample}(g) - m_{dry\ sample}(g)}{m_{dry\ sample}(g)} \qquad (3.7)$$

Moisture content of each fresh sample was determined by drying it in an oven at 105 ºC until constant weight. Organic matter was measured as volatile solids (VS) by burning 2.5 g dry sample in a muffle furnace at 550 ºC for 4 h (until constant weight). Total organic carbon (C_{org}) was determined in a Carbon-Sulphur Analyzer (Leco Instruments, model SC-144 DR), where carbon was oxidized to carbon dioxide by heating at 550 ºC. The total nitrogen content (N) was quantified by elemental analysis (EA 1108 CHNS-O-Fisons). All determinations were performed at least in triplicate.

3.3. Results and discussion

3.3.1. Characterisation of the composting materials

Physical and chemical parameters determined for the individual materials are shown in Table 3.1, where the results were reported by mean± standard deviation and the values in brackets indicate the number of samples analysed.

These determinations show that the key component under analysis, PP, is characterized by the highest moisture content and the lowest FAS. Indeed, although PP revealed a favourable

C_{org}:N ratio for microbial activity, its valorisation throughout composting may be hindered by the low inter-particle volume of voids available for oxygen diffusion.

Table 3.1: Physical and chemical properties of individual mixture components.

Property	Materials tested			
	PP	GC	RH	SD
MC (%)	85.0±2.8 (n=8)	75.5±4.7 (n=4)	10.7±0.5 (n=6)	11.6±0.4 (n=9)
VS (%)	95.0±1.0 (n=7)	84.8±6.0 (n=3)	84.1±0.0 (n=3)	99.7±0.0 (n=6)
BD (kg m^{-3})	707±71 (n=8)	149±27 (n=3)	122±3 (n=6)	256±3 (n=6)
PD (kg m^{-3})	1016±21 (n=5)	1071±87 (n=3)	902±109 (n=6)	1525±243 (n=6)
FAS (%)(a)	30.0±7.0 (n=5)	85.7±1.6 (n=3)	86.3±1.7 (n=6)	85.0±3.3 (n=6)
C_{org} (%, dm)	47.0±4.6 (n=4)	50.0±18.2 (n=3)	46.3±1.1 (n=3)	52.1±0.2 (n=3)
N (%, dm)	1.9±0.4 (n=4)	4.5±3.4 (n=3)	0.8±0.1 (n=3)	0.3±0.1 (n=3)
C_{org}:N	25.6±2.5 (n=4)	11.4±2.7 (n=3)	57.6±8.5 (n=3)	159±42 (n=3)
WAC (%)	n.d.	n.d.	350	440
% particles retained in a 2mm sieve	(b)	76	47	14

Abbreviations: MC- moisture content; VS- volatile solids; BD- wet bulk density; FAS- free air space, C_{org} - organic carbon; WAC- Water absorption capacity, dm- dry matter, n.d.- not determined.
(a)-Calculated by using Eq. (3.6); (b)-particles with dimensions less than 2 cm.

With respect to rice husks and sawdust both have been extensively used in composting as structural or drying amendments to reduce bulk density and increase air voids of the mixture (Chang et al., 1999; Chang and Hsu, 2008; Gao et al., 2010; Huang et al., 2006; Jolanun et al., 2008; Nakasaki et al., 1989, 1986). In our study, these materials exhibited low moisture content (less than 12%) and high water absorption capacity (440% for SD and 350% for RH) which are compatible to the purpose of correcting moisture excess of PP. In addition, the high FAS of these components (about 80%) indicates that they are able to maintain air spaces between particles in the composting mixture with PP waste and ensure its proper aeration. With respect to GC, this material also exhibits high FAS. However, in this case it decomposes rapidly during composting, and thus it is not a good bulking agent for composting mixtures. On the other hand, its low C_{org}:N ratio turns GC into a good nitrogen source whenever necessary. In summary, the ingredients selected in this study seem to be adequate to obtain well balanced mixtures, since their properties are complementary.

3.3.2. Response regression model analysis

To evaluate how a specific composition determines the FAS for mixtures containing PP, GC and RH or SD as bulking agents, experimental responses of FAS were obtained for each trial listed in Table 3.2. Those responses were fitted to Eq. (3.1) to (3.3) by using Design-Expert ®. Full cubic, Eq. (3.4), and special quartic, Eq. (3.5), models were excluded from the evaluation, because the number of model terms should be less than to the number of unique points in the design (10 in this experimental design).

Table 3.2: Experimental response values of FAS for set A (with RH) and B (with SD) (n=3).

	Independent variables			Set A (RH)			Set B (SD)		
Blend order	X_1 (PP)	X_2 (GC)	X_3 (RH or SD)	BD (kg m^{-3}) ±sd	PD (kg m^{-3}) ±sd	FAS (%)±sd	BD (kg m^{-3}) ±sd	PD (kg m^{-3}) ±sd	FAS (%)±sd
1	1.000	0.000	0.000	619±10	938±40	34.0±3.0	798±19	1043±32	23.5±3.0
2	0.167	0.167	0.667	134±3	1348±4	90.1±0.2	264±2	1213±55	78.2±1.0
3	0.000	0.000	1.000	122±4	833±50	85.3±1.0	257±2	1525±80	83.1±0.9
4	0.000	0.500	0.500	118±2	1022±154	88.4±1.8	233±21	1015±151	77.0±4.0
5	1.000	0.000	0.000	625±9	963±53	35.1±3.7	788±14	1053±21	25.2±0.9
6	0.000	1.000	0.000	137±2	1069±91	87.1±1.1	179±8	1264±90	85.8±1.2
7	0.000	1.000	0.000	136±2	951±75	85.7±1.1	175±8	1257±72	86.1±1.0
8	0.667	0.167	0.167	290±1	879±18	67.0±0.7	426±20	1065±59	60.0±2.9
9	0.333	0.333	0.333	168±7	1198±18	86.0±0.6	311±4	1233±5	74.8±0.3
10	0.500	0.500	0.000	267±4	1042±33	74.4±0.9	343±23	1197±23	71.4±2.0
11	0.167	0.667	0.167	151±3	1034±23	85.4±0.4	238±9	1064±40	77.6±1.2
12	0.000	0.000	1.000	122±1	1013±46	87.9±0.6	255±10	1735±97	85.3±1.0
13	0.500	0.000	0.500	178±0	1179±41	84.9±0.5	340±4	1219±64	72.1±1.5
14	0.333	0.333	0.333	186±19	1067±60	82.5±2.0	309±11	1201±20	74.3±1.0

The polynomial fitting results are presented in Table 3.3 with respect to common statistical parameters, lack of fit and sequential model sum of squares. In fact, the model summary statistics includes standard deviation, R^2_{adj} (proportional reduction in the variance resulting from fitting the model), R^2_{pred} (amount of variation in new data explained by the model) and PRESS statistic (prediction residual error sum of squares, which describes how well the model fits data).

The lack of fit compares the residual error with the error expected in the response, if the experiment is repeated (pure error). Since it is not desirable that residual error significantly exceeds the pure error, models with a p-value greater than 0.1 should be considered. Regarding sequential model sum of squares, this parameter represents the sum of the squared deviations from the mean for each model, and it indicates the overall cumulative improvement in the fit as terms are added.

Table 3.3: Model regression statistics for set A (with RH) and B (with SD).

Regression model	Model summary statistics				Lack of fit *p-value*	Sequential model sum of squares *p-value*
	Standard deviation	R^2_{Adj}	R^2_{pred}	PRESS		
Set A						
Linear	8.91	0.777	0.673	1515	0.0005	0.0001
Quadratic	1.28	0.995	0.985	69.43	0.5469	<0.0001
Special Cubic	1.12	0.996	0.990	45.01	0.8036	0.1018
Set B						
Linear	7.88	0.850	0.794	1112	0.0003	<0.0001
Quadratic	1.52	0.994	0.985	78.80	0.1226	<0.0001
Special Cubic	1.46	0.994	0.970	161.6	0.1256	0.2325

Among the regression models under consideration, the selection of the best one to predict the experimental response was based on the following criteria:

i) Low standard deviation, high R^2_{adj} and R^2_{pred} and low PRESS;

ii) Low probability of response improvement as additional terms are added (*p-value* in the sequential model sum of squares less than 0.05);

iii) High *p-value* in the lack of fit test.

Therefore, for set A, special cubic model complies with criteria i) and iii); however, the *p-value* associated to the sequential model sum of squares indicates that there is no significant improvement of the model fit due to additional terms. Hence, a quadratic model was selected in this case. For set B, criteria i) and iii) were fulfilled by quadratic and special cubic models. Nevertheless, the probability of response improvement was low for the higher order model, and thus the quadratic model was also selected.

The regression coefficients obtained for the selected models are shown in Table 3.4.

Table 3.4: Regression coefficients of the quadratic model for predicting FAS of set A (with RH) and B (with SD).

Set A			Set A (reduced model)		Set B		
Coefficient value	Independent variable	term *p-value*[*]	Coefficient value	Independent variable	Coefficient value	Independent variable	term *p-value*[*]
34.56	X_1		34.52	X_1	24.79	X_1	
86.40	X_2		86.61	X_2	85.74	X_2	
86.77	X_3		86.97	X_3	84.13	X_3	
50.41	X_1X_2	<0.0001	50.81	X_1X_2	61.55	X_1X_2	<0.0001
92.98	X_1X_3	<0.0001	93.38	X_1X_3	68.66	X_1X_3	<0.0001
3.24	X_2X_3	0.5351	-	X_2X_3	-38.85	X_2X_3	0.0002

[*] If a term is not significant for the model, then p-value is >0.05 and model reduction should be evaluated.

The second order polynomial describing FAS response for mixtures with PP:GC:RH presented a non-linear term (X_2X_3) that is not significant. Therefore, a reduced model was evaluated, by removing the term with the highest *p-value* until all *p-values* are less than 0.1 (Smith, 2005). This procedure was performed using Design-Expert ® and led to the response indicated in Table 3.4. As a result, parameters R^2_{adj} and R^2_{pred} were slightly improved (from 0.994 and 0.985 to 0.996 and 0.991, respectively).

As usual, obtained models for predicting FAS were based on the assumption that errors are independent and identically distributed with zero mean and homogenous variance (Smith, 2005). The confirmation of these conditions was performed by visual inspection of the normal probability plot of the studentised residuals (to check for normality of residuals), by comparing their values with the predicted ones. Although it was observed some scatter, normal plot of residuals followed a straight line suggesting a normal distribution.

3.3.3. Surface and response trace plots analysis

Trace and surface/contour plots were drawn to evaluate the effects of individual ingredients on FAS response. For sets A and B, surface plots are depicted in Figure 3.2 a) to b), which include the projection of the response in a 2D representation.

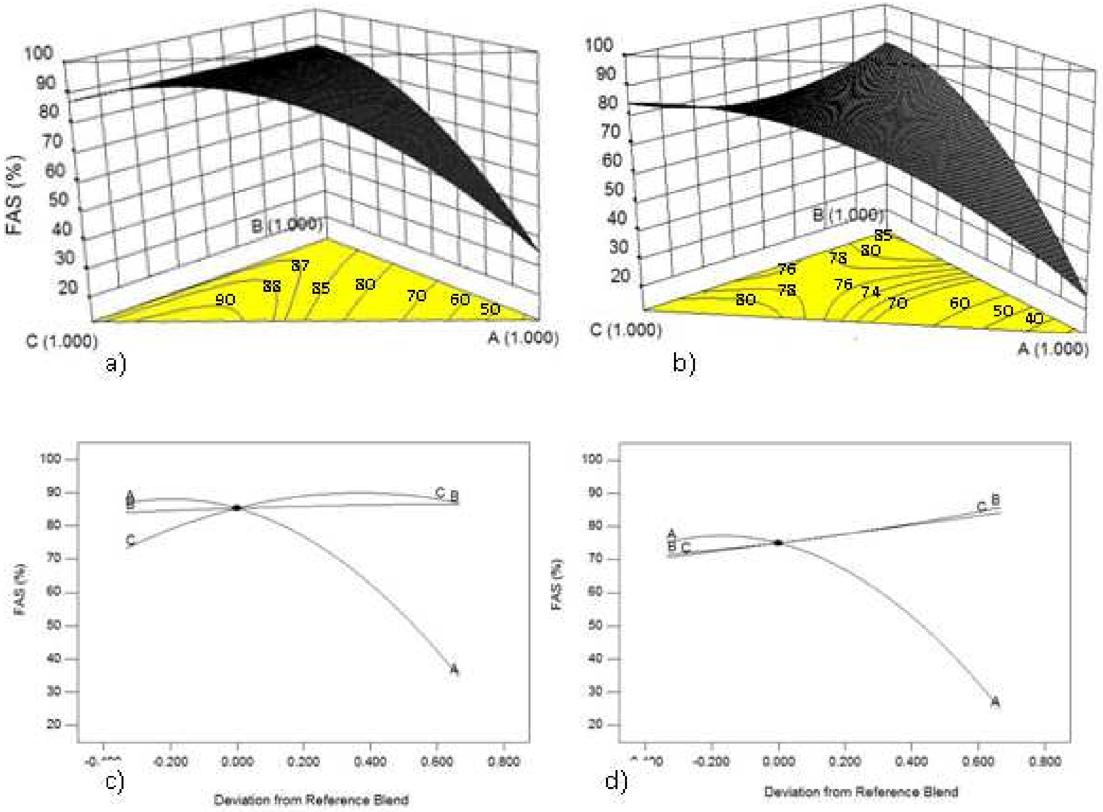

Figure 3.2: Contour plots for FAS: a)- set A (A:PP; B: GC; C: RH); b) set B (A:PP; B: GC; C: SD); Response trace plots for FAS: c)- set A; d)- set B.

In these figures, mixture compositions that produced the same response are indicated by a contour line. For both sets A and B, maximum values FAS are located towards pure blends of GC and RH/SD. In fact, contour lines in Figure 3.2a) to b) indicate a broad range of GC and RH/SD proportions where FAS assume identical values. It should be noted that large amounts of GC may seem to ensure high values for FAS. However, this material decomposes quickly and then tend to compact, reducing pores between compost particles (Rynk et al., 1992).

Moreover, the response surface in Figure 3.2 b) shows that the binary mixture of SD with GC acts antagonistically on FAS parameter, which is reflected in the negative coefficient associated to X_2X_3 (Table 3.4). The main reason for this result is the great importance of the particle size distribution of the bulking agent to the structural condition of the mixture. In our study, 86 % of SD particles are lower than 2 mm, which is a high value comparing with the one suggested by Haug (1993) (50% of particles passing a mesh of 2.23 mm). By mixing SD with GC, the interstices of GC were probably occupied by sawdust, thus reducing FAS, rather than what is expected of a good bulking agent.

Trace plots, Figure 3.2 c) to d), enable to compare the effects of all components in the design space and determine the sensitivity of the response to deviations from the formulation near the reference mixture (the centroid of the triangle, design points nº 9 and 14 from Table 3.1. The lines in Figure 3.2 c) to d) correspond to the FAS response due to variations in the proportions of a selected component, keeping constant the ratio of the others ingredients and the sum of mixture components equal to one. The results reveal that independently of the bulking agent used, PP (ingredient A) has a strong effect on the reduction of FAS as mixtures deviate from the centroid. When RH is used, Figure 3.2 c), the response trace to GC is nearly a horizontal line, meaning that it has a negligible effect on FAS. When SD is tested, Figure 3.2 d), GC and SD response curves are overlapped, and thus these components have roughly identical linear effect on FAS. This result may be explained by the particle size of the SD used in our study, as previously mentioned.

3.3.4. Validation of the response regression model

Three compositions were selected for each bulking agent, for validating regression models, Eq (3.8)-(3.9), previously obtained for FAS:

$$FAS_{Set\ A} = 34.52X_1 + 86.61X_2 + 86.97X_3 + 50.81X_1X_2 + 93.38X_1X_3 \tag{3.8}$$

$$FAS_{Set\ B} = 24.79X_1 + 85.74X_2 + 84.13X_3 + 61.55X_1X_2 + 68.66X_1X_3 - 38.85X_2X_3 \tag{3.9}$$

The selection was based on the range of ingredients proportions that are favorable to composting in what concerns MC, C_{org}:N and FAS. Restrictions imposed to C_{org}:N are in the interval 20-40:1 (Rynk et al., 1992). The upper limit was slightly broadened for MC (55-75%) due to the high absorption capacity of bulking agents involved (Barrena et al., 2011). Mixture proportions that fulfilled the requirements for MC and C_{org}:N were calculated according to the principle of mass conservation to a blend of PP, GC and RH or SD, whose characteristics were listed in Table 3.1. FAS was allowed to vary between 60-85%. By combining the constraints of the parameters MC, C_{org}:N and FAS, an interception area (shaded region in Figure 3.3) is defined for selecting the mixtures RH1 to RH3 and SD1 to SD3, for FAS model validation.

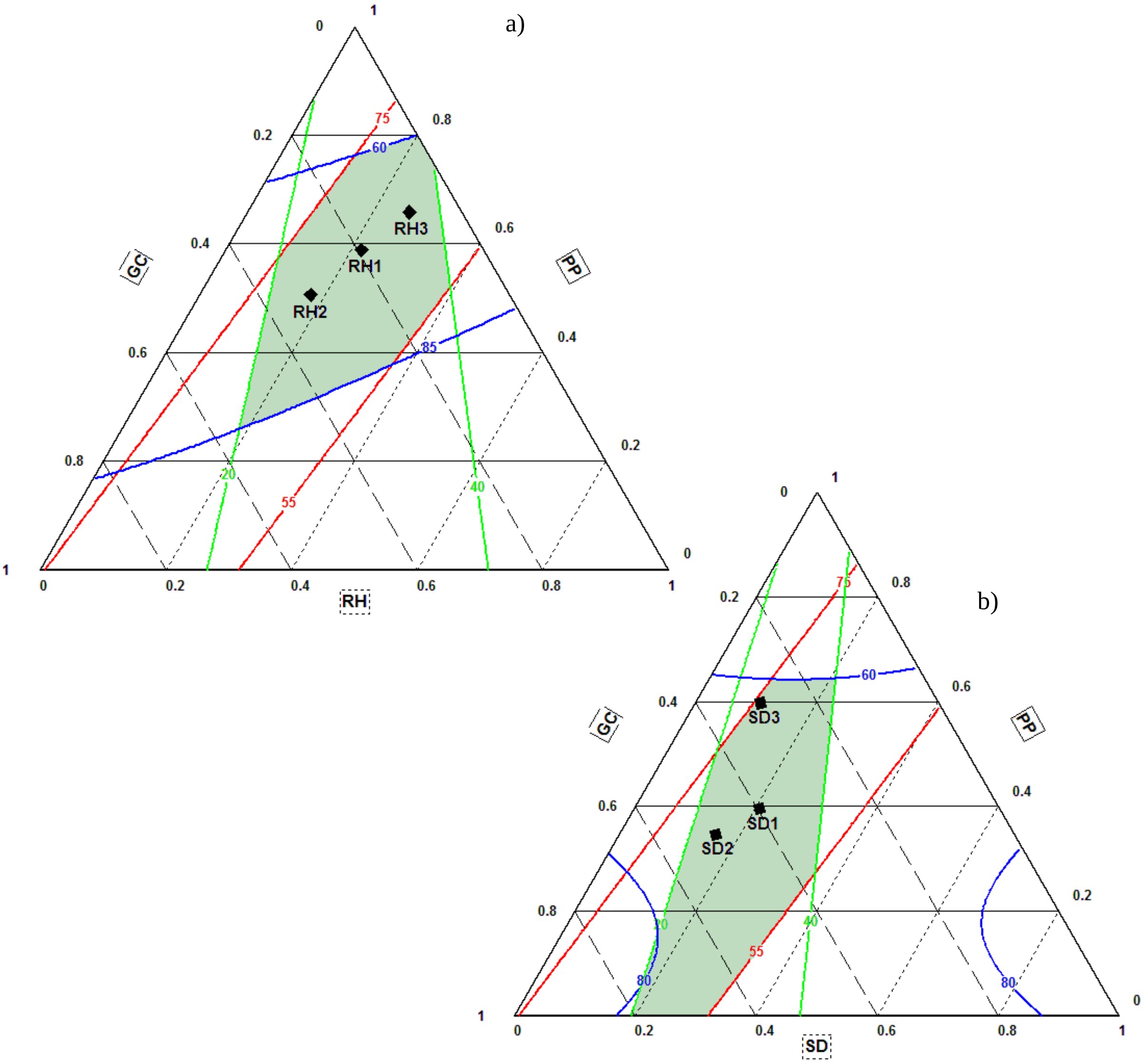

Figure 3.3: Contour and response trace plots for FAS for: a) set A (A:PP; B: GC; C: RH), RH1-RH3 are the validating mixtures; b) set B (A:PP; B: GC; C: SD), SD1-SD3 are the validating mixtures.

The numerical results for validating the models, Eq.(3.8) and Eq (3.9) are shown in Table 3.5.

Table 3.5: Experimental conditions tested and validation results.

Mixt. Ref.	X1 (PP)	X2 (GC)	X3 (RH or SD)	MC (%)	VS (%)	BD (kg m^{-3})	FAS (%)		
							Exp. result	Model prediction	Pred. Interval
RH1	0.587	0.192	0.219	70.5	86.7	240	76	74	±4.2
RH2	0.506	0.324	0.170	69.6	85.9	229	76	77	±4.0
RH3	0.660	0.081	0.259	64.3	86.2	245	74	71	±4.5
SD1	0.400	0.400	0.200	67.5	90.5	325	71	73	±5.3
SD2	0.343	0.516	0.141	71.4	88.5	268	78	76	±5.3
SD3	0.600	0.300	0.100	70.8	90.0	440	67	63	±6.5

Experimental results for FAS were found to be very close to the predictions of the models, with relative errors ranging from -2.7 to 5.8%, but within the prediction intervals. Therefore, the models developed by mixture design approach for predicting FAS, Eq.(3.8) to (3.9), can be used to properly calculate the free air space of ternary mixtures of PP, GC, RH or SD.

In this study FAS was quantified by using the simplest theoretical correlation available, Eq.(3.6), although air pycnometry is pointed out as the most reliable technique for its determination in composting matrices (Ruggieri et al., 2009). But the unavailability of commercial picnometers for this type of solid material, conditioned the cross-confirmation of our theoretical correlation based results.

In literature, FAS has been estimated by theoretical or empirical approaches (Albuquerque et al., 2008). Figure 3.4 compares FAS predictions of the models developed in our study, Eqs. (3.8)-(3.9), with the ones calculated by equations proposed by Richard et al. (2004), Eq.(3.10), and Agnew et al. (2003), Eq.(3.11):

$$\mathrm{FAS_R} = \left(1 - BD \times \left(\frac{1-DM}{D_w} + \frac{DM \times OM}{PD_{OM}} + \frac{DM \times (1-OM)}{\mathrm{PD_{ash}}}\right)\right) \times 100\% \tag{3.10}$$

$$FAS_{Ag} = 100 - 0.0889 \times BD \tag{3.11}$$

where *BD* is the wet bulk density (kg m^{-3}), *DM* the fraction of dry matter, OM the fraction of organic matter in dry basis, *Dw* the density of water (kg m^{-3}), PD_{OM} the particle density of organic matter (1600 kg m^{-3}) and PD_{ash} the particle density of ash (2500 kg m^{-3}).

In general, for mixtures RH1 to RH3, FAS calculated by Eq.(3.10)-(11) are slightly higher than the predicted by models of our study, Eq.(3.8)-(3.9). However, in the case of SD1 to SD3 (sawdust as bulking agent), correlations values fitted inside the prediction intervals. These differences might be related to the applicability conditions of theoretical and empirical approaches for FAS quantification.

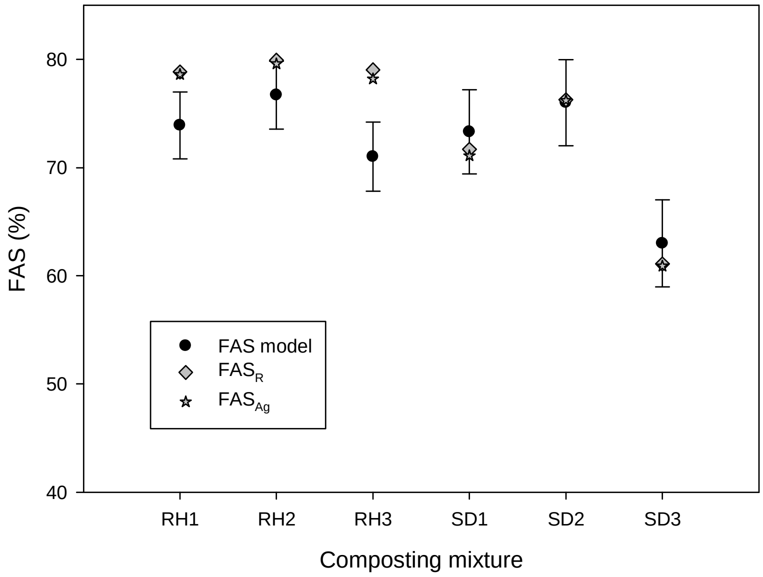

Figure 3.4: Comparison between FAS predictions Error bars indicate prediction intervals with a significance of 95%.

According to Ruggieri et al. (2009), properties such as BD and MC have a strong influence on FAS estimated by these correlations, and boundaries were established to its application. Namely, FAS$_R$, Eq.(3.10), is limited for materials where BD is superior to 400 kg m $^{-3}$; below this level FAS$_R$ may lead to misleading values. Also FAS$_{Ag}$, Eq.(3.11), seems restricted only to high-moisture and/or low-density materials where FAS$_R$ may provide misleading values. In our study, mixtures with RH have low bulk densities (<245 kg m^{-3}) and FAS$_R$ were the highest ones, over estimating FAS$_{model}$ (relative error ranged from -4.2 to -11.3%). FAS indicated by FAS$_{Ag}$ were also overestimated but within the relative errors (-3.8 to -10.1%). For mixtures with SD, FAS$_R$ and FAS$_{Ag}$ were equally consistent with FAS$_{model}$ predictions.

3.4. Conclusions

This study aimed to predict free air space of mixtures for further used in composting, based on mixture design approach. The organic materials tested were potato peel (PP), grass clippings (GC) and rice husks (RH) or sawdust (SD). The results proved that the mixture design methodology led to reliable mathematical models for predicting FAS of mixtures containing primarily PP. In particular, for mixtures with PP, GC and SD the FAS model pointed out the antagonistic effect of the binary mixture of GC and SD to the overall property. Contour plots revealed the occurrence of a wide range of GC and SD (bulking agent) proportions where FAS assume identical values; nonetheless, large proportions of GC should be avoided.

As expected, response trace plots clearly indicated that independently of the bulking agent used, the addition of potato peel has a strong effect on reducing the mixture air voids volume.

The models obtained for predicting FAS were compared with theoretical and empirical correlations proposed in literature which consider mixture properties like DM, OM and BD. For formulations with SD, correlations are consistent with our model prediction. When RH was used as bulking agent both literature correlations overestimated the FAS but even so the deviations were low.

In summary, mixture design approach led to good results to estimate FAS of initial mixtures with industrial potato peel waste, avoiding, this way, trial and error methods often used to establish the ingredients proportions. By overlapping contour plots that describe the influence of the mixture composition in FAS, it was possible to find a range that complies with requirements in what concerns moisture content, C:N ratio and FAS.

Future studies should address how model predictions are affected by two aspects: 1) variability of wet basis properties since they affect the surface areas of the feedstock particles and pore space formation and 2) biodegradability of the mixture, because when the materials decompose quickly, pores between particles are reduced and composting evolution may be hindered.

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4. Assembly and operation of lab-scale composting reactors

In this section the composting reactors lab-assembled are described and tested.

Firstly, some specific design criteria such as volume and shape definition are explored. Monitoring systems were coupled to the reactors and detailed description is indicated. Then, composting experimental procedures in terms of sampling, monitoring and homogenisation are presented.

Finally, a set of exploratory tests are described. The main goal was to provide information about the adequacy of the reactors and procedures to achieve the objective of this thesis. Bi-dimensional temperature gradients were analysed to determine the hot-spot, as well as the vertical and radial gradients. The capacity of the gas cooling system to meet the operating conditions of the O_2 sensor in terms of temperature and humidity is also explored in this section. At last, the suitability of the composting procedure to the ash conservation principle was tackled.

4.1. General design concepts of composting reactors

A significant number of lab scale composting devices have been developed with the purpose to mimic full-scale facilities conditions. In that scope, self-heating reactors (SHR) are frequently used to simulate the typical thermodynamic regime of composting process that includes mesophilic, thermophilic, cooling and maturation phases. SHR rely only on microbial heat production to attain process temperatures, possessing no temperature control apart from external insulation (Lashermes et al., 2012; Mason and Milke, 2005). On the other hand, for studying the process at a particular temperature instead of evaluating the process dynamics, fixed temperature reactors are considered more adequate, where the desired temperature is imposed by external heating or cooling devices (Mason and Milke, 2005).

Within the scope of present work, SHR were selected for evaluating the level of thermophilic sanitising temperatures achieved. The design aspects of major concern for SHR are (Mason and Milke, 2005; Petiot and de Guardia, 2004):

1) Surface area to volume ratio (SA:V), once it determines the wall heat flux; this ratio depends on the volume and shape of the system although cylindrical structure is the most used;
2) Type and thickness of insulation material used in the external surface to increase thermal resistance;
3) Reactor to particle diameter ratio (D:dp), due to the influence of wall effects on preferential airflow inside the composting matrix.

To aid the volume selection of a cylindrical SHR, an estimate of the ratio between instantaneous convective/conductive/radiative (CCR) heat losses through the reactor wall and the biological heat produced during composting can be calculated. The calculation followed the procedure suggested by Mason and Milke (2005) as described in the supplementary material (section 4.7).

In Figure 4.1 the instantaneous CCR heat loss:biological heat ratio (CCR:BH) is presented as a function of reactor volume (V) and SA:V ratio, for a cylindrical reactor with different height (H) to diameter (d) proportions. For CCR:BH calculations, an Aeroflex_EPDM rubber based elastomeric product was considered as insulation material (thermal conductivity of 0,035 W m^{-1} K^{-1}, 19 mm of thickness). This material presents a higher mechanical resistance in comparison to other insulation materials (like glass wool), thus facilitating the reactor loading and unloading, or even homogenisation phase by rolling.

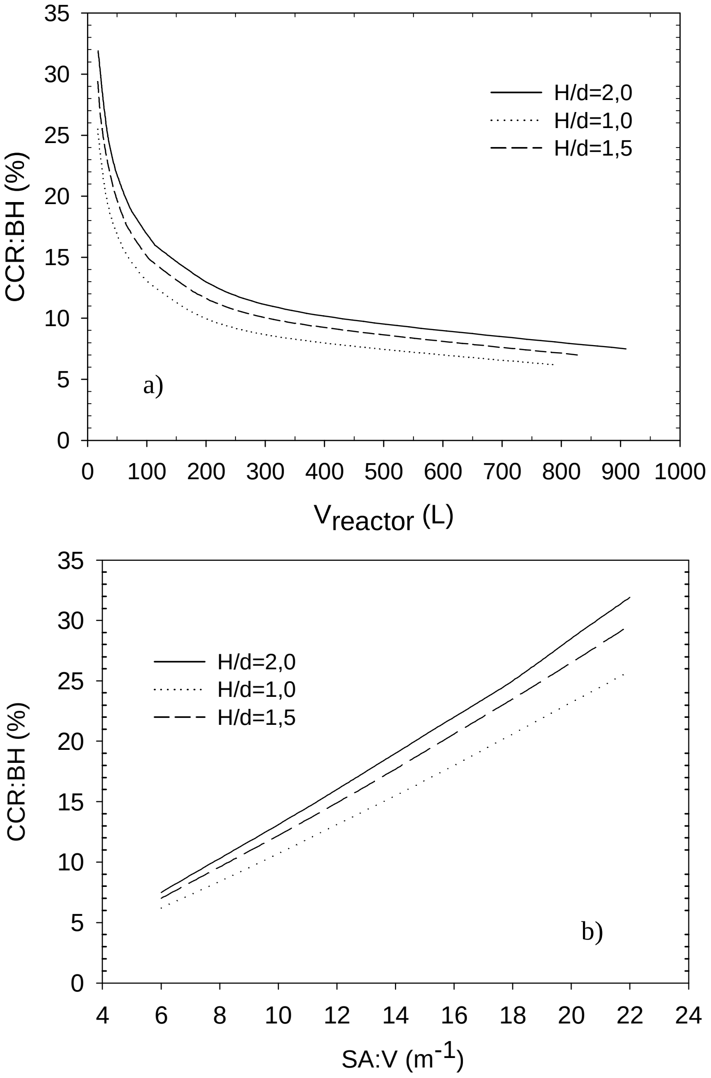


Figure 4.1: Influence of a) the volume of a cylindrical self-heating reactor, b) SA:V in CCR heat loss:BH. (Considerations: PVC cylindrical reactor, rubber based elastomeric insulation layer= 38 mm, ambient temperature=25ºC, composting mixture temperature=70ºC).

According to the data presented in Figure 4.1, the reactor volume has a significant influence on the instantaneous CCR losses, as expected. At volumes lower than 30 L, a simple insulation is not sufficient to allow proper self-heating, as indicated by Petiot and de Guardia (2004), since CCR losses represent more than 20% of the biological heat produced by the biomass contained into the reactor. Although smaller reactors are easy to handle, less expensive and easier to control, it would be necessary 320 mm layer of insulation materials to limit CCR losses to 5%, in a 21 L cylindrical reactor with H=d=0.3m. On the other hand, to restrict CCR:BH near 6% without increasing insulation thickness, large-scale reactors with working volumes higher than 785 L are necessary.

Recently, Lashermes et al. (2012) developed a SHR of 4 L where the wall temperature was controlled within 1-2ºC bellow the temperature of the compost material. That configuration allowed the study of a natural self-heating process in a small scale reactor. Nevertheless, the amount of instrumentation was significant, which raised the cost of the overall composting system.

Therefore, taking into consideration logistic issues, in this work SHR will be designed with an operating volume of between 100-130L, corresponding to a CCR:BH near 12% considering a cylindrical shape with H/d=1-1.5. This choice leads to SA:V in the range of 10-12, which is similar to some works reported by Mason and Milke (2005). In addition, the reactor to particle diameter should be higher than 10 to minimize wall effects on preferential airflow inside the solid matrix (Mason and Milke, 2005).

4.2. Description of the reactors

4.2.1. General characteristics

A system of four self-heating reactors (R1 to R4) in parallel was assembled. The reactors were identical in size, shape and other general characteristics. Figure 4.2 displays an overview of one SHR and its main components.

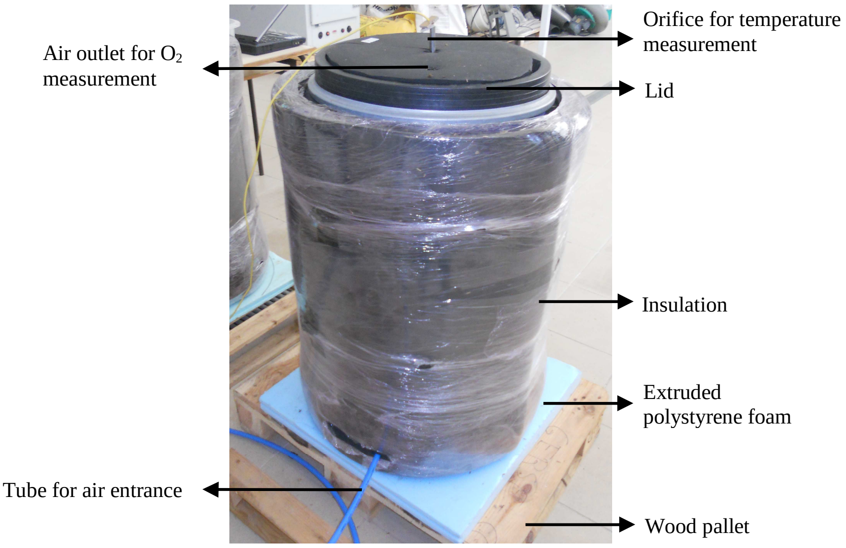


Figure 4.2: Experimental setup overview of the SHR used in this work.

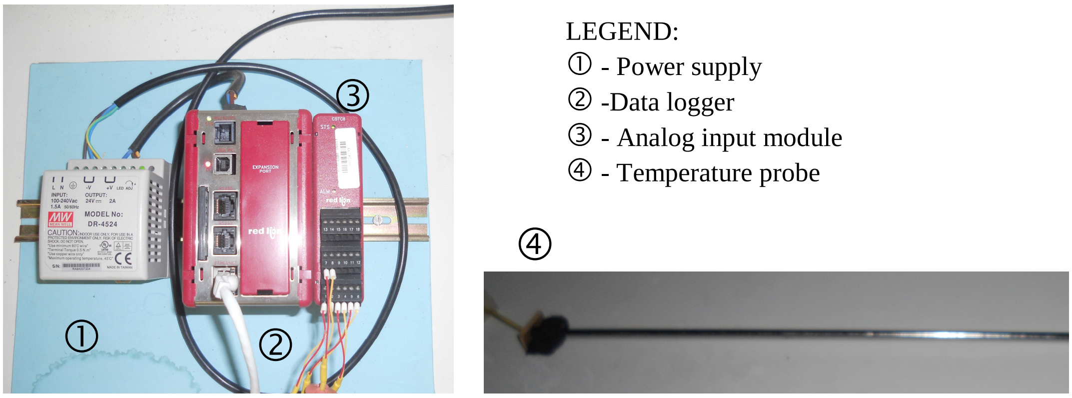
Each reactor was made of PVC with 0.8 m high, 0.48 m in diameter (total volume of 145 L) and insulated with two layers (total of 38 mm) of a rubber based elastomeric material (Aeroflex_EPDM® thermal conductivity of 0.035 W m^{-1} K^{-1}) (Figure 4.2). The lid of each reactor was externally insulated with a layer of Aeroflex_EPDM® to lessen heat losses.

At the top of the reactor there are two connection points for the temperature sensor (at the centre region) and for outlet air flow. The reactor is in contact with a board of extruded polystyrene foam to decrease heat transfer. This setup is settled on a wood pallet to allow subsequent weighing.

For aeration purposes, an air plenum with 0.10 m in height was created at the bottom of the reactors with a perforated acrylic board to promote air flow distribution. As a result, the available working volume of each reactor was set to 127 L with a H/D= 1.5. The acrylic board has 16 radial rows of orifices, totalising 145 holes with a diameter of 5 mm each. Bellow that board there is an orifice with a quick fit connection to the air supply system. That same orifice also allows the withdrawal of leachates by disconnecting the air inlet tube and tilting the reactor.

4.2.2. Temperature monitoring

Temperature monitoring system involved one thermocouple per reactor connected to an acquisition system (Figure 4.3) with the characteristics indicated in Table 4.1.



LEGEND:
① - Power supply
② -Data logger
③ - Analog input module
④ - Temperature probe

Figure 4.3: Overall view of the components of the temperature monitoring system.

Table 4.1: Temperature monitoring system: components' description.

Component	Temperature system component	General characteristics
①	Power supply Model DR-4524; (MeanWell)	Input: 100-240 V AC; 1.5 A; 50/60 Hz Output: 24V DC; 2A
②	Data logger VIRTUAL HMI CSMSTRSX (Red Lion)	Supports up to 16 Modular Controller Series Modules 2 RS-232 Serial Ports 1 RS-422/485 Serial Port 10 Base-T/100 Base-TX Ethernet Connection Can Connect To Multiple Devices Via Up To 10 Protocols Simultaneously CompactFlash ® Slot Allows Process Data to be Logged Directly to CSV Files Power supply: 24V
③	ANALOG INPUT MODULE CSTC8 (Red Lion)	Channels: 8 single-ended; Effective Resolution: Full 16-bit; Sample Time: 200 msec, for 4 inputs
④	Thermocouple BS.T.K.1.8.500.S5 (Bresimar)	Type: Ni-Cr-Ni (0-1100ºC) Sheath: Diameter= 8 mm; Length = 50 mm

This system allowed the continuous monitoring of reactor temperature every 10 min, with simultaneous record of readings in a compact flash card with 64 Mb capacity. Software Crimson 2.0 was used to configure the data logger module and provided an interface between the user and the data logger for data access and transfer.

Each thermocouple was located at the geometric centre of the composting mixture. For such, the probe was able to slide through the lid whenever adjustments were necessary. For this purpose, a quick fit *raccord* and a poliamide and inox tubes with 10 mm of internal diameter were used, as detailed in Figure 4.4.

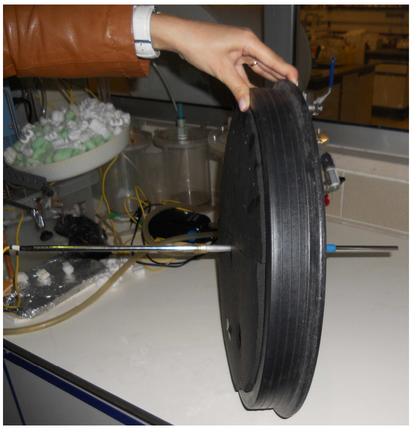
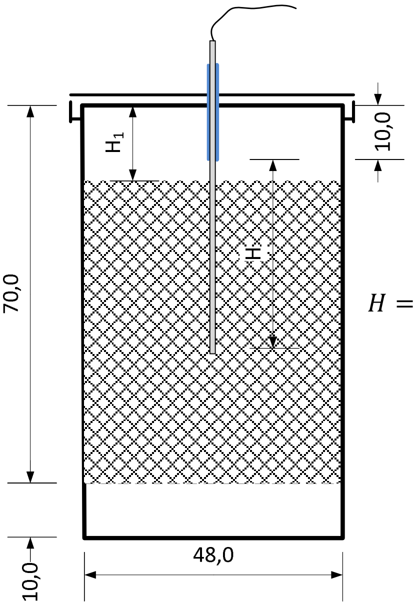


Figure 4.4: Adjustment of the temperature probe to the reactor lid.

The location of the probe according to the geometric centre of the composting mixture was adjusted as describe in Figure 4.5.



$$H = \frac{70 - H_1}{2} + H_1 - 10$$

Figure 4.5: Description of the adjustment procedure for temperature probe to the centre of the mixture (measures are in cm, composting mixture is represented by the grid mesh)**.**

4.2.3. Air flow and oxygen measuring systems

Air flow was supplied by compressed air source and measured through a meter E4K-4BR, Kytola Instruments, as indicated in Figure 4.6 a). Before passing through the air meters, the compressed air was conducted to a filter regulator lubricator with pressure gauge, for removal of liquid condensates and air pressure regulation. Calibration of the air meters was manually performed by using a home-made wet air flow meter system, Figure 4.6 b), with 1000 mL capacity. Inside the glass cylinder, a soap bubble travels a limited volume between two marks, in a measured time, thus indicating the air flow. Each air flow measurement was performed for at least five times.

Figure 4.6: a) Air flow measuring system, b) home-made wet air flow meter.

Oxygen concentration was measured in the exit gas stream of each reactor, as indicated in Figure 4.2. The gases were conducted to a water trap before analysing oxygen concentration (v/v) with a gas sensor, XLS1047, XLlogger (Figure 4.7). The water trap allowed to condensate the water vapour in the saturated hot gases and to reduce temperature before gases contact with the oxygen sensors (0-40ºC).

Figure 4.7: a) Water trap for the oxygen measuring system; b) oxygen gas sensor and measuring cell.

4.3. Composting procedure

Composting tests at laboratory scale comprised three essential steps, namely i) ingredients collection and pre-treatment, ii) mixture preparation, iii) operation of the composting reactors and sampling.

Most of the ingredients were gathered on the day before starting the composting experiments, except ES which was collected and frozen within 2-4 h at -14ºC. Then, 24 h before starting the tests, ES material was thawed at room temperature. Whenever necessary materials were sieved with a 5 cm mesh, in order to obtain a homogenous material in size and shape.

Mixture preparation consisted in weighing the ingredients according to predefined proportions and thoroughly blend for 15-20 min. Reactors were weighted after loading.

The reactors start-up procedure included air flow adjustment near to 0.5 L min^{-1} per kg of organic matter (OM), which is within the range of 0.43-0.86 L min^{-1} kg^{-1} OM used by Lu et al. (2001) for food waste. Afterwards, process control comprised manual changes of air flow rate to guarantee an outlet oxygen concentration in the range of 5 to 15% (v/v) and to maintain the thermophilic phase as long as possible. In fact, oxygen concentration lower than 5% can cause anaerobic conditions, while values above 15% are indicative of excessive aeration that favours cooling of the material (Puyuelo et al., 2010). As previously mentioned, temperature measurement was automatically performed every 10 min, while O_2 monitoring was manually executed. More specifically, oxygen was measured 6-8 times a day during thermophilic period, while monitoring frequency was reduced to 2-3 times a day, for mesophilic conditions.

In general, to prevent or reduce gradient of physical and chemical properties of the mixture, including a homogenisation phase may be an appropriate procedure (Papadimitriou et al., 2010). Some homogenisation schemes have been described in the literature: consecutive unloading, homogenisation and loading (Doublet et al., 2011) or a vertical rotating axis with blades fixed at the top of the reactor (Petric et al., 2009). The first option removes too much heat from the composting matrix, while the second scheme is more adequate for small scale reactors. In this work, the homogenisation of reactors were promoted by rolling them on the floor for a distance equivalent to 14 rotations. Axial mixing was helped by hand, without unloading the reactor. This procedure required to disconnect air supply and on-line monitoring system. Therefore, it was performed as quickly as possible, to minimize perturbation effects in the process. The reactor was homogenised on a daily basis during thermophilic conditions, every two days during mesophilic conditions and whenever samples were withdrawn.

After homogenisation, the composting mixture was weighted using an electronic weighing device, Figure 4.8 a), with a precision of 0.1 kg. A lift pallet (Figure 4.8 b)) was used to raise the reactor (Figure 4.8 c)). Then, the weighing device was placed under the reactor and the lift gently dropped the composting reactor until it was fully supported by the scale (Figure 4.8 d)).

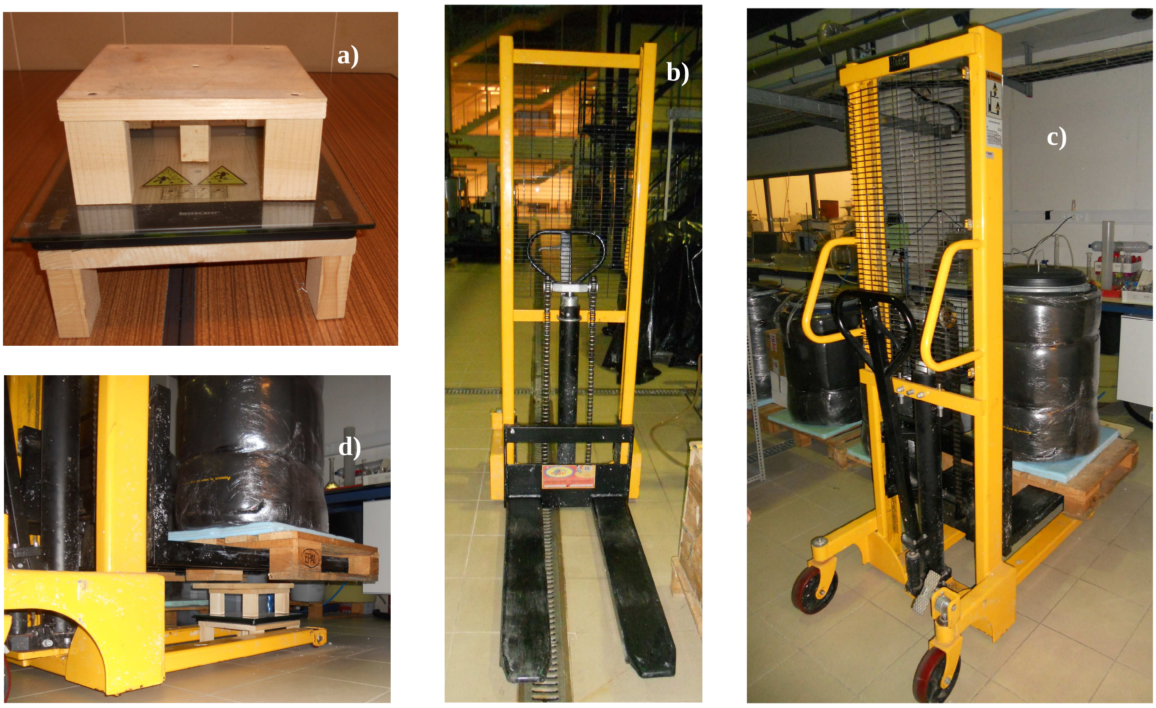


Figure 4.8: Reactor's weighing procedure: a) scale; b) pallet lift; c) raising of the reactor; d) reactor adjustment to the scale.

Leachates (if any) were drained before reactor homogenisation as previously described and solid samples collected after homogenisation. Each sample withdrawn from the reactor was collected considering three regions, as detailed in Figure 4.9, according to the mixture height. This means that a sub-sample was removed from the top of each area to obtain a composite sample for analysis.

The sample withdrawn should be small in comparison to the total amount of mixture and defined according to the envisaged number of sampling events and the mass requirements for physical, chemical and biological analysis. In the reactor composting tests performed by An et al. (2012) and Petric et al. (2012) at lab scale, the total amount of sample withdrawal during the complete composting period represented 9 to 16% (w/w) of the starting mixture. In this study, this range was a guideline for establishing the sampling size.

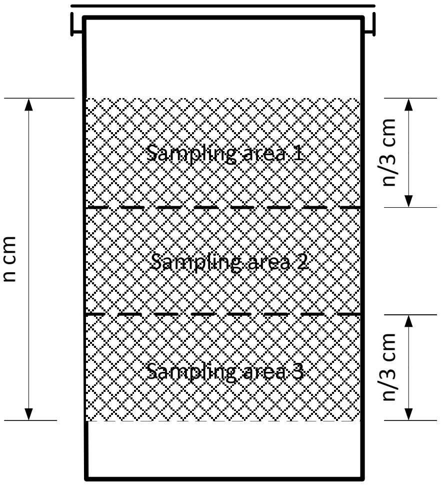


Figure 4.9: Definition of the sampling areas of the reactor.

Table 4.2 depicts the maximum amount of sample withdrawn in each sampling event, according to the mass of the starting composting mixture.

Table 4.2: Correspondence between mass of sample withdrawn and initial composting mass.

Mass in the SHR (kg)	Maximum sample withdrawn in each sampling event (kg) *
20	0.8
25	1.0
30	1.2
35	1.4
40	1.6
45	1.8
50	2.0
55	2.2
60	2.4

* Considering 4 sampling events

In addition, all samples collected were weighed and taken into consideration in the overall mass balance of the reactor. Thus, the fraction of organic matter ($\frac{VS_t}{VS_0}$) not degraded during composting was calculated as follows (Gomes, 2001):

$$\frac{VS_t}{VS_0} = \frac{m_t \times (1 - f_{H,t}) \times f_{VS,t}}{m_0 \times (1 - f_{H,0}) \times f_{VS,0} - \sum_{j=o}^{j} m_j \times (1 - f_{H,j}) \times f_{VS,j}} \quad \text{for } t\text{=1, n} \qquad (4.1)$$

where m_0 and m_t (kg) is the mass at the start and during the composting trial, respectively; m_j (kg) is the mass of sample in the j withdrawn; $f_{H,0}$, $f_{H,t}$ and $f_{H,j}$ are moisture fractions in the composting mass at the start, during composting and in sample j, respectively; $f_{VS,0}$, $f_{VS,t}$ and $f_{VS,j}$ are the fractions of volatile solids in dry basis, at the start, during composting and in sample j, correspondingly.

Before each sampling event, the height of the mixture inside the reactor was recorded for assessing the variation of mixture bulk density, along process evolution.

As the process evolved, the temperature sensor was repositioned inside the reactor to follow the compression of the mixture, by measuring the mixture height inside the reactor throughout the process, as detailed in Figure 4.5.

Composting reactor operation lasted until oxygen uptake rate (OUR) of the mixture attains values of 0.5g O_2 kg^{-1} VS h^{-1} which is the threshold for considering a composting mixture as stable (Gómez et al., 2006). After that, materials concluded maturation phase in small piles at room temperature, that were homogenised and moisture corrected, on a weekly basis.

4.4. Exploratory tests

As previously indicated, the goal of the exploratory tests conducted in SHR was to gain insight about the performance of the reactors. Temperature profiles, oxygen monitoring and application of ash mass balance were considered in these studies.

4.4.1. Temperature gradients and oxygen measuring system

An important aspect concerning temperature monitoring in composting process is the position of sensors (Ekinci et al., 2004). In general, temperature measuring points are established to obtain an overview of the temperature distribution (Papadimitriou et al., 2010). Central readings seek for hot-spots and peripheral measurements provide information about air transport throughout the solid matrix.

However in the case of unmixed SHR, the definition of an inner core volume where temperature readings are taken maybe more adequate to assess process evolution (Mason and Milke, 2005). That core volume is generally based upon the horizontal and vertical temperature profiles.

Oxygen concentration is widely used to assess the degree of aerobiosis (Papadimitñou et al., 2010). It is usually evaluated in the off gas from the composting system, though interstitial O_2 concentration can be measured depending on the available sensor technology.

Electrochemical sensors are cells mainly constituted by a Teflon membrane, anode, semi-solid electrolyte paste and cathode. The oxygen enters the sensor through the membrane, contacts with the cathode and is immediately reduced to hydroxyl ions. These ions migrate through the electrolyte to the anode, generally constituted by lead. There, hydroxyl ions react with the anode which is then oxidised to lead oxide. As reduction-oxidation reaction takes place, a current is generated which is proportional to O_2 concentration, allowing a precise quantification. These electrochemical cells are sensitive to temperature (which interferes with the output signal) and excessive moisture in the gas (which restricts the gas permeability through the membrane). Consequently, a working range in terms of temperature and moisture are generally imposed for these types of sensors. In the case of the sensors used in this work (gas sensor, XLS1047, XLlogger), operating conditions are restricted to 0-40ºC (with internal compensation of the temperature) and gas humidity <95% (non condensing).

Thus, the following exploratory test has a two-fold objective: i) to confirm that the centre temperature readings were acceptable to obtain a picture of the self-heating capacity of the mixture, by obtaining bi-dimensional temperature profiles and ii) to evaluate if the cooling and condensing system coupled to the reactors was able to fulfil the oxygen sensor requirements, in terms of gas temperature and humidity.

4.4.1.1. Material and methods

This assessment was performed by mixing industrial PP from a national industry of potato chips with grass clippings (GC). Wheat straw (WS) was used as amendments to moisture and free air space correction of the composting mixture. These materials were blended according to the formulation presented in Table 4.3. Operating conditions are also depicted in Table 4.3. Air flow rate in these experiments was controlled in order to maintain the oxygen concentration of the outlet gas in the range of 5 to 15% (v/v).

Table 4.3: Composting mixture properties and reactor operating conditions.

	Composting mixture						SHR Operating conditions	
Reactor	PP (%w/w)	GC (%w/w)	WS (%w/w)	C/N	Moisture content (%)	Initial composting mass (kg)	Inlet gas temp. (ºC)	Air flow rate
R3	52	30	18	24.9	67.2	29	17	Controlled to-maintain 5%<$[O_2]_{\text{oulet gas}}$ <15%

Temperature was recorded in the points indicated in Figure 4.10, after 10 min stabilisation of the probe. These measurements were performed in the first and fourth days of composting, and on average all measurements took about 2h30m to 3 h. During that period, the lid of reactor was removed and a 15 cm in thickness polyurethane foam, perforated as detailed in Figure 4.10 b), was placed on the surface of the mixture to minimize heat losses.

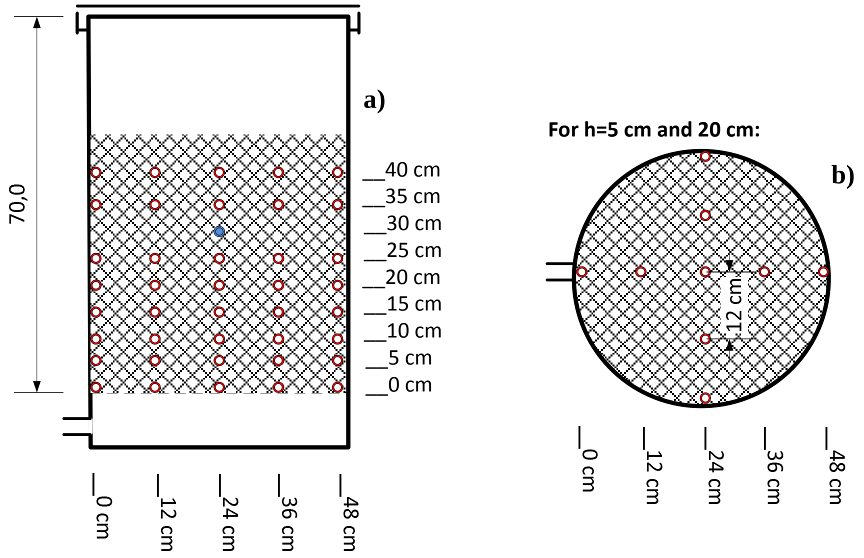


Figure 4.10: Schematic representation of the temperature measuring points a) perpendicular plane, b) radial plane (the validation point is indicated in blue, at h=30 cm).

Since this composting trial was exploratory, the operation and sampling procedure was simpler than the one described in section 4.3. Namely, no withdrawl of the matrix was performed, but homogenisation of reactor content was carried out as detailed in section 4.3.

Bi-dimensional temperature profiles were obtained by using Matlab (version 7.8.0.347, Mathworks, USA) using a biharmonic spline interpolation method (MATLAB® 4 griddata method) that only supports 2-D interpolation. These profiles were calculated between the h=0 cm (near the air distribution plate) and 5 cm beneath the mixture height. For validation purposes, the temperature was measured at the centre of the matrix (h=30cm), as indicated in Figure 4.10 a).

In what concerns oxygen monitoring, the measuring cell showed in Figure 4.7 b) and a glass thermometer (0-100ºC) were used. The formation of condensates in the measuring cell was visually evaluated.

4.4.1.2. Results

Bi-dimensional temperature profiles in the axial and radial directions observed for the first and fourth days of composting are depicted in Figures 4.11 and 4.12, respectively.

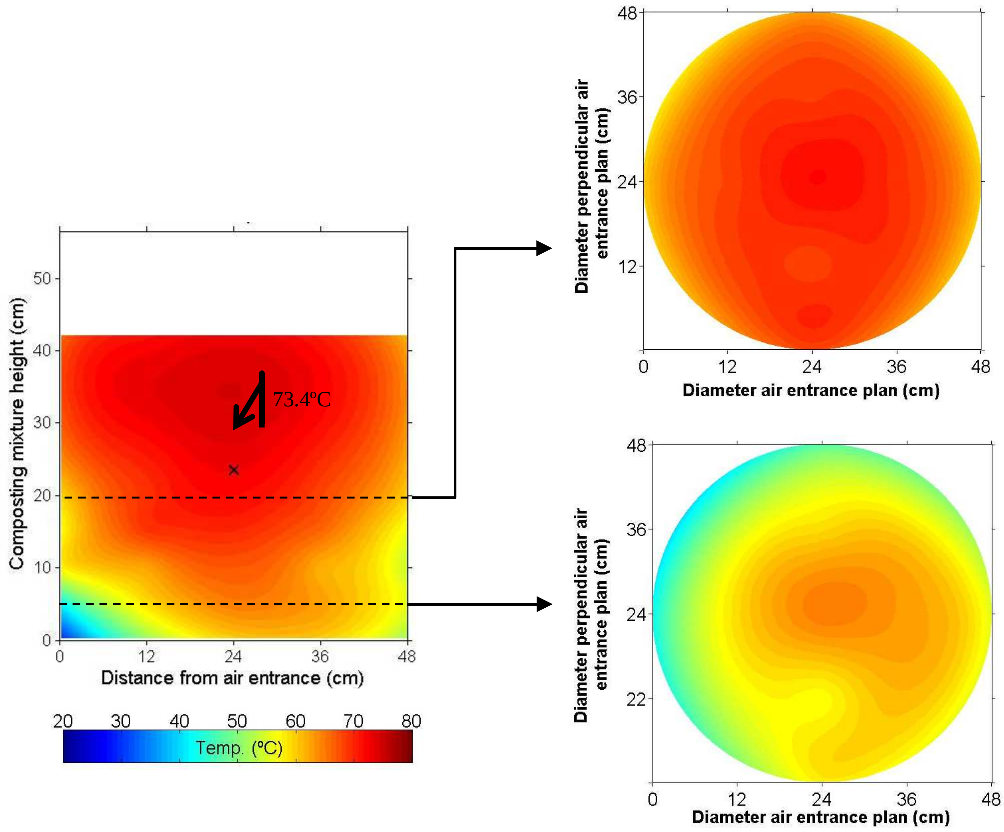


Figure 4.11: Bi-dimensional temperature profiles for the 1st day of composting (inlet air temperature: 17°C).

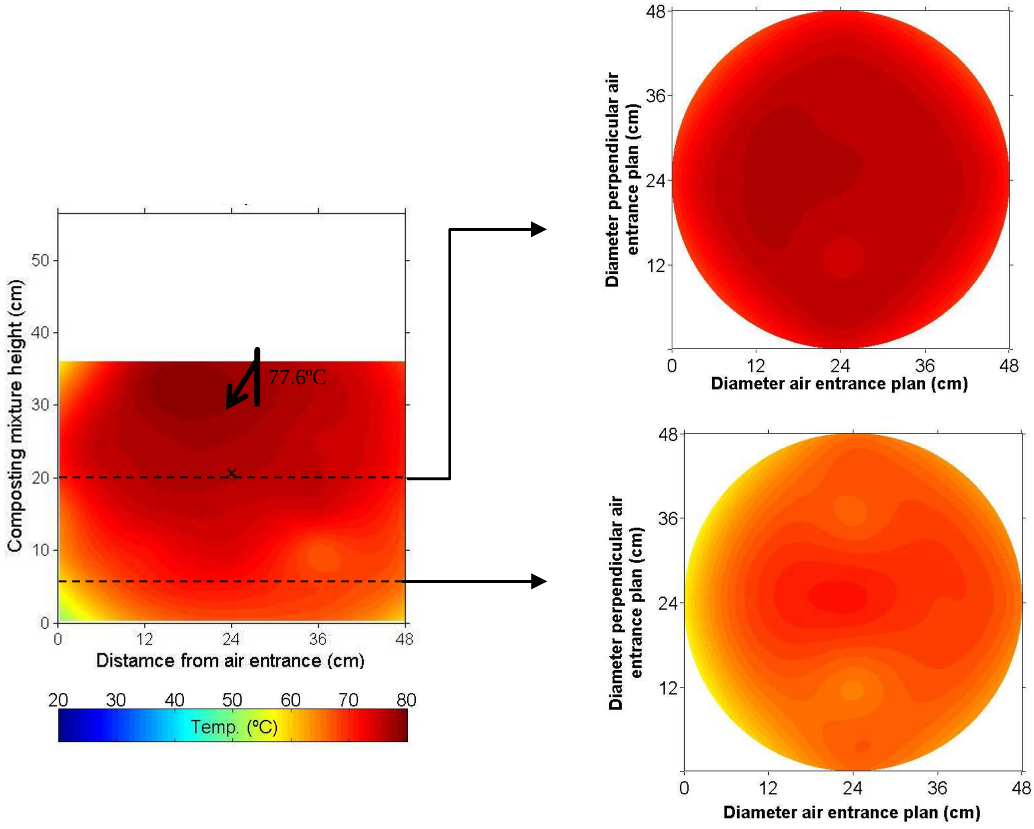


Figure 4.12: Bi-dimensional temperature profiles for the 4th day of composting (inlet air temperature: 17°C).

During the first four days of composting, the hot-spot was located about 10 cm above the geometrical centre of the matrix. However, the difference between hot-spot and temperatures observed in the centre was only near to 3%. This indicates that measurements at the centre of the matrix is a good strategy for determining the maximum temperature. Profiles calculated by using the Matlab were in very good agreement with the measures in the validation point (relative errors of 0.5% for day 1 and 0.9% for day 4, respectively).

As expected, temperatures near the air entrance were lower and the circular plots in Figures 4.11 and 4.12 show that its influence extends to the reactors surface surroundings, especially for 5 cm height. Maximum vertical temperature gradients were near 0.3 ºC cm^{-1}, considering the values recorded at 5 cm and 30 cm height and at the centre of the air entrance plan. Schloss and Walker (2000) found similar vertical gradients (0.4-0.9ºC cm^{-1}) in a composting reactor with 30 cm of diameter and an initial mixture height of 47 cm.

Table 4.4 shows the maximum radial temperature profiles recorded for each height indicated in Figure 4.10 a).

Table 4.4: Radial temperature gradients found for the self-heating reactor.

First day		Fourth day	
Height (h) (cm)	|Radial gradient| (ºC cm^{-1})	Height (h) (cm)	|Radial gradient| (ºC cm^{-1})
5	0.9	5	0.6
10	0.5	10	0.6
15	0.6	15	0.4
20	0.5	20	0.2
25	0.5	25	0.3
35	0.5	36	0.6
42	0.5		

In general, higher temperatures lessen radial temperature gradients, while at the bottom gradients are more relevant. Average radial temperature gradient is 0.5ºC cm^{-1} which is above the ones reported by Schloss et al. (2000) (0.13ºC cm^{-1}), but lower than the value found by Saucedo-Castaneda et al. (1990) (5ºC cm^{-1}). By comparing radial with vertical gradients, one can conclude that heat transport is favoured in the vertical direction.

Figure 4.13 depicts the temperature recorded in the exit gas stream, oxygen measuring system, as well as in the geometrical centre of the mixture. The grey area indicates the operating range of the O_2 sensor. The experimental data clearly show that the cooling/condensing system was adequate for the operating conditions of the O_2 sensor. Moreover, it was observed that water condensation occurred mainly in the tube connecting the reactor and the condensing flask (Figure 4.7 a)), but not in the measuring cell (Figure 4.7 b)).

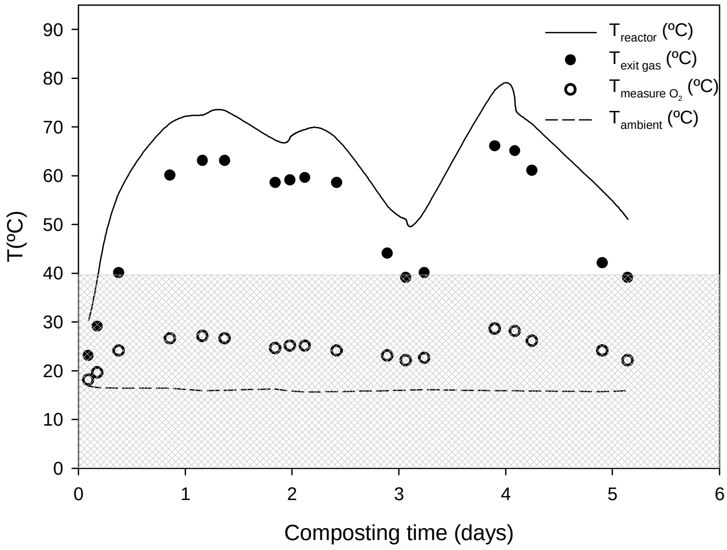


Figure 4.13: Temperature profiles during five days of composting at the mixture centre, exit gases, oxygen measuring system and ambient.

4.4.2. Ash conservation

Inorganic fraction of the composting matrix should behave has a conservative constituent throughout the process (Haug, 1993), as previously depicted in Figure 2.3.

The procedure adopted in the laboratory envisages the weighing and withdrawl of the samples during process evolution to gain insight of the organic matter and nitrogen dynamics. Thus, the following exploratory test was performed to assure that the ash conservation principle was appropriate to the experimental tests.

4.4.2.1. Material and methods

The composting mixture prepared according to the formulation in Table 4.5 was tested in three reactors (R1 to R3). Industrial potato peel (PP), grass clippings (GC) and wheat straw (WS) were the ingredients used. In Table 4.5 the initial aeration rate is also indicated, but this parameter was controlled over time to maintain the oxygen concentration of the outlet gas between 5 to 15% (v/v). The procedure for operation and sampling of the reactors was previously described in section 4.3. Moisture content and volatile solids were determined in each sample as described in section 3.2.3.

Table 4.5: Composting mixture properties and reactor operating conditions.

	Composting mixture							SHR Operating conditions
Reactor	PP (%w/w)	GC (%w/w)	WS (%w/w)	C/N	Moisture content (%)	VS (%)	Initial composting mass (kg)	Air flow rate (L min^{-1} kg^{-1} VS)
R1	52	30	18	24.9	73.6	88.1	29.5	0.30
R2							29.2	0.98
R3							29.2	1.59

4.4.2.2. Results

Ash content was determined as:

$$m_{ash} = (1 - VS)(1 - MC) \times m_t \tag{4.2}$$

where m_t (kg) is the mass of the mixture in the reactor, VS is the fraction of volatile solids in dry basis and MC is the moisture content.

Mass balance closure for ash in consecutive sampling events (period in which no sample is withdrawn) was determined by:

$$Ash\ closure_{period\ i}\ (\%) = \frac{m_{ash,init,period\ i}}{m_{ash,fin,period\ i}} \times 100\% \tag{4.3}$$

where $m_{ash,init,period\ i}$ is the mass of ash at the beginning of period i, $m_{ash,fin,period\ i}$ is the mass of ash at the end of period i and *period i* represents the interval between sampling events (*i*=1 to 4).

Experimental results suggested that the mass balance closure for ash is in general satisfactory giving rise to calculated values ranging from 95-107%, as indicated in Table S4.2 (supplementary material, section 4.7). Komilis et al. (2011) indicated values between 88-135% closure for C-CO_2 measurements in 100 L composting reactors with dewatered-sludge. Defaults in those values were attributed to the inherent heterogeneity of the composting system and, in some extent, to the analytical errors. In addition, moisture content determination is also pointed out as an error income for ash mass balances (Zhang and Matsuto, 2010). In fact, by analysing the standard deviation for moisture content in Table S.4.2 the values obtained were much higher than for VS determination. For that reason it was decided that in future studies, moisture determination would be based on five replicates and the mean value based upon the three more consistent values.

4.5. General conclusions

Exploratory tests on a composting lab installation (with four SHR) indicated that its operation was adequate, in particular with respect to the following aspects:

i) temperature in the centre of the composting mixture was representative of the hot spot;

ii) radial temperature gradients were more significant than in the axial direction;

iii) gas cooling and condensing apparatus allowed gas temperature and moisture to fit within the operating conditions of the oxygen sensors;

iv) sampling procedure reasonably fulfilled the ash conservation, though analytical error associated to the moisture determinations should be lessen by performing more replicates.

4.6. References

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4.7. Supplementary material

According to Mason and Milke (2005), steady state heat transfer across a wall, Q (W), maybe modeled by Eq (S4.1), which is equal to CCR(W):

$$Q = UA\left(T_0 - T_f\right) \qquad \text{(S4.1)}$$

For a cylinder with a two component wall, the term UA may be determines as follows:

$$\frac{1}{UA} = \frac{1}{2\pi r_1 L h_{c,i}} + \frac{ln\left(\frac{r_2}{r_1}\right)}{2\pi k_A L} + \frac{ln\left(\frac{r_3}{r_2}\right)}{2\pi k_B L} + \frac{1}{2\pi r_2 L\left(h_{c,0} + h_r\right)} \qquad \text{(S4.2)}$$

where Q is the heat flux due to convective/conductive/radiative (CCR) heat transfer through the reactor wall (W); U is the overall heat transfer coefficient (W m^{-2} .K^{-1}); A is the surface area (m^2); T is the temperature (K); h_c is the convective heat transfer coefficient (W m^{-2} .K^{-1}); k is the thermal conductivity (W m^{-1} .K^{-1}); h_r is the radiative heat transfer coefficient (W m^{-2} .K^{-1}); r is the radius (m); L is the height of cylinder (m); i,o refer to the inside and outside locations in the bulk mixture immediately adjacent to the wall boundary layers; A, B represent different types of materials (PVC and AeroFlex_EPDM); 1,2,3 represent the inner to outer radii, respectively.

Biological heat (BH) is defined by:

$$BH = BHO \times BD \times V_{reactor} \qquad \text{(S4.3)}$$

where BHO is the peak of biological heat output (W kg$^{-1}{}_{TS}$), BD is the dry mixture bulk density (kg$_{TS}$ m^{-3}), $V_{reactor}$ is the volume of the reactor (m^3) and TS represents the total solids in the mixture. For estimating instant CCR and BH, data presented in Table S4.1 were assumed.

Table S4.1: Data used for estimation of CCR heat losses and biological heat production on a cylindrical self-heating composting reactor. Adapted from Mason and Milke (2005).

Parameter	**Units**	**Value**
Peak biological heat output (BH0)	W kg$^{-1}_{TS}$	28.0
Mixture bulk density (BD)	kg m^{-3}	250
Moisture content of mixture	%	65
Compost temperature	ºC	70
Ambient temperature	ºC	25
PVC thermal conductivity	W m^{-1} .K^{-1}	0.092
Thickness of PVC	mm	5
AeroFlex_EPDM thermal conductivity	W m^{-1} .K^{-1}	0.035
Thickness of AeroFlex_EPDM	mm	36
Convective/radiative heat transfer coefficient (hc, hr)	W m^{-2} .K^{-1}	10

Table S4.2 summarizes experimental results for analysing the ash balance closure (%), which was calculated according to Eq.(4.3).

Table S4.2: Experimental results of ash conservation for SHR.

		Sampling period							
		1st		2nd		3rd		4th	
SHR	Composting days / Parameters	0	2	2*	4	4*	7	7*	10
R1	m (kg)	29.5	28.3	28.0	27.2	26.7	23.2	22.0	21.2
	MC (%±sd, n=3)	73.6±2.6	73.2±0.1	73.2±0.1	73.7±3.1	73.7±3.1	78.9±1.0	78.9±1.0	78.7±0.5
	VS (%±sd, n=3)	88.1±0.3	88.1±0.5	88.1±0.5	87.8±0.3	87.8±0.3	83.3±0.5	83.3±0.5	83.3±0.1
	m_{ash} (kg)	0.93	0.90	0.90	0.87	0.86	0.82	0.78	0.76
	Ash balance closure (%)	97		97		95		97	
R2	m (kg)	29.2	26.2	26.0	23.9	23.5	21.8	21.3	20.9
	MC (%±sd, n=3)	73.6±2.6	76.3±2.4	76.3±2.4	72.4±1.1	72.4±1.1	73.5±1.8	73.5±1.8	70.0±2.6
	VS (%±sd, n=3)	88.1±0.3	85.6±0.4	85.6±0.4	86.3±0.2	86.3±0.2	83.9±0.5	83.9±0.5	84.5±0.2
	m_{ash} (kg)	0.92	0.93	0.92	0.93	0.89	0.93	0.91	0.97
	Ash balance closure (%)	102		102		104		107	
R3	m (kg)	29.2	25.7	25.4	23.5	23.0	22.4	21.6	21.3
	MC (%±sd, n=3)	73.6±2.6	74.8±4.1	74.8±4.1	74.3±0.8	74.3±0.8	72.8±0.9	72.8±0.9	73.1±0.7
	VS (%±sd, n=3)	88.1±0.3	86.5±0.4	86.5±0.4	84.7±0.6	84.7±0.6	84.8±0.3	84.8±0.3	84.5±0.3
	m_{ash} (kg)	0.92	0.87	0.86	0.92	0.90	0.93	0.89	0.89
	Ash balance closure (%)	95		107		103		100	

*- initial conditions after sample withdrawal.

Part C. Composting trials with industrial eggshell waste

The third part of the thesis integrates the core of the work developed regarding composting studies. Firstly, in Chapter 5 a screening of starting composting mixtures is performed to select the most promising formulation in terms of self-heating potential. The influence of eggshell (up to 30% w/w) and N-rich material on the composting process evolution and end-product quality is thoroughly studied in Chapter 6. Chapter 7 is devoted to the application of Principal Components Analysis (PCA) to unravel the main relationships structuring the variability of data collected from experimental studies that addressed the incorporation of high levels (up to 60%) of eggshell waste in a composting mixture.

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5. Evaluation of self-heating potential in specific mixtures*

Industrial eggshell waste (ES) is classified as an animal by-product not intended to human consumption. For reducing pathogen spreading risk due to soil incorporation of ES, sanitation by composting is a pre-treatment option. Aiming to attain pathogen-killing requirements, the inorganic nature of ES obliges its composting with organic materials to upsurge the energy input for the process.

In this work, the selection of a starting composting mixture to allow eggshell waste recycling is addressed. Potato peel, grass clippings and rice husks were the organic materials considered.

This study showed that the presence of grass clippings enhanced the self-heating potential.

Keywords
Industrial eggshell waste, composting, self-heating potential, starting mixture

*The information presented in this chapter was based upon the publications:

Soares, M.A.R., Quina, M.J., Quinta-Ferreira, R., 2013. Co-composting of eggshell waste in self-heating reactors: Monitoring and end product quality. Bioresour. Technol. 148, 293–301.

Soares, M.A.R.; Quina, M.J.; Quinta-Ferreira, R, 2012. Selection of organic materials for co-composting industrial eggshell waste, presented at WasteEng2012- 4th International Conference on Engineering for Waste and Biomass Valorisation, 10-13th September, In Proceedings, Vol.2, pg 604-609, Porto, Portugal. (*oral communication*).

5.1. Introduction

There are millions of hen eggs that are broken every day in food plants. The eggshell (ES) is usually seen as a waste from this industry, whose production can represent from 0.03 to 0.12 of the mass of the egg products obtained from eggs (Russ and Meyer-Pittroff, 2004).

In 2011, European countries produced about 10.6 million ton of eggs from which about 30% was diverted to egg breaking processing (Agra CEAS Consulting Ltd, 2008; FAO, 2012). Since ES represents 11% of the total egg weight (Meski et al., 2011), the annual production of ES in Europe may ascend to 350 thousand ton.

The adequate handling and disposal of this waste has been considered a challenge for the food industry in what concerns environmental protection, due to not only the large amounts generated but also to its potential for microbial proliferation or growth of pathogens (Russ and Meyer-Pittroff, 2004).

Suitable management strategies have not been well established for ES and disposal in landfills has been traditionally used (Tsai et al., 2008). However, recycling and recovery should be investigated to avoid the waste of resources.

In accordance with Regulation (EC) Nº 1069/2009 of the European Parliament and the Council, the ES is nowadays classified as an animal by-product (ABP) not intended to human consumption. ABP are categorized into three categories, which reflect the level of risk to public and animal health arising from those wastes, and for each category, a list of disposal and use alternatives is imposed in that Regulation. ES is included in the inventory of Category 3, and in this case composting prior to soil incorporation is an alternative to disposal/use, in order to reduce spreading risk of pathogens to the environment and human health.

Composting may be defined as a process of biological decomposition and stabilization of organic substrates, under aerobic conditions, that allows development of thermophilic temperatures, as a result of biologically produced heat (Haug, 1993), for human and plant pathogens inactivation. It can transform organic matter of animal or vegetal origin into a final product that is stable, free of pathogens and plant seeds, rich in humus and beneficial for recycling of soil organic matter and nutrients.

Nowadays, there is a renewed attention concerning composting and its application is not limited to a farm context but is broadened to municipal and industrial frameworks. In this scope, composting has been identified as an environmentally sound alternative, for tailoring the recovery of waste organic matter and nutrients to suppress soil deficiencies (Farrell and Jones, 2009).

Due to the high nutrition content of ES, namely in calcium, its recycling by composting seems quite interesting. In fact, the application of enriched calcium compost to soils deficient in this nutrient may constitute an option to promote ES recycling into a value-added product and consequently recover a natural resource contained in this ABP.

In a composting process the major energy input is due to the organic content of the initial mixture (Haug, 1993). Since ES is mainly an inorganic material with about 94% (w/w) $CaCO_3$ (Tsai et al., 2008), it may be expected that the energy released from organic decomposition may not be sufficient to rise the temperature of the composting matrix to the thermophilic range adequate for pathogen inactivation. Consequently, composting of ES with other materials should be considered to upsurge the energy input for the process, aiming to satisfy sanitizing requirements for ES (70ºC for 1 h) (Commission Regulation (EU) Nº142/2011)."

The main objective of this work was to evaluate the self-heating ability of a set of composting mixtures, to ensure proper sanitation of eggshell. Potato peel (PP) (from the food industry), grass clippings (GC) and rice husks (RH) were the organic materials considered to seek for a balanced initial composting mixture.

5.2. Materials and methods

5.2.1. Self-heating reactors

Experiments were performed in a system of three self-heating reactors (SHR), whose characteristics were previously described in Chapter 4.2.

Biological activity of the composting mixture was assessed by calculating oxygen uptake rate (OUR) defined as (Barrena et al., 2009):

$$\text{OUR}\,(\text{g O}_2\ \text{kg VS}^{-1}\ \text{h}^{-1}) = \frac{Q \times \Delta O_2 \times 31.98}{1000 \times Vg \times VS_t} \tag{5.1}$$

where Q (L h^{-1}) is the airflow rate, ΔO_2 (mL L^{-1}) the difference in oxygen concentration between the inlet and the outlet airflow, 1000 is the conversion factor from mL to L, Vg (L mol^{-1}) the volume of one mole of gas at inlet conditions, 31.98 (g mol^{-1}) is the molecular weight of O_2, and VS_t (kg) the weight of the organic matter of the composting mixture, at instant t.

The profile of organic matter loss during experiments was fitted to a first order kinetic model (Bustamante et al., 2008):

$$\frac{VS_0 - VS_t}{VS_0} = \frac{VS_{bio}}{VS_0} \times (1 - e^{-kt}) \tag{5.2}$$

where VS_0 is the initial weight of organic matter in the mixture (kg), VS_{bio} is the maximum amount of organic matter degraded (kg), k is the rate constant ($days^{-1}$) and t is the composting time (days). After fitting, the obtained equation was used to quantify instantaneous VS_t. Cumulative OUR was calculated by numeric integration of the instantaneous OUR values recorded during composting.

5.2.2. Materials and experimental design

Potato peel (PP) was gathered at a Portuguese industry of potato chips, rice husk (RH) was provided by a rice husking factory and grass clippings (GC) were obtained from a garden maintenance service from a national football stadium. Materials were used in composting experiments immediately after their collection. Grass clippings were sieved through a 5 cm mesh, in order to obtain a homogenous material in size and shape. The other materials did not require any specific treatment.

To select a composting mixture with an adequate biodegradability level to drive the process into thermophilic conditions, three formulations were tested (Table 5.1) involving PP, GC and RH as ingredients. Since PP is a very wet material (~85% moisture) and with low inter-particle volume of voids available for oxygen diffusion, RH was added as structural and drying amendment material. Free air space (FAS) of initial mixtures was predicted according to a model based on the ingredients proportions and described elsewhere (Soares et al., 2013).

Each blend was prepared by weighing the components according to the pre-defined proportions indicated in Table 5.1, for a total load equivalent to a volume of 99 L. The ingredients were mixed thoroughly for 20 min, before loading the SHR.

Table 5.1: Composting mixtures characteristics tested in the self-heating reactors.

	SHR_1	SHR_2	SHR_3
PP (%w/w)	58.7	50.6	66.0
GC (%w/w)	19.4	32.4	8.1
RH (%w/w)	21.9	17.0	25.9
Mixture mass (kg)	23.8	22.7	24.2
Moisture Content (%)±sd	70.5±1.3	69.6±1.5	64.3±1.2
Volatile Solids (VS) (%)±sd	86.7±0.03	85.9±0.03	86.2±0.03
Predicted FAS (%)±pi	73.9±4.2	76.7±4.0	71.0±4.5

SHR: self-heating reactor; PP: potato peel; GC: grass clippings, RH: rice husk, sd: standard deviation, pi: prediction interval

The initial airflow rate was 7.5 L min^{-1}, and afterwards it was manually adjusted to guarantee an outlet oxygen concentration in the range of 5 to 15% (v/v). Composting experiments lasted 25 days and the content of the reactors was homogenised in a daily basis.

Samples were taken for analysis on day 0, 4, 11, 18 and 25, as previously described (Chapter 4.3).

5.2.3. Analytical methods

The moisture and VS content were determined as detailed in Chapter 3.2.3. FAS of the composting mixtures during aerobic biodegradation was calculated as described in the previously referred Chapter (Eq (3.6)). Additionally, during composting monitoring, FAS was also quantified by Eq (3.10) a theoretical approach proposed by Richard et al. (2004).

5.2.4. Statistical analysis of data

Statistical analyses, model fitting and numeric integrations were performed in software Matlab (Version 7.8.0.347, Math- works, USA).

5.3. Results and discussion

It is well established that the self-heating capacity of a composting mixture depends on the heat production rate during the microbial breakdown of organic materials. This parameter can be considered as linearly dependent on the oxygen consumption rate (Barrena et al., 2006) and determines the extent to which easily biodegradable organic matter has decomposed (Barrena et al., 2011). In fact, also according to Scaglia et al. (2000) higher demand of oxygen per unit of organic matter can be understood as higher release of heat produced by biodegradation.

To categorise the biological activity of organic mixtures and thus establish a comparison of the potential self-heating capacity, Barrena and co-workers proposed three main categories based on the rate of oxygen consumption (Barrena et al., 2011):

i) high biodegradable (respiration activity higher than 5 mg O_2. gVS^{-1} h^{-1});
ii) moderate biodegradable (respiration activity within 2 to 5 mg O_2. gVS^{-1} h^{-1}) and
iii) low biodegradable (respiration activity lower than 2 mg O_2. gVS^{-1} h^{-1}).

In the scope of this work, the selection of the initial mixture for subsequent composting of eggshell was performed on a high self-heating based concept, meaning that finding the composting mixture with higher oxygen consumption per unit of organic matter during thermophilic activity was the prime goal.

For each mixture tested in the reactors SHR_1 to SHR_3, whose properties were indicated in Table 5.1, Figure 5.1 a) shows the temperature and airflow profiles and Figure 5.1 b) depicts the OUR evolution, over 25 days of composting trials.

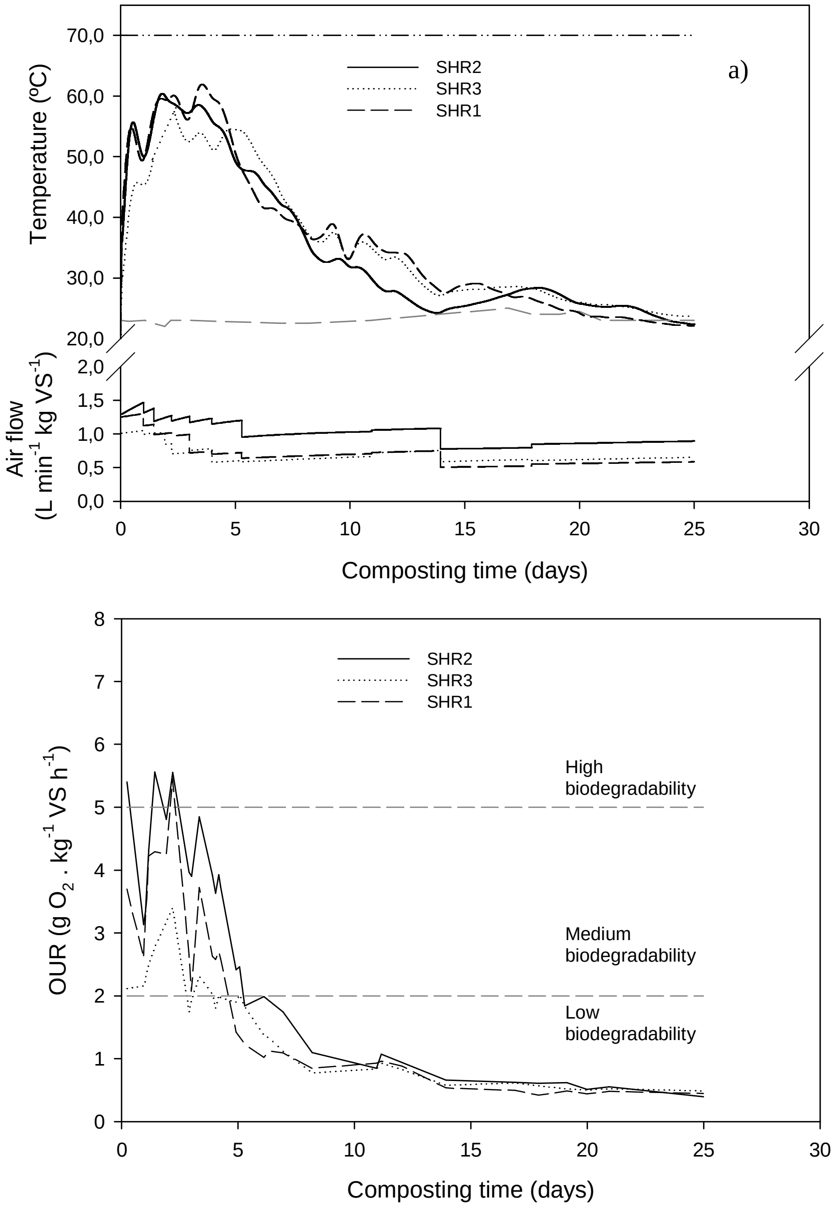

Figure 5.1: Evolution of (a) temperature and airflow rate and (b) OUR for SHR_1 to SHR_3 reactors.

The results reveal that regardless of the mixture composition, temperature profiles have the expected behaviour over time, where the three classic phases are well observed (Zhu, 2006). Indeed, a short initial phase is visible due to the activity and growth of mesophilic organisms that increase the temperature within the first 24 h of composting. Then, the thermophilic phase (T>40ºC) was developed and lasted about 7 days, promoting degradation of materials, while non-thermo-tolerant organisms were inhibited. Finally, the mesophilic activity arose once again and determined the temperature profile, reflecting the depletion of readily biodegradable components. The maximum temperatures achieved were 61.9, 60.4 and 57.5ºC for SHR_1 to SHR_3, respectively.

Differences between mixtures were also observed in terms of time/temperature relations for thermal inactivation of pathogens. In accordance to Haug (1993), most common pathogens and parasites present a minimum threshold of 55ºC during 10 to 60 min of exposure for thermal inactivation. In this study, SHR_3 presented a lower exposure period of 5 consecutive hours to 55ºC, while for SHR_1 and SHR_2 that period attained 81 and 68 hours, respectively. Nevertheless, none of the mixtures tested attained the criteria of temperature exposure (70ºC for 1 h) imposed by Commission Regulation (EU) Nº142/2011 now in force, which might be a consequence of excessive aeration (high airflow rates).

However, according to Figure 5.1 a) the heat removal by convection may have been lower for SHR_3 in comparison with SHR_1 and SHR_2, because the air flow rate applied by kg of VS was smaller in the former case. Therefore differences found in terms of time/temperature exposure should be attributed to the biodegradability of the organic matter in the starting material. The lower capacity of mixture SHR_3 to achieve and maintain higher temperatures is also observable in OUR profile. According to Figure 5.1 b) the maximum OUR reached in SHR_3 ranged the interval characterized by moderate biodegradability as indicated by Barrena et al. (2011), which points out a smaller availability of biodegradable organic matter. This is probably due to the presence of higher amounts of rice husks that contain significant levels of recalcitrant organic matter. In addition, also biodegradability of the potato peel may play a role owing to the presence of compounds like lignin and phenolic substances (Ezekiel et al., 2013) that are rather resistant to degradation and their rate of disappearance is not as fast as that of polysaccharides (Said-Pullicino et al., 2007).

For the mixtures SHR_1 and SHR_2 the maximum OUR reached the level of “high biodegradability”, but the OUR cumulative values for thermophilic phase (T>40ºC) were 432 and 624 g O_2 kg^{-1} of organic matter for SHR_1 and SHR_2, respectively. This means that the SHR_2 mixture is characterized by a higher thermal energy to drive the composting process into thermophilic temperatures and induce pathogen elimination as required for eggshell recycling by composting.

However, high biodegradable mixtures may impair composting because when materials decompose quickly, pores between particles are reduced and gas transfer conditioned (Alburquerque et al., 2008). Therefore, FAS was quantified according to Eq (3.6) and (3.10) and pore space evolution during composting is presented in Figure 5.2.

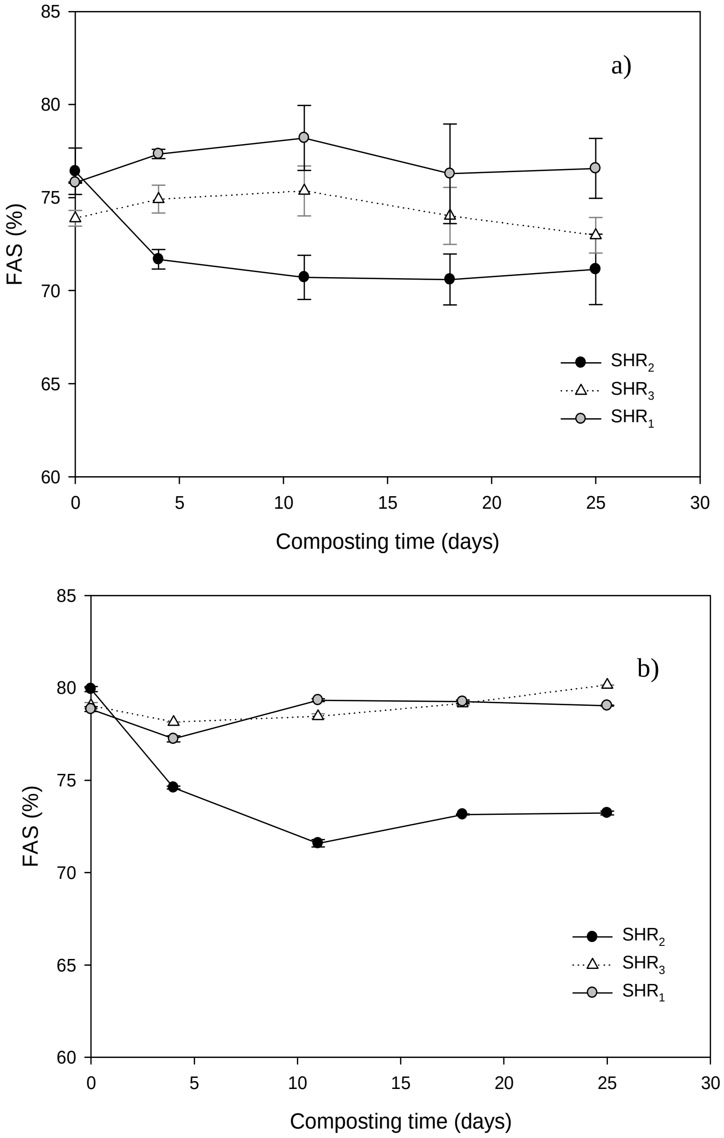

Figure 5.2: Evolution of FAS during composting of SHR_1, SHR_2, SHR_3 a) by Eq (3.6) b) by Eq (3.10) (measurements correspond to means of 3 replicates and error bars indicate standard deviation).

In general, FAS calculated by Eq. (3.6) is slightly lower than quantification provided by the theoretical approach proposed by Richard et al. (2004), Eq. (3.10), with relative errors ranging between -1.6 to -8.9%. Nevertheless, FAS evolution during composting was similarly described by the two equations used in this study, and both emphasized that SHR_2 undergoes a more prominent reduction of FAS during the most active phase of biodegradation (the initial 7 days of compost). But the proportion of rice husk in mixture SHR_2 allowed to maintain FAS far above 30%, which is commonly considered to be the limiting value (Huet et al., 2012).

Thus, it was considered that the higher biodegradability of SHR_2 places no restriction on the pore volume. Therefore, the SHR_2 mixture was considered for further experiments with eggshell waste.

The effect of air flow on reaction temperatures of the SHR_2 mixture was still evaluated. In this experiment, the mixture formulation (SHR_2+0%ES) was equal to SHR_2, a working reactor volume of 105 L was used, and the starting airflow was set to 3.5 L min^{-1}, equivalent to 0.5 L min^{-1} kg^{-1} of initial organic matter. The air volumetric rate was adjusted over time, and homogenisation and sampling procedures followed the methods aforementioned.

Figure 5.3 compares temperature, airflow rate and OUR observed in SHR_2 and SHR_2+0%ES for 25 days of composting.

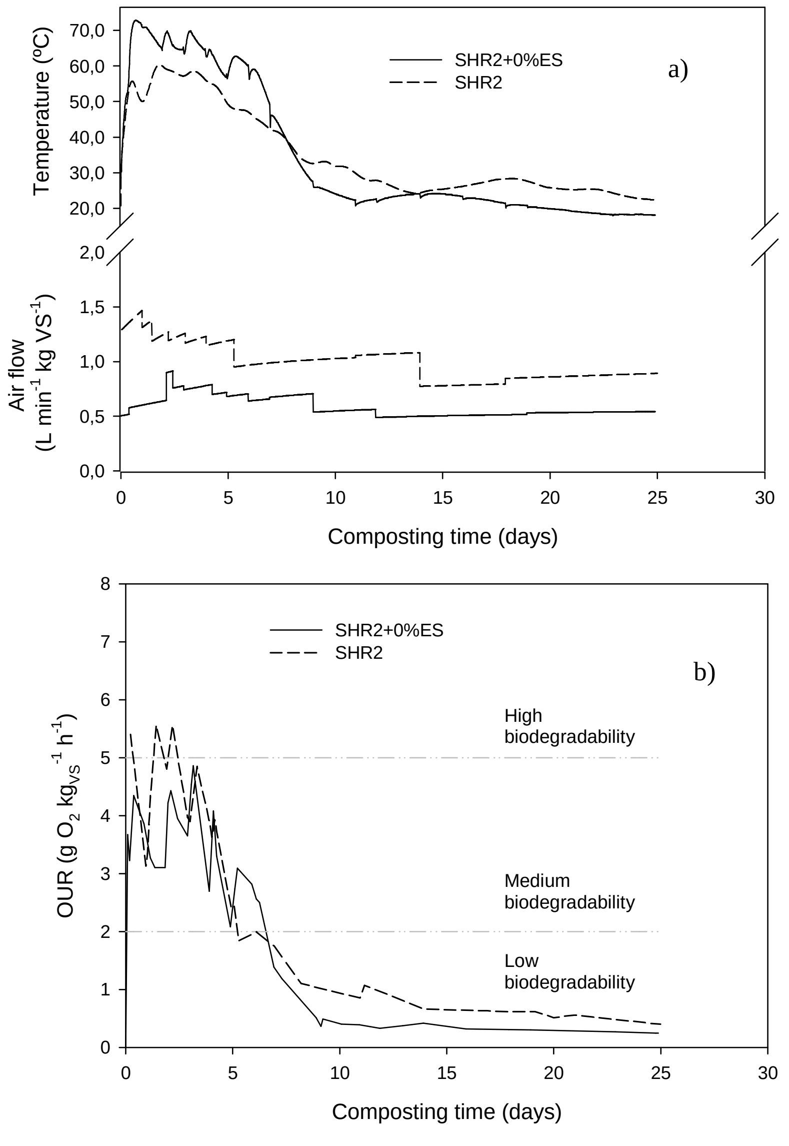

Figure 5.3: Evolution of (a) temperature and airflow rate and (b) OUR composting for SHR_2 and SHR_2 +0%ES.

By reducing the air flow initially fed to the mixture SHR_2 from 1.3 to 0.5 L min^{-1} kg^{-1} of initial organic matter, a significant increase in the maximum temperature was attained (from 60.4ºC to 72.8ºC, Figure 5.3 a)). These findings indicate that the mixture was sensitive to the air flow rate with respect to heat removal by convection during composting.

Regarding biodegradability, the maximum OUR reached was slightly reduced in the early stage of the process (5.3 to 4.8 g O_2 kg^{-1} VS h^{-1}, Figure 5.3 b)), probably due to an initial limitation in oxygen concentration during that stage, but that change was determinant to reduce convective heat losses, and therefore attain the desired sanitation temperatures needed for ES treatment. Nevertheless, seeding of the initial mixture would be a worthwhile option to evaluate in further studies. In fact, the addition of adequate inoculants could enhance global biotransformation (reaction rates) with consequent reduction of the mixture susceptibility to the air convective effects (Nakasaki and Akiyama, 1988).

A preliminary experiment was performed to address the effect of eggshell (ES) waste on composting process evolution. For this, two mixtures were tested: SHR_2-0%ES and SHR_2-30%ES involving 30% (w/w) of ES and 70% (w/w) of a mixture equal to SHR_2-0%ES. The operating conditions were the ones previously described (105 L of reacting volume, airflow of 0.5 L min^{-1} kg^{-1} of initial organic matter) and homogenisation and sampling procedures followed the aforementioned methods.

Profiles shown in Figure 5.4 reveal a rapid increase in temperature for both mixtures, mainly due to microbial metabolism favoured by the presence of readily available organic substances. The reaction temperature reached about 70ºC and remained above that value for 18 consecutive hours for SHR_2+0% ES and 8.7 hours for SHR_2+30% ES. Thus, the addition of 30% of ES allows compliance with the minimum time and temperature sanitising requirements of 1 h at 70ºC, proposed by Commission Regulation (EU) Nº142/2011.

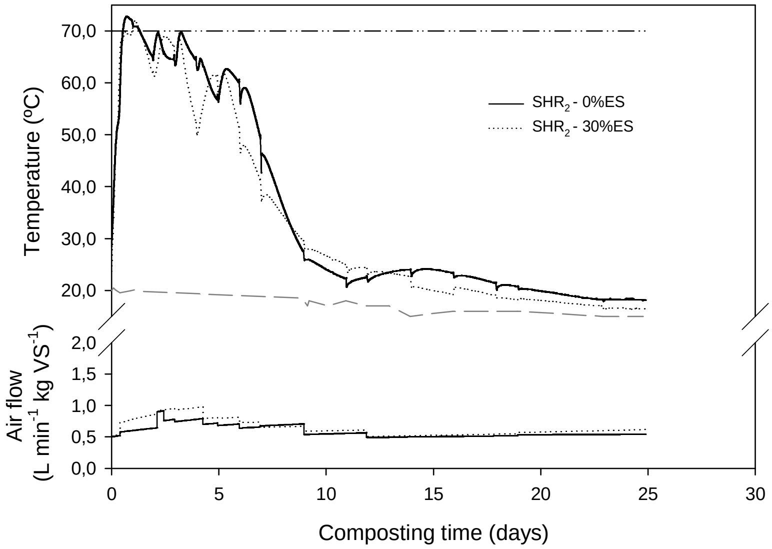

Figure 5.4: Evolution of temperature and air flow rate, during composting of SHR_2 with 0 and 30% ES (w/w).

5.4. Conclusions

The objective of this work was to select a mixture composition of potato peel (PP), grass clippings (GC) and rice husks (RH) with self-heating potential enough to promote sanitation of eggshell waste (ES). The composting experiments showed that the three organic mixtures tested with PP, GC and RH ranged from moderate to highly biodegradable. The mixture with higher content in RH and lower proportions of GC presented the smaller biological activity. In opposition, upper GC fractions enhanced the self-heating potential, therefore SHR_2 was suggested for future experiments with ES. Results indicated the air flow rate may be controlled to avoid excessive heat removal.

Preliminary experimental tests showed that ES incorporation did not affect significantly the capacity of the composting mixture to reach sanitising conditions. Nevertheless, the higher content of nitrogen in SHR_2, due to the upper fraction of grass clippings, may arise some issues regarding nitrogen conservation during composting that will be addressed in the next chapter.

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6. Valorisation of industrial eggshell by co-composting: influence of eggshell content and N-rich material*

Industrial eggshell (ES) is an animal by-product (ABP) involving some risk if not properly managed. Composting is a possible treatment approved for its safe use. This study aims to assess the influence of using N-rich material (grass clippings) to improve co-composting of ES mixtures for reaching sanitising temperatures imposed by the animal by-product regulation from the European Union.

Two sets of mixtures (M_1 and M_2) were investigated each containing industrial potato peel waste, grass clippings and rice husks at 3:1.9:1 and 3:0:1 ratios by wet weight. In each set, ES composition ranged from 0 to 30% (w/w). Composting trials were performed in self-heating reactors for 25 days, followed by maturation in piles. Results showed that only M_1 trials attained temperatures higher than 70ºC for 9 consecutive hours, but N-losses by stripping on average were 4 to 5-fold higher than M_2.

In the absence of N-rich material, biodegradability of mixtures was “low” to “moderate” and organic matter conversion was impaired. Physical, chemical and phytotoxic properties of finished composts were suitable for soil improvement, but M_1 took more 54 days to achieve maturity. In conclusion, co-composting ES with N-rich materials is important to assure the fulfilment of sanitising requirements, avoiding any additional thermal treatment.

Keywords
Eggshell waste, co-composting, N-rich ingredient, self-heating potential, sanitising

*The information presented in this chapter was based upon the following manuscripts:

Soares, M.A.R., Quina, M.J., Quinta-Ferreira, R.. Influence of N-rich material in valorisation of industrial eggshell by co-composting. Submitted to Environmental Technology, in October 2015.

Soares, M.A.R., Quina, M.J., Quinta-Ferreira, R., 2013. Co-composting of eggshell waste in self-heating reactors: Monitoring and end product quality. Bioresour. Technol. 148, 293–301.

6.1. Introduction

Composting is an ancient practice that has been used for centuries by man, as a mean of recycling organic matter to improve soil physical and chemical properties (Rynk et al., 1992). Although this technology was first developed for use in farming activities, nowadays its application has broadened to municipal and industrial wastes. In fact, regulatory targets are limiting the amount of biodegradable waste sent to landfill, promoting the retrieval of organic matter and nutrients that can be tailored to suppress soil deficiencies. In this scope, composting has been identified as an environmentally sound alternative (Chroni et al., 2012; Farrell and Jones, 2009; Gajalakshmi and Abbasi, 2008; Sharma et al., 1997; Tuomela et al., 2000).

Composting has been used to recycle several types of wastes such as municipal solid wastes (Castaldi et al., 2005; Gestel et al., 2003; Kumar and Goel, 2009), sewage sludges (Kang et al., 2011; Manios, 2004; Paredes et al., 2005; Wei and Liu, 2005), agro-food residues (Grigatti et al., 2011; Pepe et al., 2013; Sellami et al., 2008) and many others. More specifically, composting is also amongst the list of options for use and disposal of animal by-products defined the European Union legislation (Regulation (EC) Nº 1069/2009 of the European Parliament and of the Council of 21 October 2009), which imposes as operating condition a temperature-time relation of 70ºC for 60 min, to achieve successful sanitation. In the literature, some studies have already addressed the viability of this sanitizing requirement by performing composting tests with animal carcasses (Barrena et al., 2009), poultry manure (Bijlsma et al., 2013) and hydrolysed leather or blood meal (Cayuela et al., 2012).

Eggshell (ES) is a by-product of egg processing industry that is categorised as an animal by-product (ABP). Actually, this ABP is mainly inorganic (94% $CaCO_3$) (Tsai et al., 2008) and its production may annually ascend to 350 thousand tonnes in Europe (Soares et al., 2013a) which constitutes a huge environmental issue (Ibrahim et al., 2015). Raw ES typically has a low C:N ratio (≈2), mainly due to the organic content of protein, glycoproteins and proteoglycans fibres present therein (Baláž, 2014; Cordeiro and Hincke, 2011; Guru and Dash, 2014). Thus, mixture formulation must be tailored for promoting ES composting with effective retention of N, as well as for having enough self-heating capacity to allow the fulfilment of sanitizing requirements.

The N availability in composting starting mixtures is reported to have a relevant influence on organic matter degradation rate, chemical evolution, gaseous losses and likely in quality of the end-product composts (Cayuela et al., 2012, 2009; Pandey et al., 2009; Sanchez-Monedero et al., 2001; Sánchez-Monedero et al., 2010). On the other hand, self-heating capacity is intrinsically related to the feedstock biodegradability, which depends upon the materials origin (de Guardia et al., 2010a).

Due to these issues, a comprehensive understanding of N influence in the physical and chemical changes occurring during co-composting of ES is required and yet not addressed. Thus, this study highlights the effect of N-rich material (grass clippings, GC) in ES composting mixtures, where potato peel (PP) is the starting organic feedstock and rice husk (RH) is the bulking agent. The influence of GC on ES co-composting was considered at four levels: self-heating capacity, physical changes, nitrogen transformation and end-product quality.

6.2. Materials and methods

6.2.1. Composting materials and experimental design

Potato peel (PP) was collected from a potato chips industry, rice husk (RH) was provided by a rice husking factory and grass clippings (GC) were obtained from a national football stadium. Materials were used in composting experiments in less than 24 hours after their collection. GC was sieved through a 5 cm mesh, but the other materials did not require any treatment. The eggshell waste was collected in a national industry of pasteurized liquid eggs and boiled eggs, and immediately preserved at -12 ºC until further use. ES was thawed at room temperature before use. The main properties of the composting ingredients are reported in Table S6.1 in section 6.7.

The experimental design consisted in eight quaternary composting mixtures divided in two sets (M_1 and M_2, with and without GC, respectively), where ES ranged from 0 to 30%. In all mixtures, the ratio RH/PP was kept constant (≈0.34). Homogeneous mixtures according to the predefined proportions were obtained (Table 6.1) and no water was required. Total mass loaded to each reactor varied between 25.7 to 35.9 kg.

Table 6.1: Composition of mixtures and composting experimental conditions**.**

Parameter	M_1 +0%ES	M_1 +10%ES	M_1 +20%ES	M_1 +30%ES	M_2 +0%ES	M_2 +10%ES	M_2 +20%ES	M_2 +30%ES
PP (%w/w)	50.6	45.6	40.5	35.4	74.5	67.1	59.6	52.2
GC (%w/w)	32.4	29.1	25.9	22.7	0.0	0.0	0.0	0.0
RH (%w/w)	17.0	15.3	13.6	11.9	25.5	22.9	20.4	17.8
ES (%w/w)	0.0	10.0	20.0	30.0	0.0	10.0	20.0	30.0
Total mass (kg)	25.7	27.8	30.5	33.9	26.9	29.7	32.6	35.9
Initial air-flow (L h^{-1})	204	215	215	212	228	234	223	234

6.2.2. Experimental set-up and composting tests

Co-composting experiments were performed in four self-heating reactors with a working volume of 105 L. The characteristics of each reactor, as well as the air distribution system, O_2 and temperature monitoring devices were previously described in Chapter 4.

Composting trials lasted for 25 days in self-heating reactors. Airflow was initially set from 204 to 234 L min^{-1}, which is equivalent to 0.5 L min^{-1} per kg of organic matter (OM). This aeration rate is within the range of 0.43-0.86 L min^{-1} kg^{-1} OM used by Lu et al. (2001) for food waste. The air flow was adjusted to assure an outlet oxygen concentration in the range of 5 to 15% (v/v) and sustain the thermophilic phase as long as possible (Puyuelo et al., 2010). To ensure homogeneous conditions during tests, reactors were rolled for a distance equivalent to 14 rotations. The homogenisation procedure was performed on a daily basis during thermophilic conditions, and whenever samples were withdrawn (days 4, 12, 20, 25). After 25 days, mixtures were allowed to conclude maturation phase in small piles that were homogenised and moisture corrected (≈50%) every week, until 90 days of composting for M_2 and 144 days for M_1. During this stage, sampling was performed at days 61, 90 and 144.

All samples withdrawn were weighed and the mass of each was considered in the overall mass balance of composting, as detailed in Chapter 4. The fraction of organic matter $(\frac{VS_t}{VS_0})$ not degraded during composting was calculated according to Eq. (4.1)

6.2.3. Monitoring parameters

During composting trials in self-heating reactors, biological activity of the mixture was assessed by calculating oxygen uptake rate (OUR) defined as previously indicated in Eq. (5.1). The profile of organic matter loss during experiments was fitted to a first order kinetic model (Eq. (5.2)).

Wet bulk density (BD, kg m^{-3}) was determined by weighing the reactor content (kg) and calculating the mixture volume from the height of the mass inside the reactor (H1) measured as established in Chapter 4.2.2 (Figure 4.5). Free air space (FAS) of the composting mixtures during aerobic biodegradation was calculated according to Eq. (3.6).

The moisture content was determined as the loss in weight after samples were dried in an oven at 105ºC to constant weight. Water holding capacity was determined as described in section 3.2.3 (Eq. (3.7)).

pH and electrical conductivity (EC) were measured in water extracts 1:5 (v/v) for 1 h. pH was measured directly in suspensions, but a filtration was performed in the case of EC and the contribution of water, glassware and filter paper (blank essay) was subtracted.

The calcium carbonate content was assessed by suspending 1 g dry basis of compost in 50 mL 0.5 N HCl (with heat) and back-titrating to pH 7 with 0.25N NaOH (Kopčić et al., 2014).

Organic matter was estimated as volatile solid (VS) by measuring the fraction of sample (2.5 g) loss at 550 ºC for 4 h. Organic carbon was determined with a Carbon-Sulphur Analyzer (Leco Instruments, model SC-144 DR).

Kjeldhal nitrogen ($N_{kjeldhal}$) was quantified on 0.50 g of sample dried and ground, by mineralizing it with sulfuric acid 98%, followed by steam distillation and then titrimetric determination with HCl (McGinnis et al., 2011). $N\text{-}NH_4^+$ was extracted with KCl (2M) (1:10w/v) and nitric nitrogen ($N\text{-}NO_3^-$+ $N\text{-}NO_2^-$) with water (1:10 w/v) for 2 h (Tremier et al., 2005). Mineral nitrogen concentration was determined by colorimetry with a segmented continuous flow analyzer (Skalar SAN Plus) using a method based on the modified Berthelot reaction for $N\text{-}NH_4^+$ and promoting the reaction with sulfanilamide and N-1-naphthyl ethylenediamine dihydrochloride for nitric nitrogen (Benito et al., 2009).

The organic nitrogen content (N_{org}) of the samples was calculated by Eq (6.1):

$$N_{org} = N_{kjeldhal} - N_{NH_4^+{}_{\text{air-dried}}} \qquad (6.1)$$

where $N_{kjeldhal}$ is the sum of organic nitrogen compounds and ammonia of air dried sample and $N\text{-}NH_4^+{}_{\text{air-dried}}$ refers to ammonia content of air dried samples.

The capability of the compost to reheat was evaluated by using the Dewar self-heating test. Vessels of about 1.8 L were filled with compost samples moisture corrected to 50% of their water holding capacity, as described by Woods End Research Laboratory (2005). During the test, external temperature of the vessels was kept at 20ºC. Maximum temperature of the compost mass was daily recorded, until heat production had ceased. The degree of stability was attributed according to the maximum temperature achieved, as detailed in Table 6.2.

Table 6.2: Interpretation of Dewar self-heating test.

Maximum temperature in vessel (ΔTmax, ºC)	Class of stability	Description of maturity/stability
20-25	A	Mature, curing compost
25-45	B	Mesophilic, active
45-70	C	Thermophilic, very active

The phytotoxicity was assessed through germination tests using aqueous extracts from composts. About 5 mL of extract (1:10, dry weight: v; 1 hour of agitation) was placed in a petri dish containing 10 seeds of *Lepidium sativum.* Three replicates per sample were incubated at 25 ºC in dark conditions for 48 h. After this period, the number of germinated seeds and their root length were measured. A control test was prepared with deionized water (Komilis & Tziouvaras, 2009). Germination index (GI) was calculated by Eq.(6.2) as a percentage of a control experiment (with deionized water):

$$\mathrm{GI}\,(\%) = \frac{RSG \times RRG}{100} \tag{6.2}$$

where RSG is the relative seed germination (%) and RRG the relative root growth (%).

Fourier transform infrared spectroscopy (FTIR) analysis was performed using KBr pellets (2 mg of dried samples per 200 mg of KBr) in a 4200 Jasco model. FTIR spectra were acquired by averaging 32 scans in the range of 400-4000 cm^{-1}, resolution of 4 cm^{-1} and scan speed of 2 mm s^{-1}. The spectra absorption bands were correlated with the main infrared absorption bands of composts presented in Table S6.2 – supplementary data.

Cation exchange capacity in composting samples was accomplished according to Harada and Inoko (1980). About 200 mg of sample is washed with 0.05 N HCl to replace the exchangeable cations. The adsorbed H^+ was displaced by 1 N barium acetate and then titrated with 0.05 N sodium hydroxide (NaOH) to pH 8.9, in the presence of thymol blue indicator.

6.2.4. Statistical analysis of data

Statistical analysis, model fitting and numeric integrations were performed with software Matlab (version 7.8.0347, Mathworks, USA).

6.3. Results and discussion

6.3.1. Characteristics of the starting composting mixtures

Properties of the starting quaternary composting mixtures are shown in Table 6.3. Mixtures without eggshell (M_1+0%ES and M_2+0%ES) have similar moisture content (≈67%) but very distinct C:N (23.2 and 68.6, respectively), mainly due to the lack of GC in M2, but also to the higher C:N of PP used for this set of blends. Generally, the addition of ES to M_1 and M_2 increased the wet bulk density and reduced the water holding capacity of the mixture. Moisture content and C:N ratio were lowered by the incorporation of ES. However, initial moisture was always above 50% and pH near neutrality (between 6.5 and 7.3) which are considered adequate to support growth and activity of microbial populations (Liang et al., 2003; Bernal et al., 2009).

Table 6.3: Properties of the starting composting mixtures (mean±standard deviation, n=3).

Property		M_1+ 0%ES	M_1+ 10%ES	M_1+ 20%ES	M_1+ 30%ES	M_2+ 0%ES	M_2+ 10%ES	M_2+ 20%ES	M_2+ 30%ES
MC (%)		67.9±0.8	62.8±0.5	56.0±1.2	52.8±0.4	67.1±0.2	64.6±1.5	57.4±0.3	52.5±1.0
WHC (g_{water} 100 g_{dm}^{-1})		241	193	157	130	303	243	197	162
Available WHC (% of WHC)		12.2	12.5	18.9	13.9	32.7	24.9	31.6	31.8
BD (kg m^{-3})		245	265	290	323	256	283	310	342
pH		6.6	6.7	6.8	6.9	6.5	6.7	6.9	6.9
VS ($\%_{dry\ basis}$)		85.0±0.3	67.2±0.1	51.6±0.8	43.2±0.2	88.3±0.1	68.1±0.4	55.2±0.9	42.9±0.5
VS_{source} (% total VS content)	PP	27.1	26.6	26.0	25.3	32.2	31.6	30.8	30.0
	GC	25.1	24.6	24.1	23.4	0.0	0.0	0.0	0.0
	RH	47.8	47.0	46.0	44.7	67.8	66.4	64.9	62.9
	ES	0.0	1.8	3.9	6.6	0.0	2.0	4.3	7.1
C_{org} ($\%_{dry\ basis}$)		40.3±0.	32.6±0.1	25.1±0.2	21.8±0.2	41.7±0.1	33.0±0.2	29.0±0.2	21.6±0.4
N_{org} (g N kg_{dm}^{-1})		15.3±0.4	13.5±0.1	11.6±0.3	11.2±0.2	6.7±0.2	8.6±0.1	8.4±0.1	8.1±0.2
N-NH_4^+(g N kg_{dm}^{-1})		2.1±0.2	1.9±0.1	0.9±0.03	0.9±0.2	0.04±0.02	0.03±0.01	0.12±0.05	0.03±0.02
N-NO_3^-(mg N kg_{dm}^{-1})		<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
N-NO_2^-(mg N kg_{dm}^{-1})		<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
C:N		23	21	20	18	62	38	34	27

Abbreviations: BD: bulk density; C_{org}: organic carbon; dm: dry matter LOD: limit of detection (2.4 mg N kg_{dm}^{-1} for N-NO_3^- and N-NO_2^-); MC: moisture content; N_{org}: organic nitrogen; VS: volatile solids; VS_{source}: contribution of each material to VS in the mixture; WHC: water holding capacity.

6.3.2. Composting trials

6.3.2.1. Temperature profiles

Thermal inactivation is considered the main mechanism for pathogens suppression throughout composting, important to obtain a hygienic and safe end-product (Farrell and Jones, 2009). Thermal exposure of the mixtures tested is illustrated in Figure 6.1 a) and b). Table 6.4 summarises the influence of GC incorporation in some parameters of the thermal pattern found for each mixture. Throughout 25 days of composting in self-heating reactors, oxygen concentrations (%v/v) depicted in Figure S6.1 (supplementary data) were comprised between 7 and 20 % (v/v) assuring aeration rate levels were adequate to maintain aerobic conditions (Puyuelo et al., 2010).

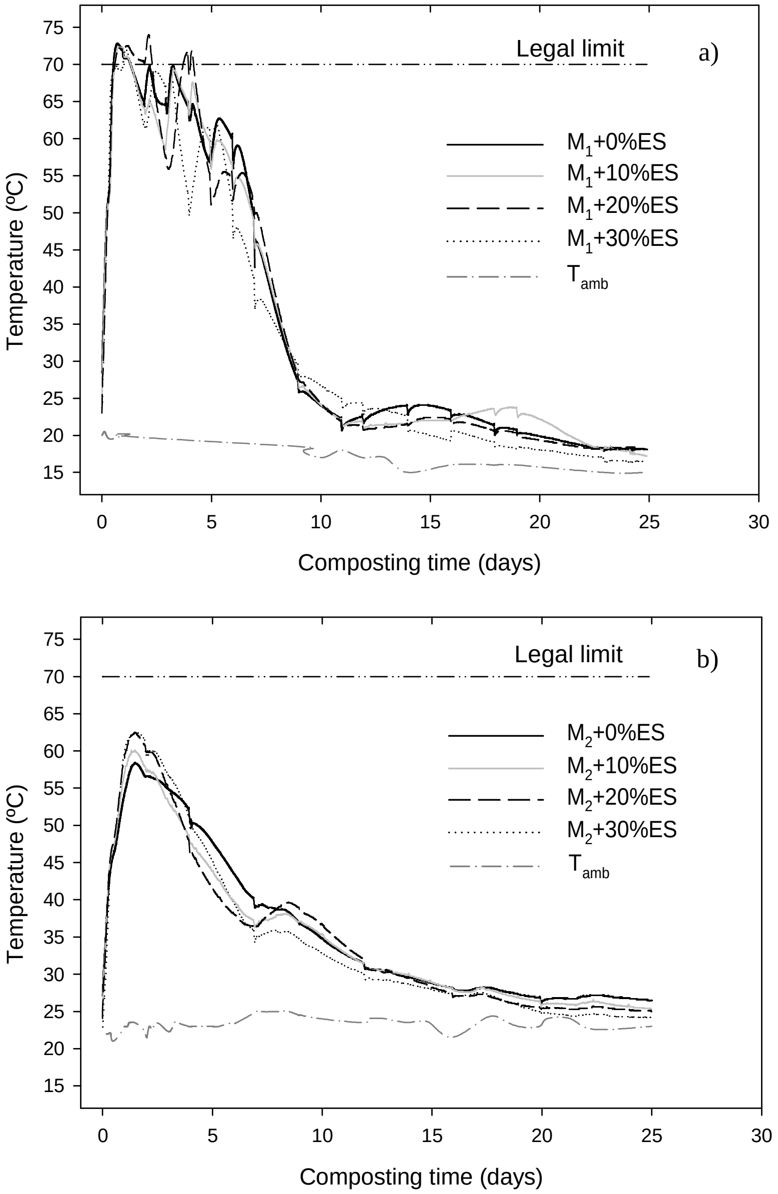

Figure 6.1: Evolution of core centre and ambient temperatures of mixtures a) M_1 and b) M_2, during first 25 days of composting.

Table 6.4: Influence of GC in thermal parameters of composting trials.

Mixture	T_{max} (ºC)	t_{max} (days)	Consecutive period with T>70ºC (h)	Consecutive period with T>55ºC (d)	Thermophilic period with T>40ºC (days)
M_1+0%ES	72.8	0.7	18	6.0	7.7
M_1+10%ES	72.3	0.7	16	5.4	7.7
M_1+20%ES	72.3	0.8	18	5.2	7.8
M_1+30%ES	72.0	1.1	9	4.8	6.9
M_2+0%ES	58.4	1.5	0	1.8	6.8
M_2+10%ES	60.1	1.5	0	1.8	5.9
M_2+20%ES	62.5	1.5	0	2.2	5.4
M_2+30%ES	62.4	1.5	0	2.5	5.9

Independently of the mixture composition, the typical temperature-dependent phases were observed (Figure 6.1 a) and b)). Initially, a short mesophilic phase (less than 7 h in all cases). Then a themophilic phase (T>40ºC) was developed, and finally another mesophilic phase arose again indicating that thermotolerant organisms were inhibited due to the depletion of readily available organic substances.

For all M_1 mixtures, temperature reached 70ºC and remained above for 9 to 18 consecutive hours (Table 6.4, Figure 6.1 a)) thus fulfilling the minimum sanitizing requirement of 70ºC for 1 h proposed by the Commission Regulation (EU) No. 142/ 2011 applicable to ES waste. Maximum temperature (1st peak) ranged from 72 to 72.8ºC. In specific for M_1+30%ES, exposure to more than 70ºC only for 9 h (50% less than the other M_1 mixtures), was probably due to the higher airflow rate and thus the heat removal by convection was enhanced.

The lack of nitrogen amendment (GC) in M_2 hindered their ability to release heat and reach pathogen-killing temperatures. As depicted in Figure 6.1 b), M_2+0%ES was able to achieve thermophilic temperatures, but the heat produced during breakdown of organic matter was short to accomplish the temperature target (>70ºC). This might be a consequence of the high contribution (68% - Table 6.3) of rice husks to the overall content of VS, by adding recalcitrant organic matter. In addition, the lack of nitrogen source in M_2 to fit C:N ratio of M_2+0%ES within 25-30 may have impaired the development of microbial populations. As a result, maximum temperature (T_{max}) attained was 58,4º C but only for 1.8 days above 55ºC (Table 6.4). Nevertheless, increasing ES in M_2 mixtures promoted an upsurge in the T_{max} and extended the period with T>55ºC (Table 6.4).

6.3.2.2. Oxygen uptake and organic matter conversion

Figures 6.2 a) and b) show the biological activity measured as OUR of the mixtures during composting process.

The OUR profiles found for M_1 and M_2 are in accordance with the temperature behaviour (Figure 6.1 a) and b)). In fact, temperature and instantaneous OUR revealed a very high positive correlation with $0.894<r<0.942$, $P<0.0001$ and $0.815<r<0.838$, $P<0.0001$ for M_1 and M_2, respectively. According to the criteria proposed by Barrena et al. (2011) to categorize the biological activity of organic mixtures, the OUR profiles for M_1 mixtures remained near to the high biodegradable level during the first 3 to 4 days of composting (Figure 6.2 a)). This means that the ability to generate heat due to microbial breakdown of the organic materials was significant and pathogen-killing temperatures were able to be reached effortlessly, as long as convective removal of heat was kept lower than reaction heat.

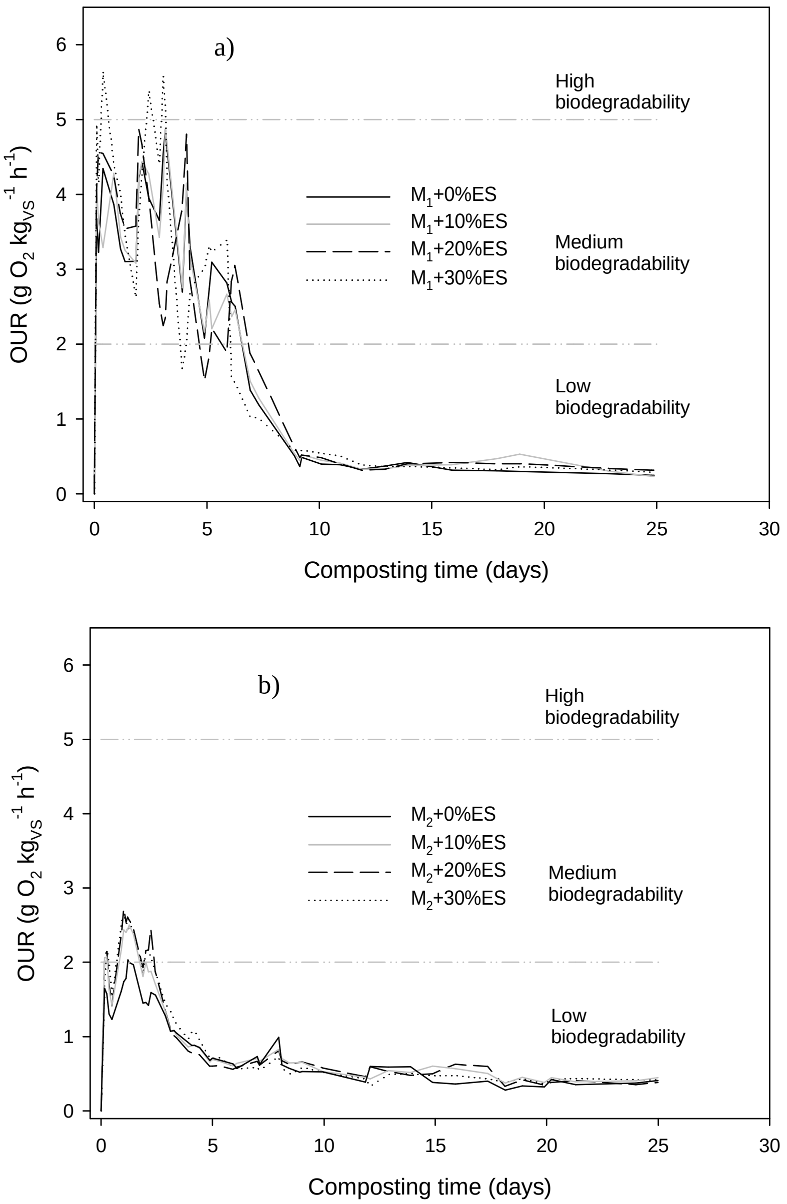

Figure 6.2: OUR evolution during composting in self-heating reactors for a) M_1 mixtures and b) M_2 mixtures.

When GC is absent (M_2), biological activity is significantly impaired, reaching values on the transition between “low” to “moderate” biodegradability and then sanitizing conditions for ES waste were compromised. Nevertheless, after 25 days of composting all mixtures were able to attain an OUR lower than 0.5 g O_2 kg^{-1} $_{VS}$ h^{-1}, which is the maximum threshold for stable composts (Adani et al., 2004; Gea et al., 2004; Scaglia et al., 2000).

Moreover, the small influence of ES on the thermal behaviour and biodegradability of M_1 and M_2 is somewhat unforeseen. In fact, since ES contains low VS its incorporation would reduce the organic matter available for degradation, thus affecting the self-heating. However, ES is high density material (≈760 kg m^{-3}) which increases wet bulk density of the mixtures. As a result, the amount of organic matter in the working volume of the reactors (105 L) was practically identical (≈7 kg VS). In addition, organic matter from ES was low (1.8 to 7.1% of the total VS – Table 6.3) and PP and GC were the main sources of biodegradable organic matter. Although it is claimed that organic matter of ES is readily available for microbial breakdown (Girovich, 1996), it was not incorporated in high quantity to overcome the intrinsic biodegradability of the organic matter provided by GC and PP. Thus, no relevant effect in the overall biodegradability of the starting mixtures was found due to ES, and the biological activity/ self-heating ability was dictated by the other components of the mixture.

Organic matter mineralisation was reasonably described by a first order kinetic model, independently of GC amount used. During the first 25 days of composting, VS conversion nearly attained a plateau (Figure S6.2 to S6.3-supplementary data). Total oxygen consumed within that period is proportional to the cumulative VS degraded, as expected (Figure S6.4 to S6.5 and Table S6.3 – supplementary data). Cumulative oxygen consumption pattern highlighted the lack of a lag phase in the process, even when a rich N-source was absent (Figure S6.2 to S6.3-supplementary data). In general, Figure 6.3 shows that incorporation of GC triggered an increase of 45% in X_{vs}, which is reflected by an average upsurge of the same level (43%) in the total oxygen consumed by unit of VS degraded. This behaviour has not been reported for other composting mixtures. For example, GC incorporation (15% w/w) in a blend of sewage sludge with wood chips and rape straw can impair VS conversion (lessening from 0.51 to 0.33) with consequent reduction of temperatures (Kulikowska and Klimiuk, 2011).

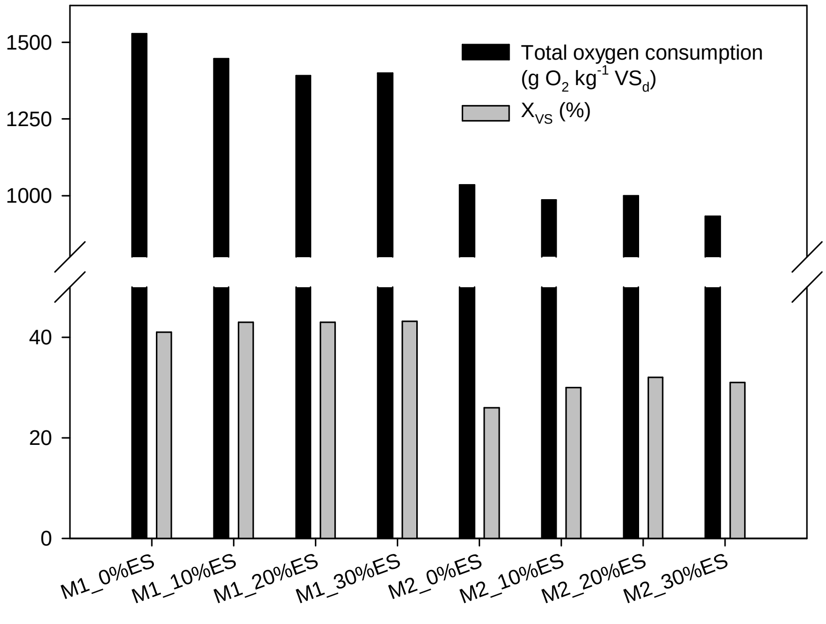

Figure 6.3: Influence of GC in organic matter conversion and total oxygen consumption by unit of organic matter degraded.

For M_1, total oxygen consumption (g O_2 kg^{-1} VS_d) is comparable to the aerobic degradation of separated pig solids with wood chips (1432 g O_2 kg^{-1} VS_d), while M_2 was similar to household waste (1050 g O_2 kg^{-1} VS_d) (de Guardia et al., 2010a). However, in the latter case, VS conversion was much more expressive (0.95) than observed in M_2 (≈0.30), which highlights the importance of waste nature on the biodegradation potential (de Guardia et al., 2010a).

6.3.2.3. Moisture and physical changes

Moisture content is considered a key factor for efficient composting. Some studies reported a wide range from 25% to 50% on a wet basis (Ahn et al., 2008). In general, composting proceeds better at moisture content of 50-70% (Liang et al., 2003). However, each mixture may have singular properties that can affect the optimum relationship between moisture content, water availability and porosity (Ahn et al., 2008). Figure 6.4 indicates moisture content, wet bulk density and FAS over 25 days.

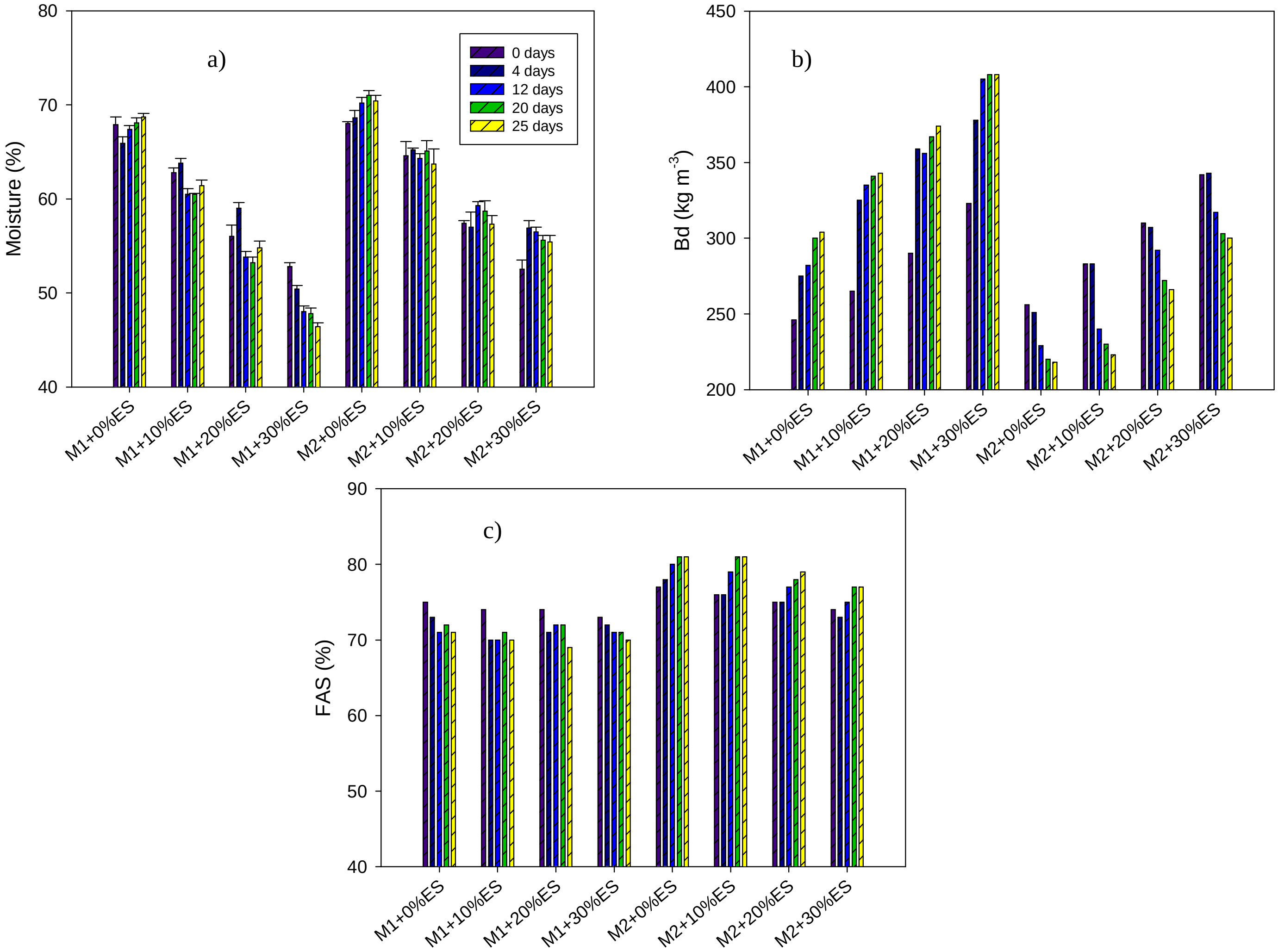

Figure 6.4: Influence of GC on a) moisture, b) wet bulk density and c) FAS evolution during composting (mean±standard deviation, n=3).

In M_1 mixtures, moisture fluctuations over 25 days of composting were not very expressive. Normally high thermophilic temperatures favor water evaporation and require external water correction (Brito et al., 2012). But in the present study no correction was necessary because part of the vapor was allowed to condensate and drop back to solid matrix. Frequent mixing of the reactors redistributed condensed water in the composting matrix. Though the initial available water capacity of M_1 was between 12 and 19% of the maximum WHC (Table 3) no leachates were formed, and composting evolved as expected. For M_2 there is a slight tendency to increase moisture, but it was probably damped due to the higher available water capacity of the initial mixtures (25 to 32.2 % of maximum WHC) (Table 6.3).

As previously mentioned, ES incorporation affected the wet bulk density of the starting mixtures, but during composting different trends for BD were observed, depending on the proportion of GC used. While for M_1 (with GC) BD increases as composting evolves, for M_2 (without GC) the opposite was observed (Figure 6.4 b)). In the case of M_1, a significant increase was noticed during the first four days, which corresponds to the period of higher OUR (Figure 6.2 a)). This fast breakdown of organic matter provoked a sudden reduction of pores volume. In fact, for ternary mixtures of PP:GC:RH, GC contributes to the increase of pore volume of a blend (Soares et al., 2013b), but as it decomposes interparticle pore volume is reduced. As a result, M_1 suffered a small FAS reduction (Figure 6.4 c)). However, composting was not hindered, since FAS was kept well above 30%, which is commonly considered the limiting value (Huet et al., 2012). The reduction of BD for M_2 as biodegradation progresses, highlights that RH provided enough structure to support the solid matrix, as organic substrates were transformed in a low to moderate rate, Figure 6.2 b). Consequently, FAS suffered a slight increase (Figure 6.4 c)).

6.3.2.4. Nitrogen transformation and estimation of N losses

Mechanism of nitrogen transformation and transfer during composting are complex and may lead to significant N losses, thus affecting the agronomic value of the composting end-product with a negative impact for the environment (Hubbe et al., 2010). During aerobic degradation, ammonification corresponds to the transformation of organic nitrogen into ammoniacal nitrogen. This fraction is added to the initial content, thus constituting a pool (N pool) that can be either lost through leaching or released as ammonia by stripping. Recent studies have shown that N pool is positively correlated to overall N losses during composting (Zeng et al., 2012a) and ammonification is the most contributing process (de Guardia et al., 2010b; Zeng et al., 2012a). Figure 6.5 presents the ammonification rate and N pool associated to N transformation during composting of mixtures M_1 and M_2 for 25 days. Ammonification rate (AR) was calculated as:

$$AR\ (\%) = \frac{\Delta N_{org}}{N_{org,0}} \times 100\% \qquad (6.3)$$

where ΔN_{org} corresponds to the mass of organic N removed during the first 25 days of composting (g N) and $N_{org,0}$ is the mass of organic N in the starting mixture (g N).

The N pool was quantified as (de Guardia et al., 2010b):

$$N\ pool = N\text{-}NH_{4,0}^{+} + \frac{\Delta N_{org}}{VS_0} \qquad (6.4)$$

where $N\text{-}NH^{+}_{4,0}$ is the total ammoniacal nitrogen present in the starting composting mixture (gN kg^{-1} VS_0) and VS_0 is the mass of volatile solids in the starting mixture. The parameters ΔN_{org}, $N_{org,0}$ and VS_0 were corrected with sample withdraws during process monitoring. Detailed calculations are indicated in Table S6.4 – supplementary data. Figure 6.5 clearly illustrates that the ammonification rate significantly differs between M_1 and M_2.

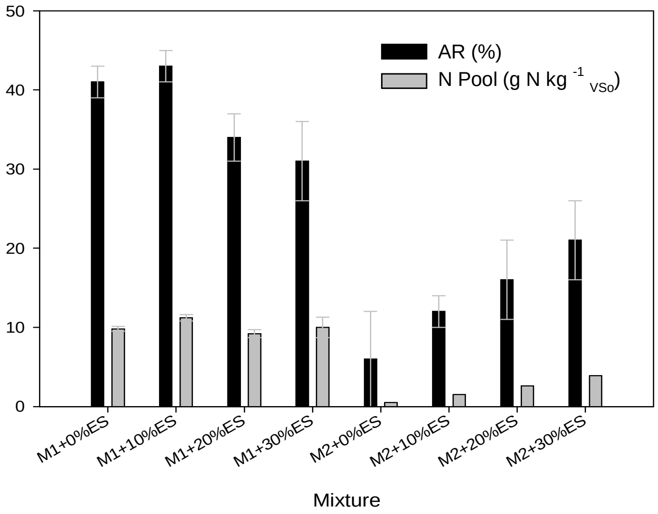

Figure 6.5: Ammonification and N pool for M_1 and M_2 mixtures during 25 days of composting (error bars standard deviation)

Thus, GC is the component whose biodegradation was responsible for the general upsurge of the ammonification rate in M_1. As a result, N pool is significantly higher and it can be hypothesized that N losses by stripping (since no leachates were produced) were stimulated by GC. In fact, M_1 involved higher temperatures (Figure 6.1) and biodegradability (Figure 6.2) thus creating operational conditions that favour ammonia removal through that route. The levels of ammonification recorded for M_1 are comparable to the ones obtained by de Guardia et al. (2010b) for composting household waste (36-47%).

In addition, ES incorporation seems to affect in some extent the ammonification rate of M_2, thus leading to an increase of the N pool with the consequent slightly higher potential for N losses, though this potential is always smaller than in the case of M_1 (Figure 6.5). Supposing that ammonification is associated to biodegradation (de Guardia et al., 2008) no alterations would be recorded for M_2, since biodegradation attained for that mixtures is quite similar, independently of ES incorporation (Figure 6.3). On the other hand, Guardia et al. (2010) also showed that ammonification ability differs significantly from one waste to another. Thus in this study, ES is presented as an ingredient that alters the ammonification ability of M_2, by contributing to an increase of the amount of N ammonified per unit of organic carbon degraded. Nevertheless, the understanding of the mechanisms behind this behaviour should be adequately evaluated in further studies.

Figure 6.6 shows the evolution of mineral N during 90 days of composting. In general terms, differences amongst mixtures are mainly related to the concentration of N species which are higher when GC is present.

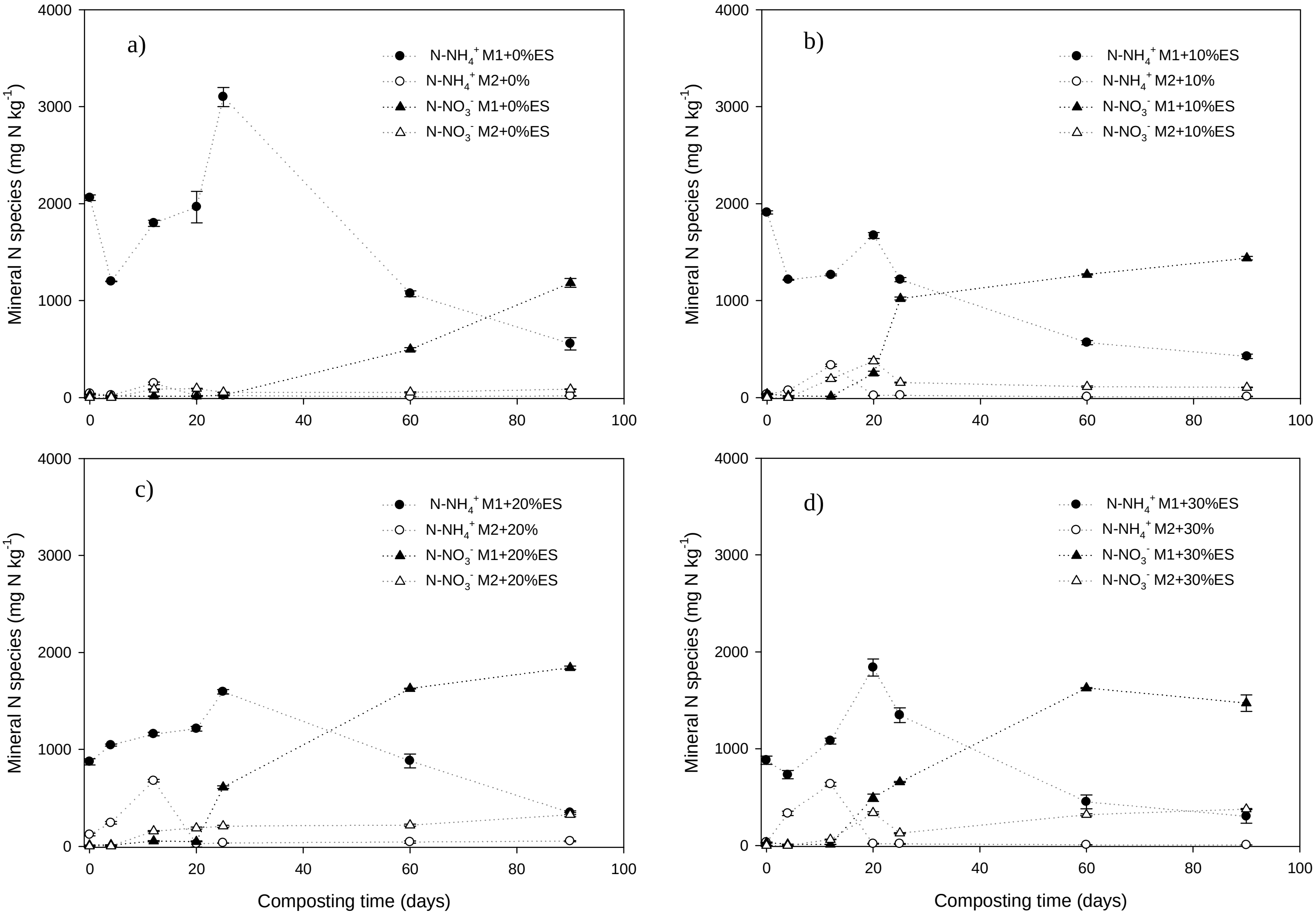

Figure 6.6: Evolution of mineral N during M_1 and M_2 for 90 days of composting (error bars indicate standard deviation, n=3): a) 0% ES, b) 10% ES, c) 20% ES, d) 30% ES.

In particular for M_1, N-NH_4^+ values are significant, but as maturation evolves N-NH_4^+ tend to 400 mg kg^{-1} which is the maximum recommended value for mature composts (Zuconni and de Bertoldi, 1987). Regardless the mixture composition, nitrification seems to be inhibited during the thermophilic phase, since temperatures higher than 40ºC impair the ammonia-oxidizing bacteria responsible for nitrification (Sanchez-Monedero et al., 2001). Additionally, those bacteria grow slowly and can be inhibited by the competitive activities of heterotrophic microorganisms (Zeng et al., 2012b). Thus, little accumulation of N-NO_3^- was observed in the early stages of M_1 and M_2 (Figure 6.6). However, as the process evolves to maturation phase nitrification became significant with consequent build-up of NO_3^- in the compost.

6.3.3. Compost quality

The feasibility of composting as a waste management option for ES is also deeply associated to the value of the final compost. The self-heating potential of the mixtures is of special concern to meet European regulation, but the ability to obtain final compost with quality level is also relevant. The main properties of the final composts obtained in this study are indicated in Table 6.5. For mixtures with GC, a longer period of time of maturation was required (144 days) since after 90 days composts were still phytotoxic (data not shown).

All composts revealed to be alkaline with a low electrical conductivity, despite composts obtained from M_1 presented a higher salinity. The neutralizing capacity (evaluated as equivalent $CaCO_3$) was not affected by the presence of GC, but this property was dose-responsive to ES in the starting mixtures.

The resistance of composts against further microbial decomposition was high, as the stability class of the obtained for end-products was A indicating cured composts (Tables 6.2 and 6.5). In addition, all end-products were phytotoxin-free and thus safe for soil application, since germination indices were higher than 50% (Zuconni and de Bertoldi, 1987). Moreover, the use of final composts as soil improvers was not conditioned by the presence of heavy metals, since concentrations were small and beneath the limits imposed by the European proposed quality criteria for composting products (Table 6.5).

Nitrification ratio (NH_4^+/ NO_3^-) was less than 0.5 as expected from mature composts (Bernal et al., 2009). Humification was indirectly assessed by CEC, by considering that humus build-up increases the functional groups. However, that quantification was not possible for composts with ES, since the carbonate present therein may undervalue that property. Both M_1+0%ES and M_2+0%ES have a CEC higher than 60 cmol kg^{-1}_{dm} which is indicative of maturity (Harada and Inoko, 1980), but this limit was already reached by the starting mixtures before composting (95 and 54 cmol $kg^{-1}{}_{dm}$ for M_1+0%ES and M_2+0%ES, respectively). Thus, in this case, CEC is not a good indicator for maturity concerning humification (Bernal et al., 2009).

Table 6.5: Main properties of the final composts, after 144 days of composting (mean±standard deviation, n=3)

Property		M1+ 0% ES	M1+ 10% ES	M1+ 20% ES	M1+ 30%ES	M2+ 0% ES	M2+ 10% ES	M2+ 20% ES	M2+ 30%ES
pH		9.3±0.1	9.2±0.0	9.1±0.0	8.9±0.1	9.3±0.1	9.4±0.0	9.4±0.0	9.5±0.1
EC (µS cm^{-1})		846			1028	503	540	508	555
Equivalent $CaCO_3$ (g $CaCO_3$ 100$g^{-1}{}_{dm}$)		5.0±0.1	30.5±1.0	50.2±2.6	61.4±1.7	8.6±1.1	34.7±0.3	47.8±1.4	60.0±1.9
OM (g VS 100 $g^{-1}{}_{dm}$)		79.4±0.5	55.0±1.6	40.5±0.8	28.5±0.3	82.8±0.7	58.7±1.5	45.9±0.4	38.6±1.1
TOC/N		21.0	18.4	15.3	11.9	46.7	31.6	26.8	21.1
NH_4^+/ NO_3^-		0.10	0.15	0.12	0.23	0.19	0.09	0.02	0.06
GI (%)		75±9	83±7	78±10	76±11	96±4	104±4	96±7	104±9
CEC (cmol $kg^{-1}{}_{dm}$)		85	nd	Nd	nd	66	nd	nd	nd
Stability	ΔT_{max} (ºC)	2.5	0.3	0.5	3.6	2.1	2.3	2.6	1.2
	T_{max} (ºC)	22.5	20.3	20.5	23.6	22.1	22.3	22.6	21.2
	Class	A	A	A	A	A	A	A	A
Ca (g $kg^{-1}{}_{dm}$)		4.0±0.0	69.2±2.1	158±8	223±3	1.4±0.1	177±1.4	281±11	319±26
Cd (mg $kg^{-1}{}_{dm}$) (limit:1.5)*		0.70±0.01	0.80±0.01	0.50±0.01	0.40±0.01	0.5±0.0	0.5±0.0	0.5±0.0	0.5±0.1
Cr (mg $kg^{-1}{}_{dm}$) (limit:100)*		12.4±1.0	11.3±0.7	7.6±0.3	4.2±0.0	2.1±0.4	1.5±1.0	1.7±0.1	3.2±0.1
Pb (mg $kg^{-1}{}_{dm}$) (limit:120)*		12.7±0.0	13.5±0.0	8.7±0.0	7.3±0.0	9.3±0.0	9.1±0.6	9.0±0.0	7.9±0.9
Cu (mg $kg^{-1}{}_{dm}$) (limit:200)*		8.2±0.2	9.0±0.0	8.5±1.3	5.2±0.2	4.2±0.5	3.4±0.2	1.1±1.4	2.0±0.3
Zn (mg $kg^{-1}{}_{dm}$) (limit:600)*		47.4±0.3	27.5±0.3	17.8±0.2	11.9±0.1	16.6±1.8	14.2±1.6	10.5±0.2	7.5±0.6
Ni (mg $kg^{-1}{}_{dm}$) (limit:50)*		0.60±0.05	7.3±1.4	3.8±0.1	5.4±0.0	6.9±0.0	6.8±0.4	6.7±0.0	5.9±0.7

EC: electrical conductivity; OM: organic matter; VS: volatile solids; TOC: total organic carbon; TN: total nitrogen; GI: germination index; CEC: Cation exchange capacity; dm: dry matter; nd: not determined; T_{max}: maximum temperature * values in brackets represent the limit concentration according to end-of-waste criteria for biodegradable waste subject to biological treatment (draft final report).

FTIR spectra for all composts are depicted in Figure S6.6 to S6.8 – supplementary data, where spectra for the starting mixtures are also shown for comparison. The infrared absorbance regions of composts and starting mixtures are similar whether or not GC is present, though bands intensity can be different. The main infrared peaks for composts without ES included O-H and N-H bands in 3400 cm^{-1}, C-H stretch of aliphatic compounds in 2920-2850 cm^{-1}, C=O bands in 1740 cm^{-1} due to aldehydes, ketone, carboxylic acids and esters, C=C of aromatic skeleton in 1595 cm^{-1}, lignin in 1510 cm^{-1}, aliphatic C-H in 1460 cm^{-1}, C-N stretch of amines in 1320 cm^{-1}, C-O and C-N vibration of carboxylic acids and amides in 1240 cm^{-1} and C-O stretching of polysaccharides, polysaccharides-like substances and Si-O silicate impurities in 1160-1060 cm^{-1}. When ES is in mixtures, peaks at 2515, 1800, 1420 and 875 cm^{-1} are also evident, which are ascribed to carbonates (Smidt and Meissl, 2007). Changes in the spectra during composting indicate that easily degradable organic constituents such as short aliphatic chains (bands at 2920-2850 cm^{-1}) and polysaccharides (bands at 1160-1060 cm^{-1}) were biooxidised. In addition, the peaks reduction at 1740 cm^{-1} and 1240 cm^{-1} for M_1+0%ES and M_2+0%ES are indicative that early metabolic products like aldehydes, ketones and esters are also further biodegraded as well as amides.

In particular for M_1, organic constituents show a more intensive biodegradation of aliphatic structures than M_2 (higher reduction of peaks at 2920-2850 cm^{-1}) which is consistent with higher reaction temperatures and oxygen consumptions previously found for M_1 (Figure 6.1 a) and 6.2 a)). The increase of aromatic C=C when compared to aliphatic carbon (illustrated by ratio of peaks 1650/2920 and 1650/2850 (Ouatmane et al., 2000) in Table S6.5 – supplementary data) reflects that final composts contained more aromatic structures of higher stability and humification has occurred at some extent for all mixtures.

6.5. Conclusions

The influence of N- rich material in the physical and chemical changes taking place during co-composting of eggshell waste (ES) with potato peel (PP) was considered in this study. Grass clippings (GC) were selected as N-rich source and rice husks as bulking agent. Experimental tests in self-heating reactors showed that GC were determinant to attain the sanitizing conditions imposed by animal by-product regulation (T>70ºC for 1 h). Temperature and instantaneous OUR revealed a positive correlation. M_1 mixtures (with GC) remained near to the high biodegradable level during the first 3 to 4 days of composting, while for M_2 (without GC) aerobic degradation was significantly impaired and OUR became “low” to “moderate” biodegradability.

Physical changes during composting did not lessened the process evolution, though GC may favour the reduction of the interparticle pore volume, as it decomposes.

For M1, organic matter biodegradation was enhanced and ammonification occurred in higher extent, thus leading to a higher potential for N-losses. Mineral nitrogen transformations were distinct in terms of concentration levels, but followed identical transformation routes.

End-product quality assessment evidenced that all composts obtained were stable and mature, but pathogen inactivation was not assured when GC was absent. Nevertheless, maturity achievement took longer for end-products with GC.

In summary, ES recycling through composting process is adequate for obtaining neutralizing Ca-rich soil improver, but the presence of N-rich material may be a determinant factor to reach pathogen-killing temperatures as legally required. Strategies to overcome the potential of N-losses associated to M_1 mixtures should be further investigated for optimising N conservation.

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6.7. Supplementary material

Table S6.1: General properties of the composting ingredients used for mixtures set M_1 and M_2 (mean±standard deviation for n=3).

Composting ingredient	Mixture	pH	MC (%)	VS (% dry basis)	C_{org} (% dry basis)	N (% dry basis)	C:N ratio
PP	M_1	5.1±0.2	85.3±0.8	97.3±1.0	48.4±0.3	1.90±0.2	25.5
	M_2	5.7±0.1	87.0±0.6	94.2±0.5	47.1±0.4	1.00±0.3	47.1
GC	M_1	6.9±0.2	72.0±1.0	84.6±0.3	42.1±0.4	3.5±0.3	12.0
	M_2	-	-	-	-	-	-
RH	M_1	6.8±0.1	10.9±0.1	84.4±0.2	42.0±0.3	0.64±0.4	65.6
	M_2	6.7±0.1	10.7±0.1	84.0±0.1	42.0±0.2	0.60±0.2	70.0
ES	M_1	8.3±0.1	15.8±0.2	5.2±0.8	2.6±0.6	0.9±0.3	2.9
	M_2	8.7±0.1	15.7±0.3	6.0±0.4	3.0±0.9	1.1±0.4	2.7

Abbreviations: PP – potato peel; GC – grass clippings; RH – rice husks; ES – eggshell; MC – moisture content; VS-volatile solids; C_{org}-organic carbon; N-total nitrogen.

Table S6.2: Main infrared absorption bands of waste materials and composting end-products. Adapted from Smidt & Meissl (2007).

Wave number (cm^{-1})	**Assignment**
3400	O-H stretching, N-H stretching (trace)
2920	C-H stretching of aliphatic methylene
2850	C-H stretching of aliphatic methylene
2515	Carbonate
1800	Carbonate
1740	C=O stretching of aldehyde, ketone, carboxylic acids and esters
1650	C=O stretching of amide groups (amine I band), quinone C=O and/or C=O of H-bonded conjungated ketones; C=C associated to aromatic ring modes, alkenes
1595	Aromatic C=C
1510	Aromatic skeletal- lignin
1460	Alkyl bending
1420	Carbonate
1320	C-N stretch of aromatic primary and secondary amines
1240	C-O and C-N vibration of carboxylic acids and amide III
1160-950	C-O; stretching of polysaccharide or polysaccharide-like substances, Si-O of silicate impurities
875	Carbonate

Table S6.3: Linear relation between cumulative oxygen consumed (y, in g) and cumulative VS degraded (x, in kg) for the first 25 days of composting.

Composting mixture	Fitting equation	R^2
M_1+0% ES	$y = 1528.4\ x$	0.997
M_1+10% ES	$y = 1446.1\ x$	0.999
M_1+20% ES	$y = 1391.4\ x$	0.997
M_1+30% ES	$y = 1399.7\ x$	0.969
M_2+0% ES	$y = 1035.1\ x$	0.990
M_2+10% ES	$y = 985.9\ x$	0.993
M_2+20% ES	$y = 1000.3\ x$	0.989
M_2+30% ES	$y = 933.2\ x$	0.994

Table S6.4: Intermediate calculations for quantification of ammonification rate and N pool (mean±standard deviation for n=3).

	Composting time =0 days					Composting time =25 days						
Mixture	Norg (gN/kg)	N-NH$_4^+$ (gN/kgM$_0$)	VS$_0$ (kg)	ms (kg)	m Norg (g)	Norg (gN/kg)	ms (kg)	m Norg (gN)	m Norg total withdrawn (gN)	Δ Norg (gN)	Ammonification (%)	N pool (gN/kgVS$_i$)
M_1+0% ES	15.3±0.2	2.4±0.0	6.5	8.3	127±1	14.4±0.2	4.9	70±1	9±0	48±2	38±2	9.8±0.3
M_1+10% ES	13.5±0.2	2.8±0.0	6.5	10.3	139±2	11.0±0.2	6.6	73±10	12±0	54±3	39±2	11.2±0.4
M_1+20% ES	11.6±0.2	1.7±0.2	6.4	13.4	156±2	10.3±0.2	9.2	95±2	13±0	48±3	31±2	9.2±0.5
M_1+30% ES	11.2±0.4	2.0±0.4	6.4	16.0	179±7	9.9±0.3	11.5	114±4	14±0	51±9	29±5	10.0±1.3
M_2+0% ES	6.7±0.4	0.1±0.0	6.9	8.6	57±3	8.2±0.2	5.9	48±1	6±0	3±3	5±6	0.5±0.0
M_2+10% ES	8.0±0.5	0.1±0.0	6.4	10.5	84±1	9.1±0.1	7.3	66±1	12±0	9±1	11±2	1.5±0.0
M_2+20% ES	8.4±0.2	0.2±0.0	6.7	13.9	118±3	8.5±0.5	10.5	89±6	12±0	17±7	14±5	2.6±0.0
M_2+30% ES	8.1±0.3	0.1±0.0	7.0	17.1	138±5	8.1±0.4	12.3	100±5	12±0	26±7	19±5	3.9±0.0

Table S6.5: Ratio of peaks 1650/2920 and 1650/2850 for FTIR spectra from M_1 and M_2 mixtures.

	Ratio $\frac{1650}{2920}$		Ratio $\frac{1650}{2850}$	
Composting mixture	Starting mixture	Mature compost	Starting mixture	Mature compost
M_1+0% ES	1.20	1.39	1.00	1.60
M_1+10% ES	1.10	1.47	1.46	1.78
M_1+20% ES	1.40	1.97	1.74	2.15
M_1+30% ES	1.60	2.19	1.99	2.46
M_2+0% ES	0.98	1.03	1.28	1.42
M_2+10% ES	1.28	1.88	1.37	1.90
M_2+20% ES	1.33	1.42	1.90	1.89
M_2+30% ES	1.88	2.04	2.13	2.35

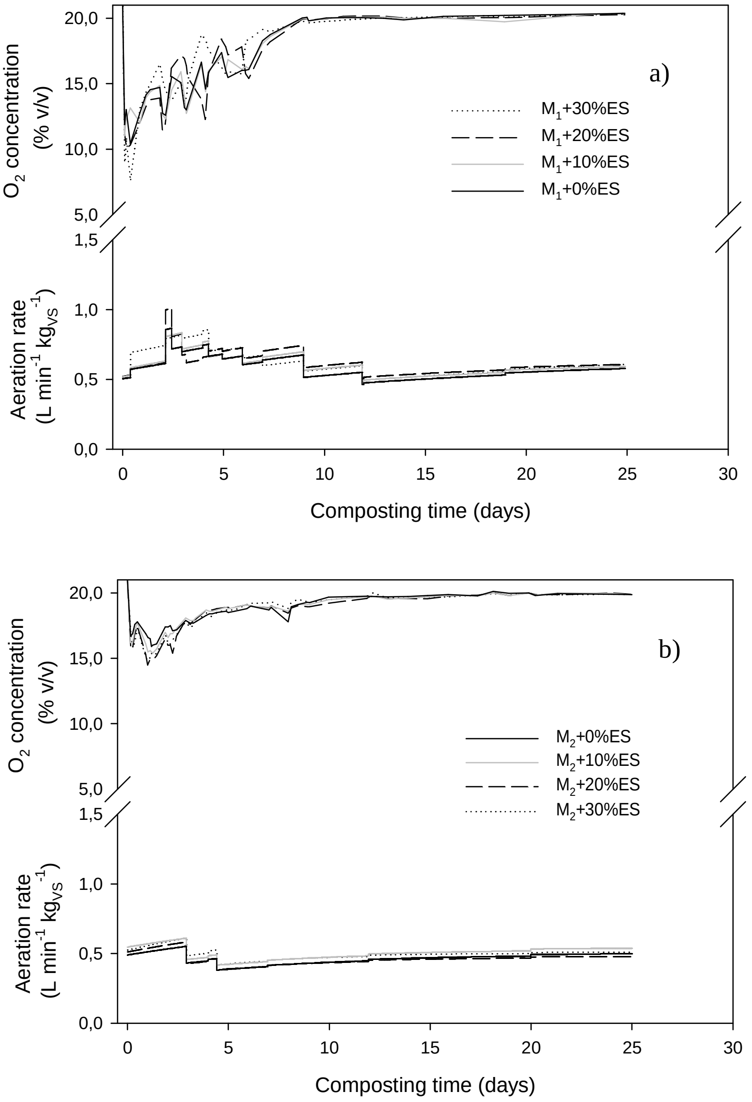


Figure S6.1: Aeration rate and oxygen levels during composting in self-heating reactors: a) M_1 mixtures; b) M_2 mixtures.

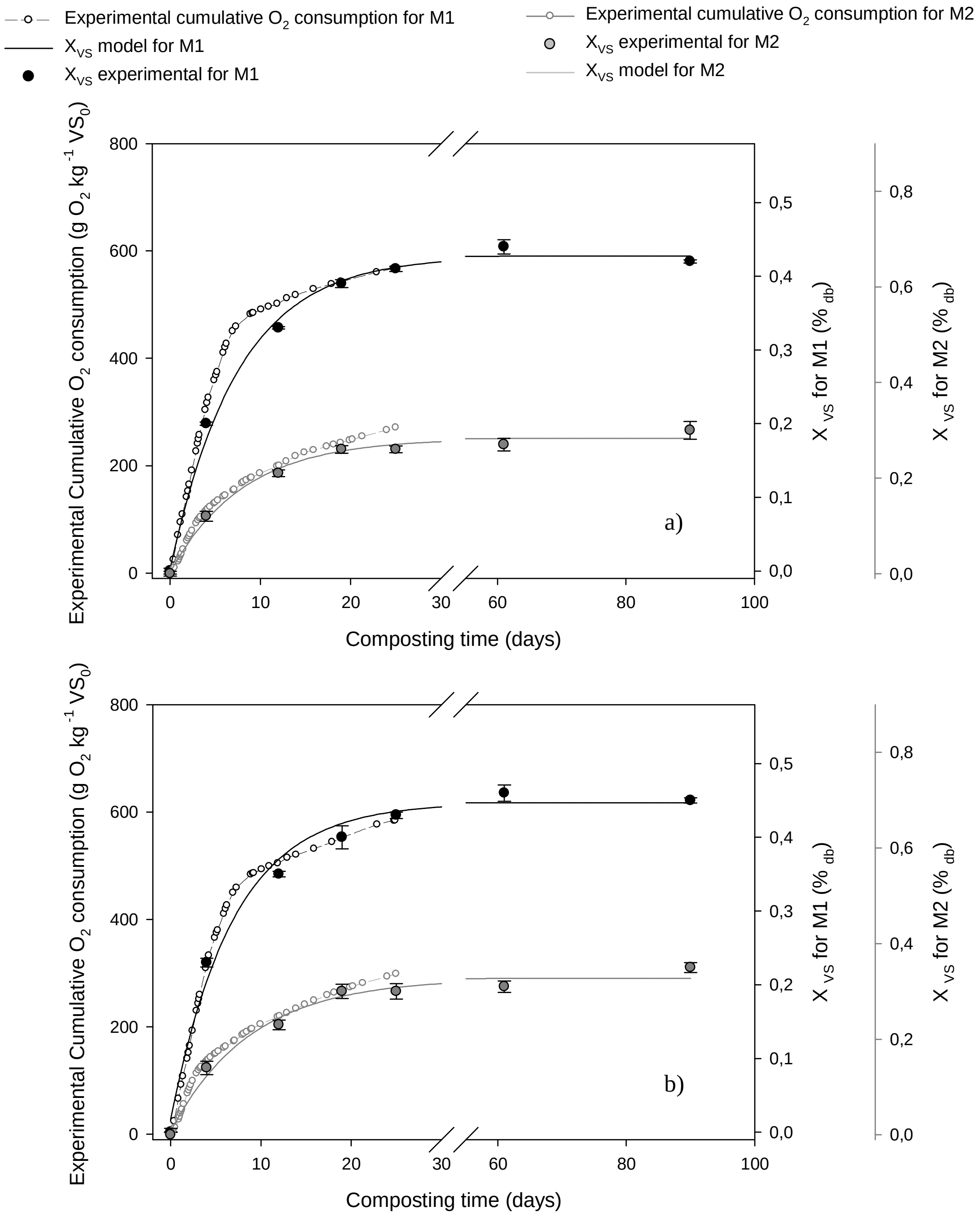


Figure S6.2: Profile of organic matter mineralisation and cumulative O_2 consumption for M_1 and M_2 mixtures with a) 0%ES, b) 10%ES.

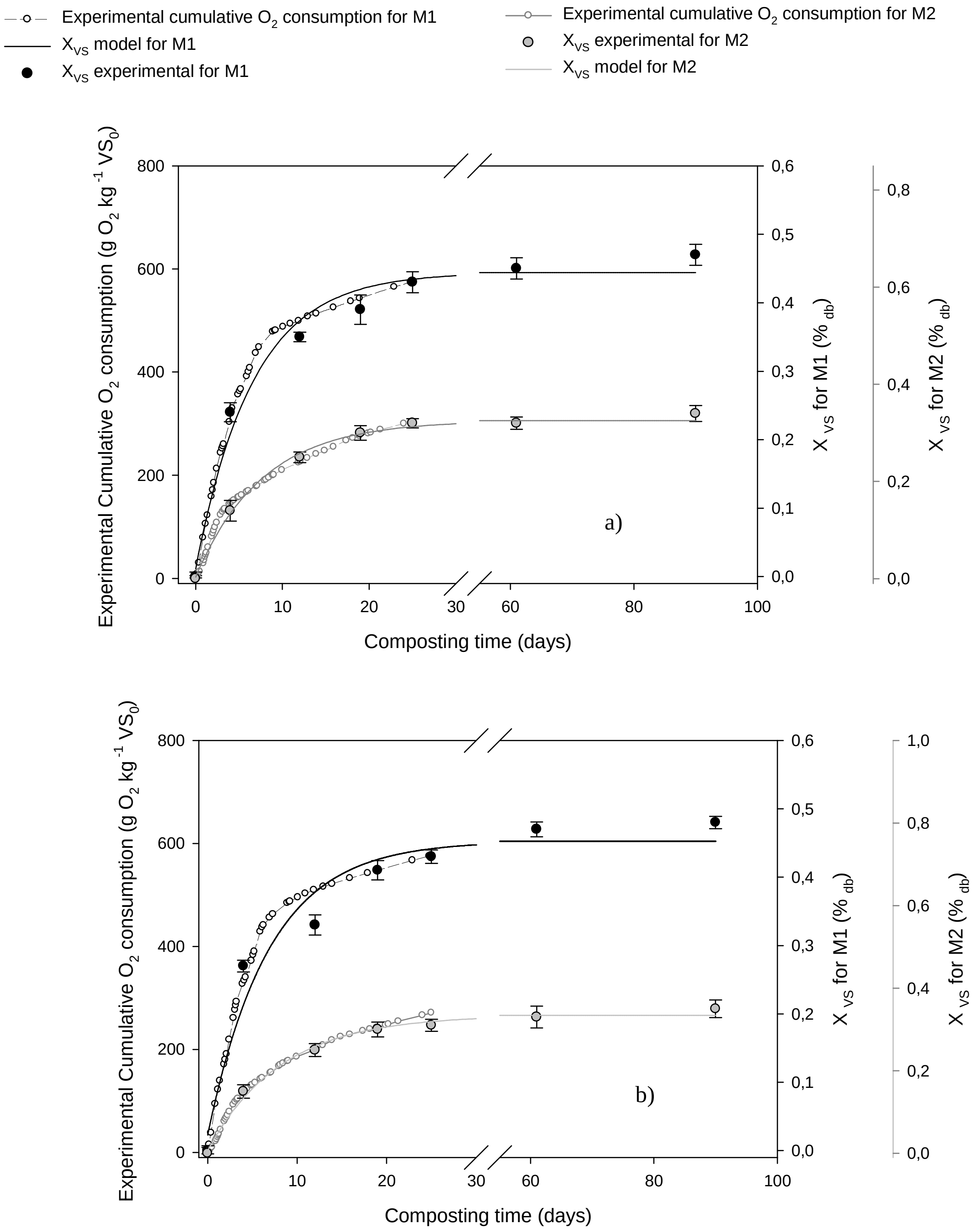


Figure S6.3: Profile of organic matter mineralisation and cumulative O_2 consumption for M_1 and M_2 mixtures with a) 20%ES, b) 30%ES.

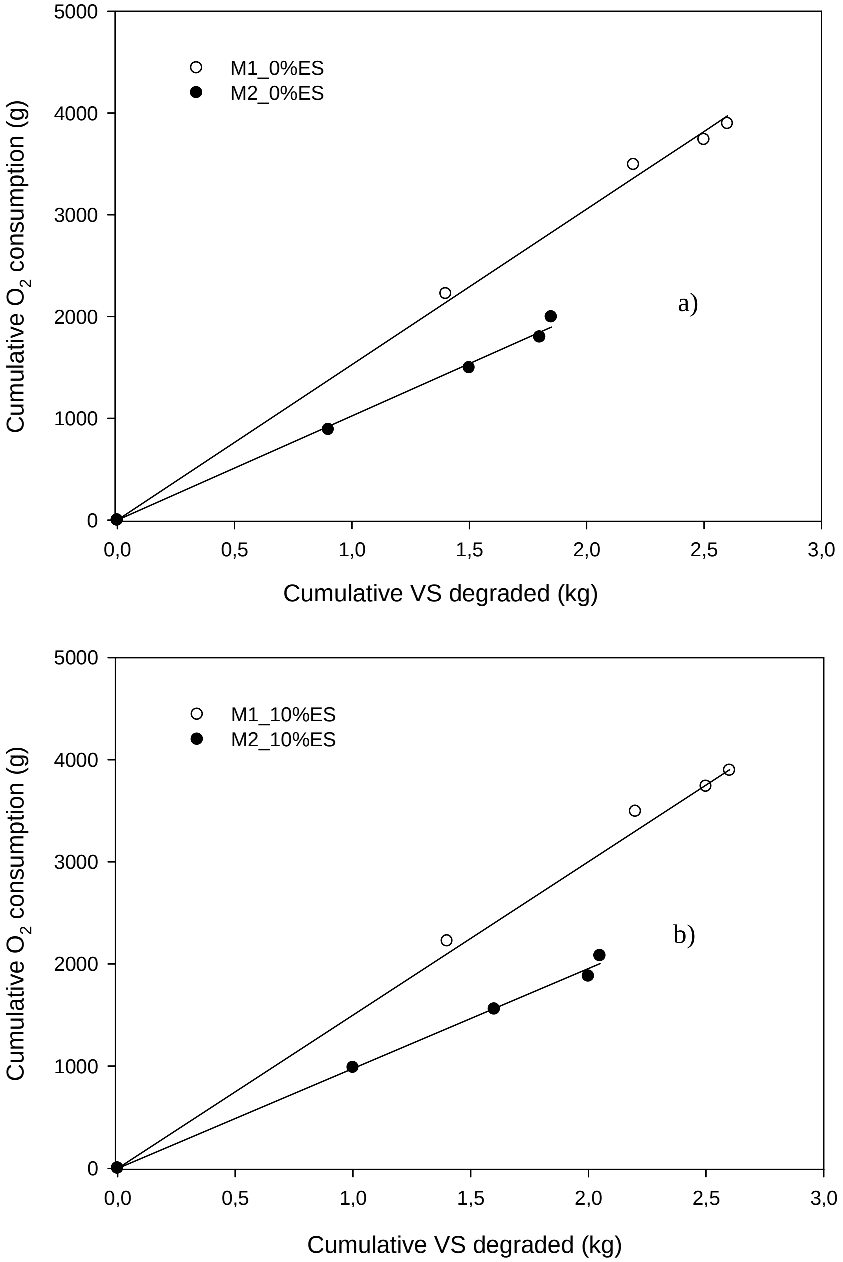
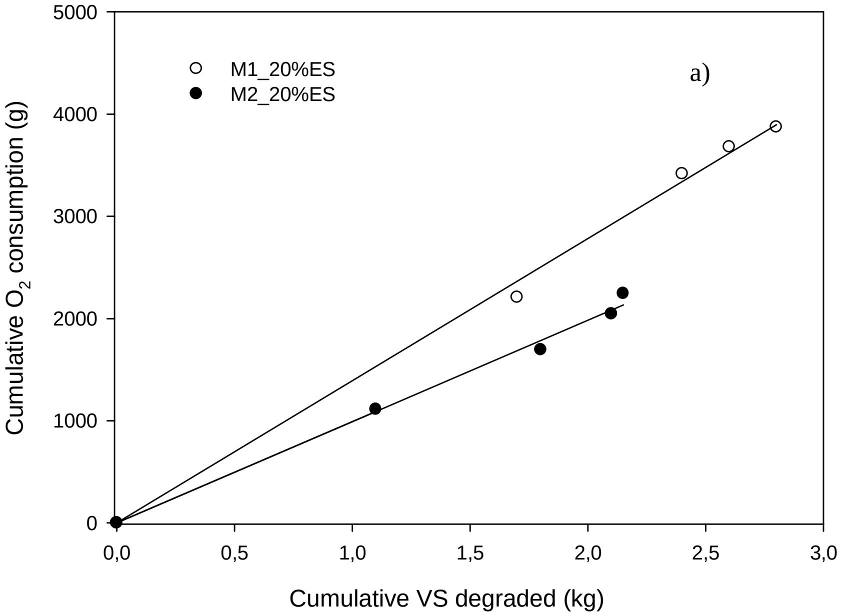


Figure S6.4: Relation between cumulative O_2 consumption and VS degraded for M_1 and M_2 mixtures with a) %ES, b) 10%ES.



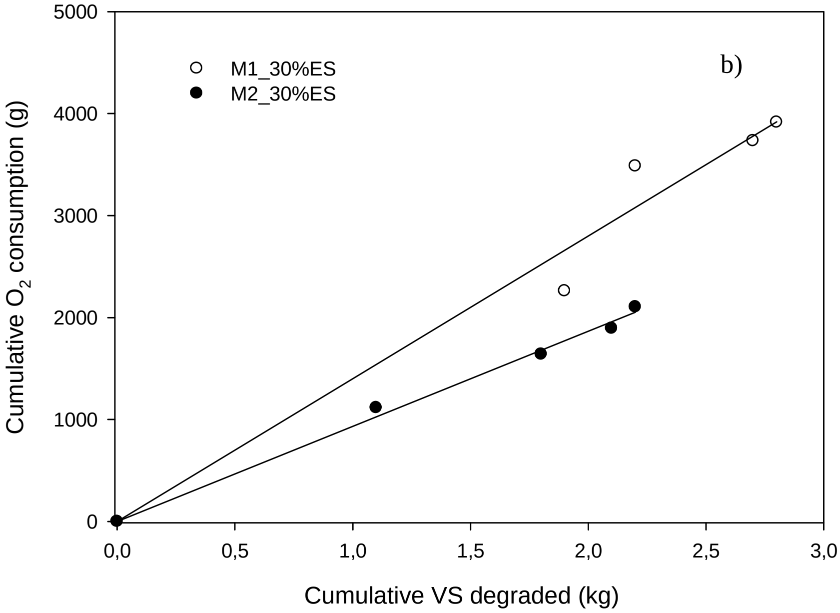


Figure S6.5: Relation between cumulative O_2 consumption and VS degraded for M_1 and M_2 mixtures with a) 20%ES and b) 30%ES.

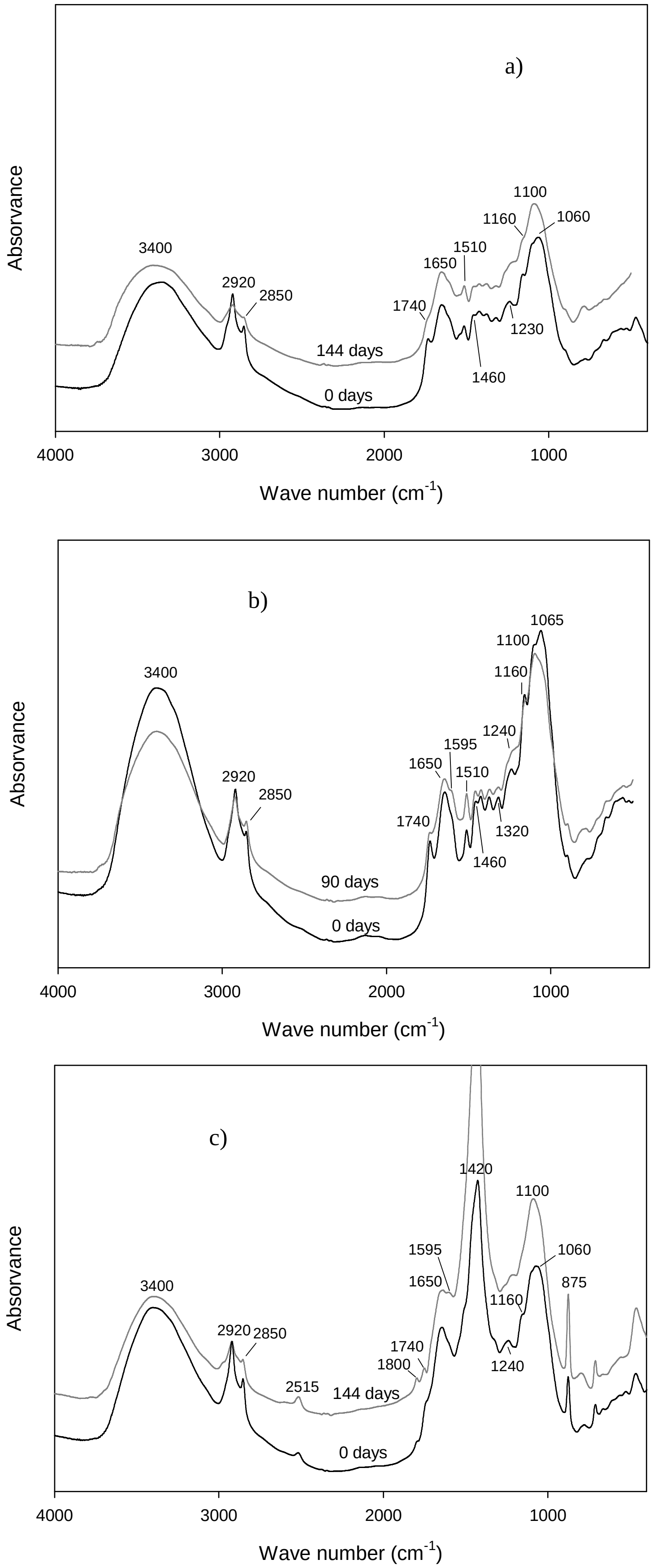


Figure S6.6: FTIR spectra for a) M_1+0%ES, b) M_2+0%ES, c) M_1+10%ES

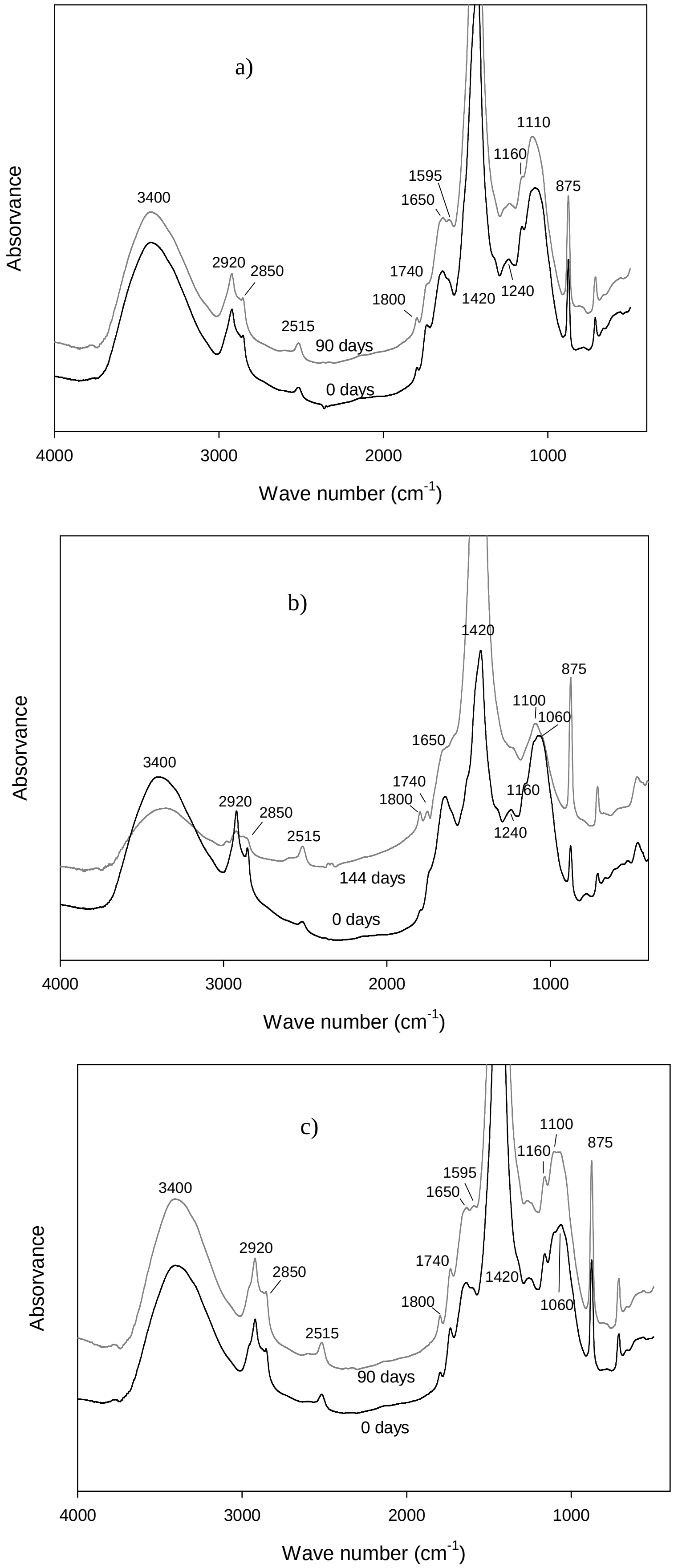
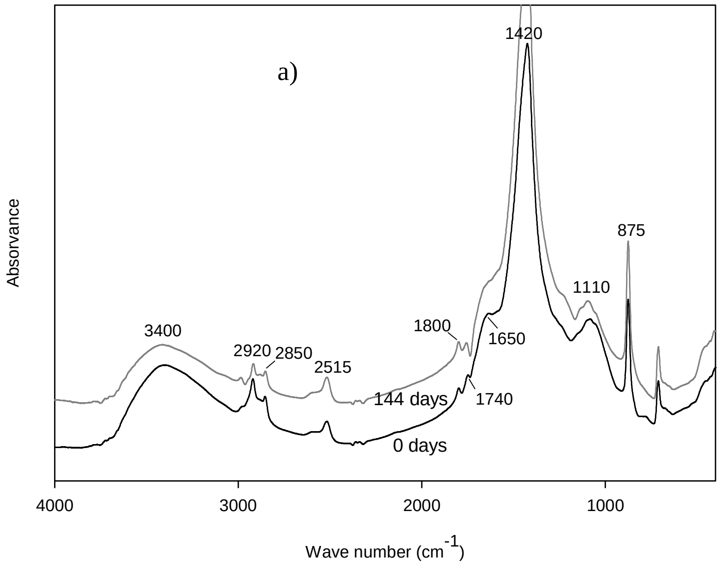


Figure S6.7: FTIR spectra for a) M_2+10%ES, b) M_1+20%ES, c) M_2+20%ES.



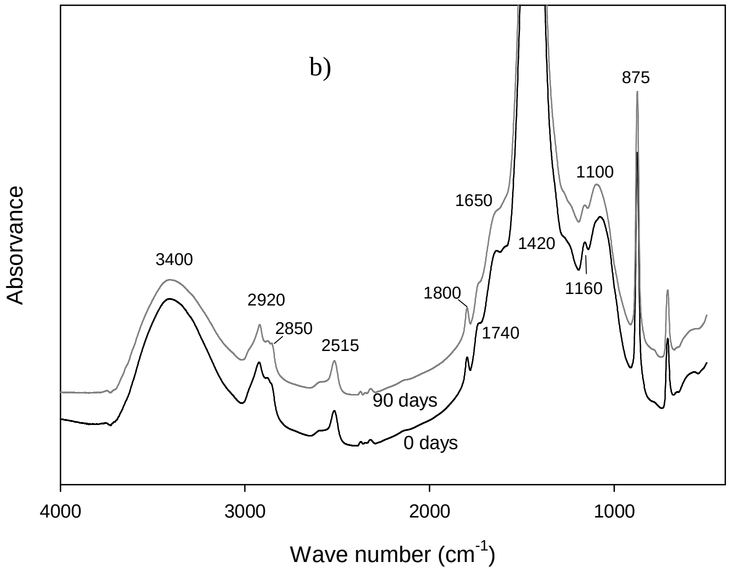


Figure S6.8: FTIR spectra for a) M_1+30%ES, b) M_2+30%ES.

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7. Analysis of eggshell co-composting process by using principal component analysis and data visualisation*

Waste management is a critical activity in modern society. Composting enables the retrieval of nutrients from some industrial wastes for obtaining a product able to suppress soil deficiencies. This study aims to investigate industrial eggshell (ES) co-composting in very high levels (up to 60% w/w) and the obtained results were evaluated by data visualisation and multivariate statistical analysis, namely involving principal components analysis. These methodologies were used to unravel the main relationships structuring the variability associated with the longitudinal composting experiments.

The experiments were designed with increasing quantities of ES (0, 10, 20, 30 and 60%ES w/w) mixed with industrial potato peel and rice husks. Co-composting tests were performed in self-heating reactors with forced aeration for 25 days. A variety of thermal, physical, chemical and phytotoxicological parameters were collected and recorded. Principal component analysis showed that physical properties like free air space, bulk density and moisture are the most relevant variables for explaining the variability due to ES content. On the other hand, variability in time dynamics is mostly driven by some chemical and phytoxicological parameters, such as organic matter decay, nitrate content and germination index. Higher ES incorporation (60% ES) enhanced the initial biological activity of the mixture, but the higher bulk density and lower water holding capacity had a negative effect on the aerobic biological activity, as the process evolved. Nevertheless, since convective heat removal was lower in mixture with 60%ES, pathogen-killing temperatures (>70ºC for 11h) were attained. Despite such findings, products obtained after 90 days were stable and non-phytotoxic.

In conclusion, we have demonstrated that valorisation of high amounts of eggshell by co-composting is feasible, but prone to be influenced by the physical properties of the mixtures. Both principal component analysis and data visualisation tools prove to be valuable aids for understanding the implications of co-composting inorganic materials on the design and operation of the process.

Keywords
Eggshell waste, co-composting, principal component analysis, data visualisation

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7.1. Introduction

Eggshell (ES) is a by-product of egg processing industry that is categorised as an animal by-product (ABP). Its annual production ascends to 350 thousand tonnes in Europe (Soares et al., 2013), thus constituting a very significant environmental issue, not only because of the large amounts generated, but also for creating conditions for the proliferation of pathogens in the environment (Ibrahim et al., 2015; Russ and Schnappinger, 2007). The use and disposal of industrial eggshell waste (ES) is regulated by European legislation, namely Regulation (EC) Nº 1069/2009 of the European Parliament and of the Council, of 21 October 2009. According to this in force legislation, composting is among the eligible options to provide prior thermal treatment to ES, in order to prevent the risk of spreading pathogens into the environment.

Though composting is a well-known process that has been used in waste management for more than 2 decades (Farrell and Jones, 2009; Gajalakshmi and Abbasi, 2008; Giusti, 2009; Schaub and Leonard, 1996), there is still a considerable lack of information regarding the application of this technology to promote the sanitation of inorganic wastes in large quantities (>30% w/w).

Analysing the existing the literature, some studies can be referred where a variety of inorganic wastes were employed as amendments to foster the composting dynamics. Gabhane et al. (2012) studied the effect of fly ash on green waste composting. Fly ash addition (5% on a dry weight basis) inhibited the thermophilic phase by microbial biomass and enzymatic activity, but showed an upsurge of N, P, and K levels in the final compost product. Moreover, Wong et al. (2009) evaluated the outcome of using coal fly ashes (CFA) with lime on the decomposition of food waste by composting. The decomposition efficiency of organic matter was enhanced and the composting period shortened when CFA additions were between 5-10% (w/w). On the other hand, Koivula et al. (2004) found that the incorporation of 20% ash from waste co-incineration in composting of source-separated catering waste boosted the temperature, mineralisation and humification of composting. Still, higher incorporation levels were not recommended due to the high specific gravity of ash. In a previous study, Soares et al. (2013) highlighted the feasibility of co-composting ES with organic wastes from the food industry to promote sanitation, despite the high inorganic content of ES. The authors also verified that the incorporation of 30% (w/w) of eggshell waste in the composting mixture did not affect negatively the mixture biodegradability, nor its capacity to reach pathogen-killing temperature (70ºC for 1 hour, as demanded by European legislation). In addition, the final product obtained may be used as a high neutralising Ca-rich improver. Nevertheless, at industrial scale it is desirable to understand the process behaviour under conditions where ES incorporation levels are above 30%, in order to maximize the potential that composting can offer to thermal treatment for ES valorisation.

Composting involves a variety of elements interacting in a complex way, namely organic and inorganic matter composition, microorganism population, operating conditions and environmental factors. The complex nature of these relationships precludes a complete and detailed mechanistic analysis of all the phenomena involved, in order to fully understand the system and derive optimal operation policies using first principles approach. However, data collected from composting processes provides another viable way to accumulate information and knowledge that could also lead to useful insights to achieve optimal operation conditions. This inductive approach starts with a careful planning of data that should be collected based on well-designed experiments. In addition, suitable data analysis frameworks to extract the fundamental aspects contained in the collected data sets is highly recommended. In this scope, several visual and analytical methodologies for highly multidimensional problems have been employed, with particular emphasis to Principal Components Analysis (PCA) (Jackson, 1991; Jollife, 2002; Reis and Saraiva, 2008). As explained in Section 2.4, PCA is a multivariate technique that seeks to unravel the main relationships structuring the variability of a data set. It operates by compressing the analysis space into a reduced set of latent variables (the principal components) which still explain a large portion of the original data variability, but that are orthogonal between each other (therefore, uncorrelated) and fewer in number (hence, easy to analyse). In this context, PCA is a natural candidate to analyse the relationships connecting the different elements playing an active role in the development of a composting process and, in particular, in signalling the dominant ones and the associated dynamics (Ho et al., 2010; Karak et al., 2014; Zbytniewski and Buszewski, 2005). PCA will also be extensively explored in this work as an exploratory data analysis tool for multidimensional complex processes, in order to efficiently guide the analysis to the key elements structuring the experimental data collected in the battery of longitudinal studies conducted in parallel (longitudinal, is a statistical term indicating that the outputs of an experiment were recorded over time).

This study aims at acquiring rigorous quantitative information and knowledge of the composting process regarding the effect of incorporating high levels of ES (until 60%ES) in composting mixtures with potato peel (PP) and rice husk (RH) as the starting organic feedstock. For such, a longitudinal experiment was conceived and a variety of quantities were collected and recorded, including thermal, physical, chemical and phytotoxicological parameters, as well as the end-product quality information. All this information was analysed with proper multivariate data analysis techniques and informative visualisation tools.

7.2. Materials and methods

7.2.1. Composting materials and experimental design

The experimental design involved five ternary composting mixtures for testing the influence of ES. The ES composition was varied from 0 to 60%. Mixtures (M_2) were prepared according to predefined proportions indicated in Table 7.1. In the case of M_2+60%ES, water was added during the mixing phase, in order to obtain an adequate moisture content near 50%.

Before loading the reactors, 1 kg of sample was withdrawn for further analysis and then each composting mixture was weighed. According to the mixtures composition, the total mass loaded to each reactor varied between 25.7 to 59.8 kg for a reactor working volume of 105 L.

Table 7.1: Mixtures composition and composting experimental conditions.

Parameter	M_2+0%ES	M_2+10%ES	M_2+20%ES	M_2+30%ES	M_2+60%ES
PP (%w/w)	74.5	67.1	59.6	52.2	29.8
RH (%w/w)	25.5	22.9	20.4	17.8	10.2
ES (%w/w)	0.0	10.0	20.0	30.0	60.0
Total mass (kg)	26.9	29.7	32.6	35.9	59.8
Water addition (L)					8.5
Initial air-flow (L h^{-1})	228	234	223	234	226

Potato peel (PP) was collected in an industry of potato chips and rice husk (RH) in a rice husking factory. Materials were feed to the composting experiments in less than 24 h after their collection. Eggshell waste was gathered at an industry of pasteurized liquid eggs and boiled eggs and immediately preserved at -12 ºC. ES was defrosted for 24 h at room temperature, before use. Properties of the starting ternary composting mixtures are presented in Table 7.2.

Table 7.2: Properties of the starting composting mixtures (mean±standard deviation, n=3)

Property		M_2+ 0%ES	M_2+ 10%ES	M_2+ 20%ES	M_2+ 30%ES	M_2+ 60%ES
MC	(%)	67.1±0.2	64.6±1.5	57.4±0.3	52.5±1.0	45.7±0.3
WHC	(g $_{\text{water}}$ 100 g $_{\text{dm}}^{-1}$)	303	243	197	162	91
Available WHC	(% of WHC)	32.7	24.9	31.6	31.8	7.1
BD	(kg m^{-3})	256	283	310	342	570
pH		6.5	6.7	6.9	6.9	7.3
VS	(% $_{\text{dry basis}}$)	88.3±0.1	68.1±0.4	55.2±0.9	42.9±0.5	21.0±0.8
C_{org}	(% $_{\text{dry basis}}$)	41.7±0.1	33.0±0.2	29.0±0.2	21.6±0.4	10.0 ±0.1
N_{org}	(g N kg_{dm}^{-1})	6.7±0.2	8.6±0.1	8.4±0.1	8.1±0.2	7.4 ±0.1
N-NH_4^+	(g N kg_{dm}^{-1})	0.04±0.02	0.03±0.01	0.12±0.05	0.03±0.02	0.49±0.09
N-NO_3^-	(mg N kg_{dm}^{-1})	<LOD	<LOD	<LOD	<LOD	<LOD
N-NO_2	(mg N kg_{dm}^{-1})	<LOD	<LOD	<LOD	<LOD	<LOD
C:N		62	38	34	27	12

Abbreviations: BD: bulk density; C_{org}: organic carbon; dm: dry matter; LOD: limit of detection (2.4 mg N kg_{dm}^{-1} for N-NO_3^- and N-NO_2^-); MC: moisture content; Norg: organic nitrogen; VS: volatile solids; WHC: water holding capacity.

7.2.2. Experimental set-up and composting procedure

Four self-heating reactors (SHR) with a working volume of 105 L were used for co-composting experiments. Reactors layout, as well as the air distribution system, O_2 and temperature monitoring devices were previously described in Chapter 4.

Composting trials lasted for 25 days in self-heating reactors. Airflow was initially set near to 0.5 L min^{-1} per kg of organic matter (OM), which is within the range of 0.43-0.86 L min^{-1} kg^{-1} OM used by Lu et al. (2001) for food waste. Air flow rates were adjusted to assure an outlet oxygen concentration in the range of 5 to 15% (v/v) and sustain the thermophilic phase as long as possible (Puyuelo et al., 2010). To ensure homogeneous conditions during tests, reactors were rolled for a distance equivalent to 14 rotations. The homogenisation procedure was performed on a daily basis during thermophilic conditions, and whenever samples were withdrawn (days 4, 12, 20, 25). After 25 days, mixtures were allowed to conclude maturation phase in small piles that were homogenised and moisture corrected (≈50%) every week, until 90 days of composting.-All samples withdrawn were weighed and the mass of each was considered in the overall mass balance of composting, as detailed in Chapter 4. The fraction of organic matter $(\frac{VS_t}{VS_0})$ not degraded during composting was calculated according to Eq. (4.1).

7.2.3. Monitoring parameters

During composting trials in SHR, biological activity of the mixture was assessed by calculating oxygen uptake rate (OUR) defined as previously indicated in Eq. (5.1). The profile of organic matter loss during experiments was fitted to a first order kinetic model (Eq. (5.2)).

Wet bulk density (BD, kg m^{-3}) was determined by weighing the reactor content (kg) and calculating the mixture volume from the height of the mass inside the reactor (H1) measured as established in Chapter 4.2.2 (Figure 4.5). Free air space (FAS) of the composting mixtures during aerobic biodegradation was calculated according to Eq. (3.6).

The moisture content was determined as detailed in the previous chapter. Water holding capacity was determined as described in section 3.2.3 (Eq. (3.7).

All the following parameters were determined according to the description presented in Chapter 4: pH, electrical conductivity (EC), calcium carbonate content, organic matter, Kjeldhal nitrogen ($N_{kjeldhal}$), N-NH_4^+, nitric nitrogen (N-NO_3^-+ N-NO_2^-) and germination index.

7.2.4. Statistical analysis of data

Several data analysis techniques were employed in this study, with special emphasis to multivariate statistical methodologies that are able to extract the main relationships between the quantities under analysis (thermal, physical, chemical and phytotoxicological parameters) and provide information about their evolution along time, at days 4, 12, 19, 25.

Furthermore, a variety of informative graphical tools were also used to facilitate the analysis of patterns of variation and potentiate the recognition of clear trends found in the data sets, such as stratified time-series plots and scatter plots, dot plots, bar charts and bubble plots. In the next subsection, a brief overview will be provided regarding PCA in order to introduce the basic nomenclature followed later on when addressing this technique, and to set the stage for the analysis of results from its implementation.

Let us consider a data set containing *n* observations and *m* observed variables, $X_{n\times m}$, which may result from an observational data collection process or a planned experimental design (the case of the present study). Due to the nature of the phenomena involved, the use of partially redundant instrumentation or even from operational reasons (such as control procedures), these variables, disposed along the columns of X matrix, often present strong mutual associations whose presence is important to detect and analyse. On the other hand, the observations may also include trends, such as drifts, oscillations or clustering behaviour, that are worthwhile to detect and analyse. When the number of variables is moderate or large, the number of pairwise combinations of variables to consider in the analysis of associations rapidly grows, even though one is still leaving aside all the interactions involving more than two variables. This means that analysing such data sets with resort to classical univariate or bivariate statistics is greatly inefficient and prone to miss relevant information. In this context, multivariate statistical techniques propose alternative ways of analysing the same data, which overtake the fundamental limitations of the classical low dimensional methods and still allow extracting the fundamental aspects regarding the two modes of the data set: the variables mode and the observations mode.
Among the multivariate methodologies, PCA assumes a prominent role, given its clear purpose, well-established body of theory and the existence of efficient algorithms to compute its quantities (Jackson, 1991; Jollife, 2002; Reis and Saraiva, 2008). In brief terms, PCA addresses the problem of finding a reduced set of *p* variables, the principal components or latent variables (as they are not really observed), that explain most of the variability exhibited by the *m* observed variables ($m >> p$). These new variables, or principal components, are computed as linear combinations of the observed variables. The basic idea is that each linear combination

maximizes the amount of variability possible to explain from the original X matrix. So, the first linear combination, conducting to the first principle component, PC1, will be the one explaining the largest portion of the original matrix, the second linear combination, corresponding to the second principal component, PC2, explains the second largest fraction, and so on. Each linear combination is orthogonal to the previous ones (therefore uncorrelated) and the combinations weights, called loadings, are bound to have unit norm. The relevance of each component is directly associated with the amount of variability it is able to explain from the original data, and is provided by the eigenvalue associated with it (PCA amounts to solving an eigenvalue problem over the covariance matrix of the X matrix). If one computes has many components as original variables, the entire data set can be exactly explained in terms of the new variables – in this case we have just rotated the original axis to a new Cartesian coordinate system, which is aligned with the dominant directions of variability. However, most often the first few *p* components already explain a large portion of the variability of X, namely the structural variability one wants to address, and discarding the last components ones would not compromise the analysis, as they are essentially composed by noise and unstructured sources of variation. These leads to the following PCA decomposition of the original data set:

$$\mathrm{X}=\mathrm{T\,L}^{\mathrm{T}}+\mathrm{E} \qquad (7.1)$$

where, L is the $(m\times p)$ loading matrix, with the coefficients for each linear combination disposed along its columns; T is the $(n\times p)$ matrix with the scores, that represent the values of the new set of uncorrelated variables (the principal components) for each observation; and E is $(n\times m)$ residual matrix, which is in general a non-zero matrix when $p<m$, being the $\mathbf{0}$ matrix when $p=m$.

From these definitions, it stems that the loadings (L) contain information about the variables that are associated (especially the first ones), whereas as the scores (T) describe the behaviour along the observations mode. The residual matrix (E) signals out observations (or variables) that are not well described by the *p*-dimensional PCA model. In the results section, these quantities will be used to explore the data set obtained from the longitudinal experiments carried out in this work. Several approaches exist for defining the number of principal components to retain (*p*), such as selecting the number of components that conduct to a given minimum fraction of explained variability (e.g. 80% or 90%), the Kaiser criteria, Scree test, parallel analysis or cross-validation, among others (Jackson, 1991; Valle et al., 1999; Vogt and Mizaikoff, 2003; Wold, 1978).

The statistical analysis was carried out with the JMP® Software, Ver 11 (SAS Institute, Software, Lda).

7.3. Results and discussion

The experimental results obtained in this study are analysed firstly by focusing on the individual parameters and their variability across the different experimental trials. Then, in a second stage, a general Multivariate Exploratory Data Analysis (MEDA) is conducted over all the parameters measured along the longitudinal studies, for different levels of incorporation eggshell waste (%ES), and the main patterns of variation are extracted and explored. In particular, the dynamics of the observations are analysed with resort to the PCA scores and the main associations are identified using the loadings from this technique.

7.3.1. Composting trials

7.3.1.1. Temperature profiles

Suitability of eggshell valorisation by composting is intrinsically related to the ability in eliminating potentially harmful pathogens by ensuring the necessary requirement of temperature-time exposure (70ºC for 1 h). During composting, reaction temperatures are dictated by the heat generated by microbial breakdown of organic materials and heat loss (Barrena et al., 2011). The influence of ES increasing content on the reaction temperature and biological activity (expressed in terms of oxygen uptake rate (OUR)) is presented in Figure 7.1 and Figure 7.2 a), respectively. Table 7.3 summarises some parameters of the thermal behaviour found for each mixture.

Although the time required to attain maximum temperature (Tmax) was identical, the increase of ES content promoted an upsurge in Tmax and extended the period with T>55ºC (Table 7.3). Nevertheless, only 60%ES mixture was able to fulfil the sanitising requirements of temperature-time exposure by ensuring T> 70ºC for 11 h. (Figure 7.1 a), Table 7.3). In addition, ES co-composting had a positive effect on OUR and on the ability to generate heat. In fact, OUR shifted from the upper level of “low biodegradability” depicted by 0% ES to “medium” level attained by 60%ES, presenting values near 4 g O2 kg VS h^{-1} within the first day of composting (Figure 7.2 a)).

Although the higher ES incorporation could enhance the initial biological activity of the mixture, OUR rapidly fell down to a “low” level after 48 h, thus conditioning the thermal behaviour of that mixture by the prevalence of heat convective losses.

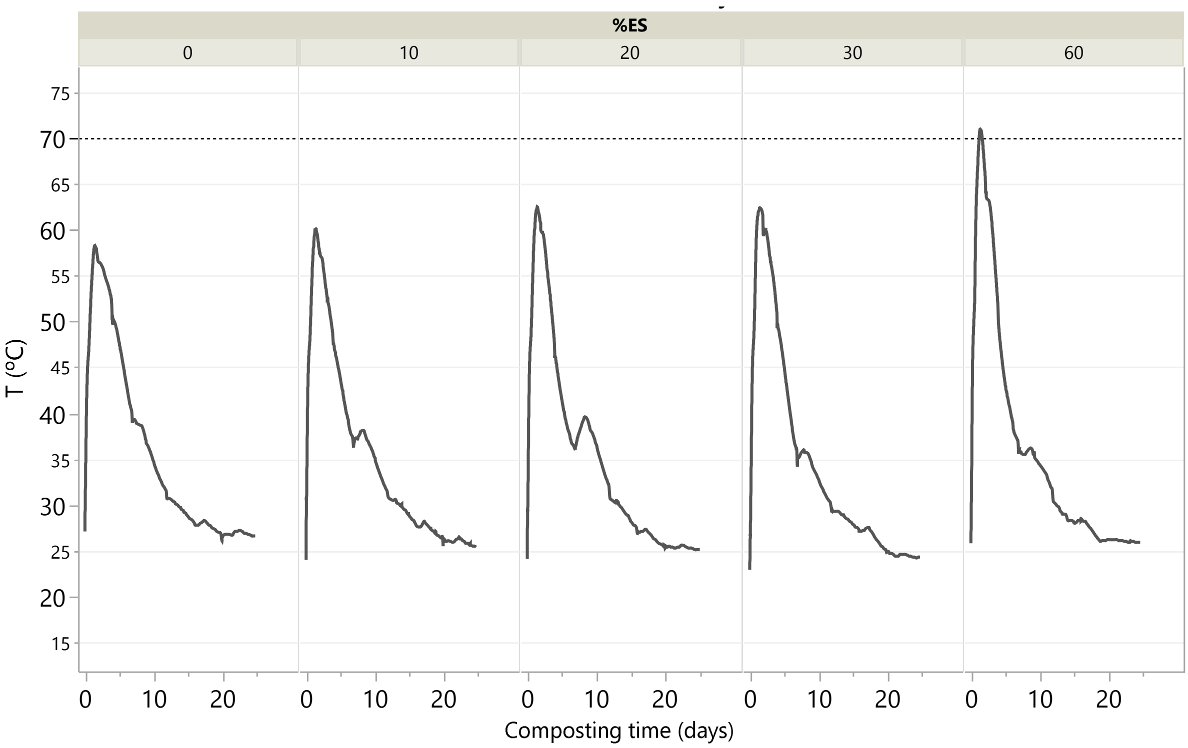


Figure 7.1: Evolution of core centre temperature of mixtures M_2+0%ES to M_2+60%ES, over 25 days of composting.

Table 7.3: Influence of ES percentage in some thermal parameters of composting process.

	Maximum T				
Mixture	Value (ºC)	Time to achieve (days)	Consecutive period with T>70ºC (h)	Consecutive period with T>55ºC (d)	Thermophilic period with T>40ºC (days)
M_2+0%ES	58.4	1.5	0	1.8	6.8
M_2+10%ES	60.1	1.5	0	1.8	5.9
M_2+20%ES	62.5	1.5	0	2.2	5.4
M_2+30%ES	62.4	1.5	0	2.5	5.9
M_2+60%ES	71.1	1.4	11	3.0	5.9

These results could be a consequence of two physical characteristics of the initial 60%ES mixture (Table 7.1): i) only 7% of maximum water holding capacity was available and ii) high wet bulk density (570 kg m^{-3}). Both aspects might had a negative impact on the biodegradability of the mixture by i) limiting homogeneous gas diffusion in the composting matrix and ii) increasing compaction of the mixture specifically in the lower layer of the reactor bed (Das and Keener, 1997). As a result, air channelling in the composting matrix could have occurred. Thus, aerobic degradation of organic matter may have hampered the characteristic smelly odours to came out. Also the formation of leachates that were drained at 3rd and 5th day of composting, indicates that the mixture presented a low ability to retain water. This aspect enhances the likelihood of anaerobic pockets, due to pore space water clogging (Liang et al., 2003).

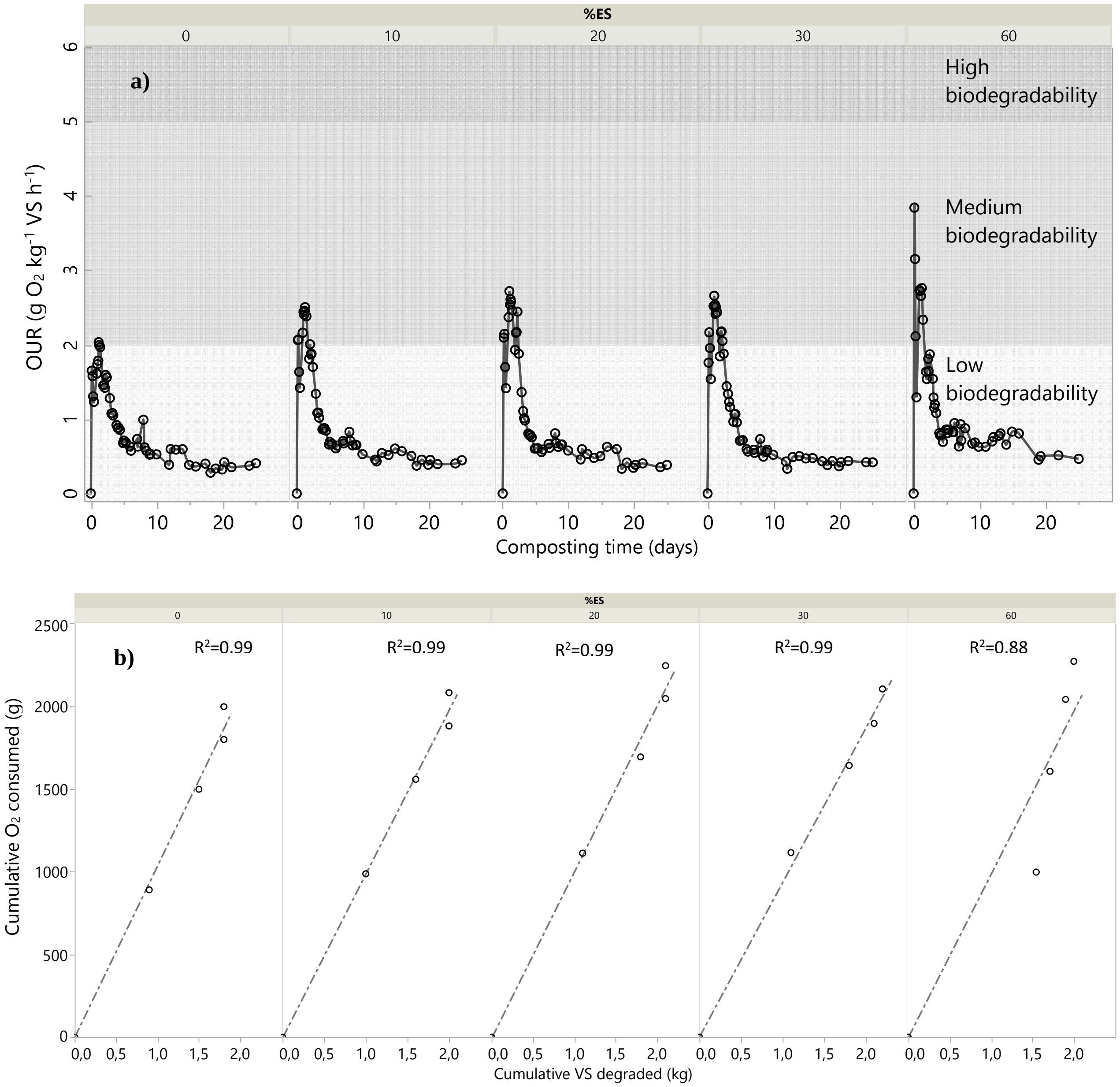


Figure 7.2: a) OUR evolution during composting in self-heating reactors, b) Relationship between O_2 consumption and VS degradation.

Figure 7.2 b) depicts the total oxygen consumed during the 25 days of composting in function of the cumulative VS degraded within the same period. Mixtures with 0% to 30% ES present good linear relation (R>0.99) between those variables, whereas for 60%ES mixture a deviation from linearity is featured. This might be associated with the development, at same extent, of anaerobic conditions in the reactor, and thus VS consumption without consuming oxygen.

7.3.1.2. Physical, chemical and phytotoxicological evolution

Some physical parameters of the composting matrix, directly influence the efficiency of the composting process and the end-product quality (Mohee and Mudhoo, 2005). In particular, the adequate relation between moisture content, water availability and porosity is depend upon the ingredients used and their relative proportion within the blend (Ahn et al., 2008). Figures 7.3, 7.4 and 7.5 indicate moisture content, wet bulk density and FAS, respectively, over 25 days.

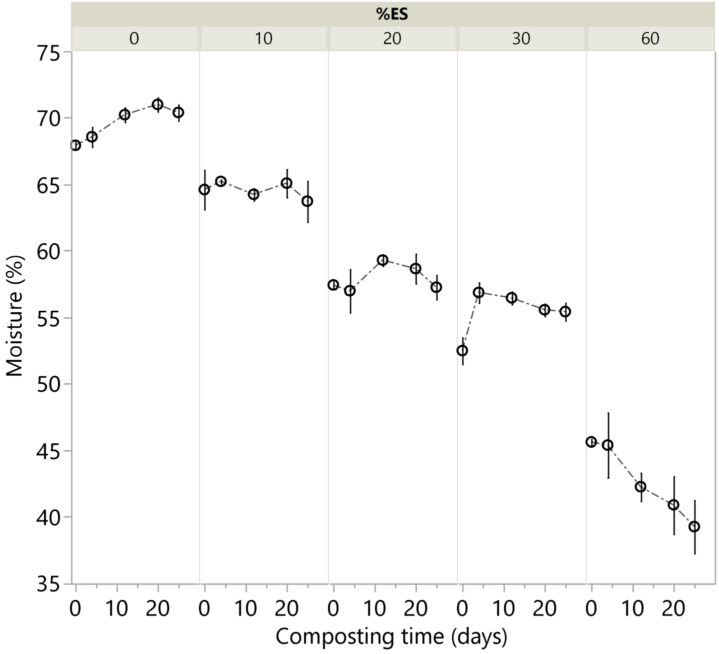


Figure 7.3: Influence of ES on moisture during composting (mean±standard deviation, n=3).

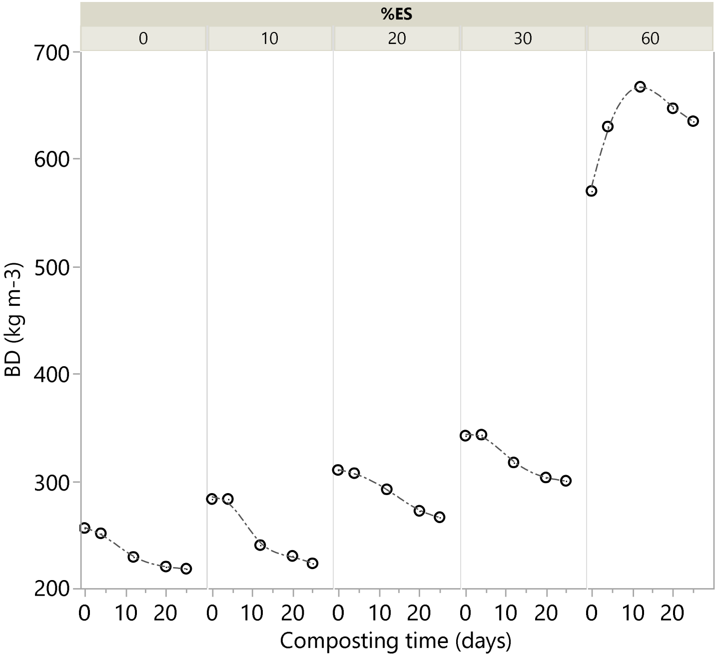


Figure 7.4: Influence of ES on wet bulk density evolution during composting (mean±standard deviation, n=3).

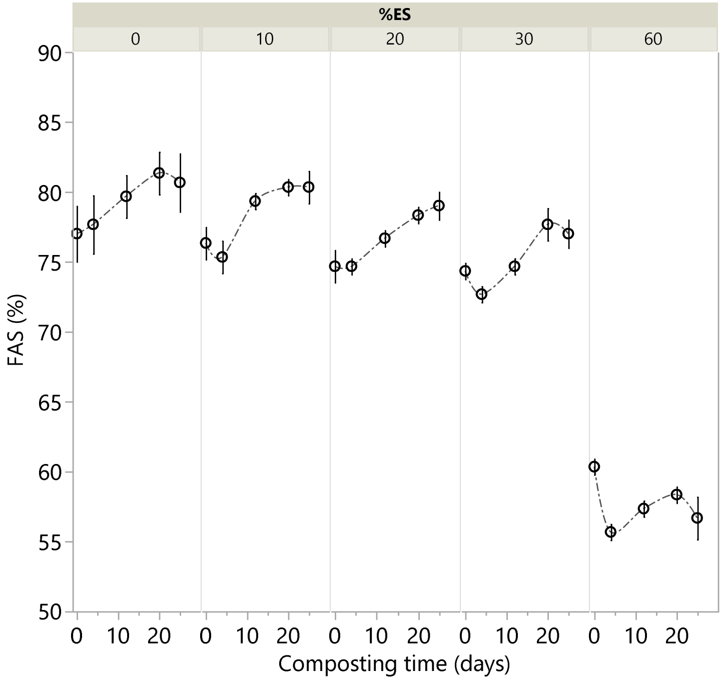


Figure 7.5: Influence of ES on FAS evolution during composting (mean±standard deviation, n=3).

Moisture content of the starting composting mixtures was lowered by the incorporation of ES (Figure 7.3). In addition, due to the inorganic nature of ES the water holding capacity was also reduced (Table 7.2). ES is a dense material, thus its incorporation intensifies the wet bulk density of the starting blend, thus lowering the available air voids (Figure 7.4 and 7.5).

As composting proceeds, there is a slight tendency to increase the moisture level (Figure 7.3) for mixtures with 0 to 30%ES, but the available water capacity of the initial mixtures (≈25 to 33% of maximum WHC) (Table 7.3) could endure that behavior. The most recommended moisture levels are between 50-70%, (Liang et al., 2003) and the moisture content of 0-30%ES mixtures fitted that interval without leachates formation, meaning that moisture provision was adequate to maintain microbial activity throughout the composting process. However, for M_2+60%ES, the initial moisture level of 45.7% (wet basis) proved to be higher than the necessary to support the increase of moisture during the biodegradation of organic matter. As a result, leachates were formed and their drainage contributed to the decrease of the mixture moisture content.

For mixtures 0%ES to 30%ES, bulk density evolution presented a tendency to be reduced as biodegradation progresses. This aspects highlights that RH provided enough structure to support the solid matrix, as organic substrates were transformed in a low to medium rate as it can be found in Figure 7.2 c). Consequently, FAS suffered a slight increase.

More specifically, a significant rise in the bulk density to 630 kg m^{-3} was observed for M_2+60%ES, which was maintained for the rest of the composting period. Though that compaction still allowed a FAS of near 60% it is believed that air permeability might have been restricted. According to Das and Keener (1997) high moisture solid beds with high wet bulk density can cause high compressive stresses. Additionally, the compaction of the matrix due to its own weight can create vertical profiles of air permeability. Particle size distribution also plays a role in air permeability. By increasing the particle dimensions, inter-particles spaces increase and air permeability rises (Huet et al., 2012). In fact, in the present study for M_2+60%ES mixture it was visible that the center region of the bottom layer (20 cm) of the reactor was characterized by crushed finer particles of eggshell. That probably resulted from the compressive stress that the nearly 60 kg of material generated inside the reactor.

The impact of these aspects on air distribution should be further investigated, once the effects of compaction on porosity and permeability have important implications on the design and operation of a composting process (Richard et al., 2004).

Figures 7.6 to 7.10 present the evolution of chemical and phytotoxicological parameters.

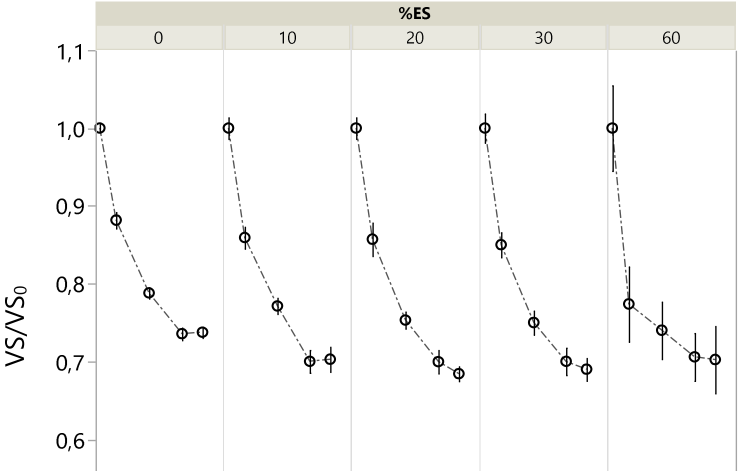


Figure 7.6: Effect of ES incorporation on volatile solids decay (mean±standard deviation, n=3).

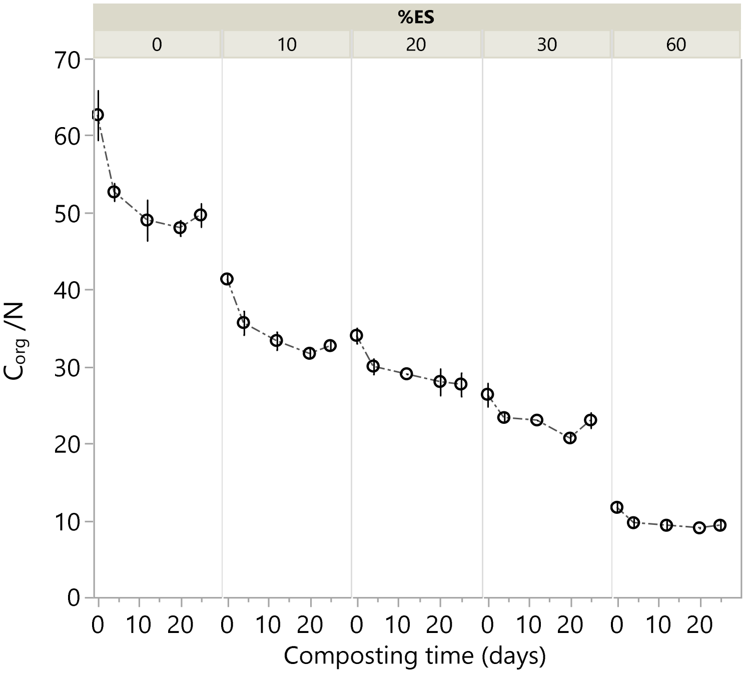


Figure 7.7: Effect of ES incorporation on C_{org}/N evolution (mean±standard deviation, n=3).

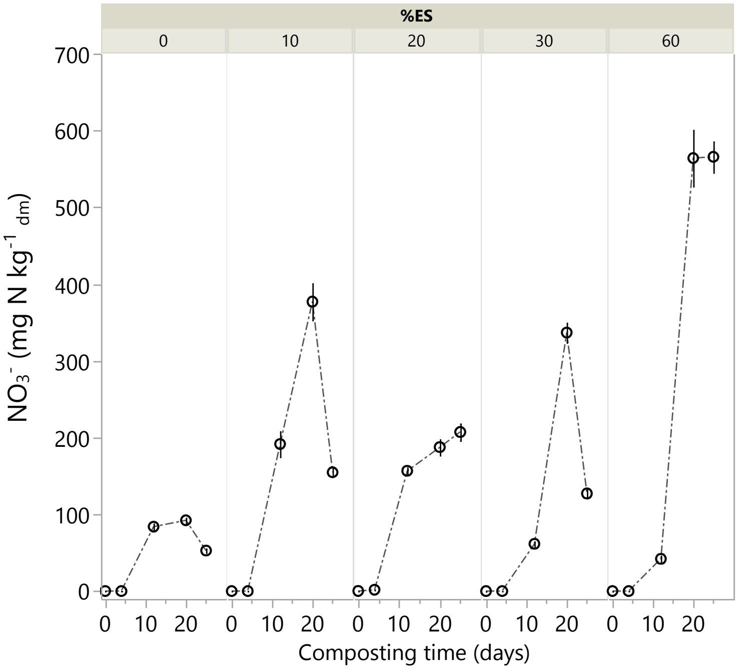


Figure 7.8: Effect of ES incorporation on nitrate formation during composting (mean±standard deviation, n=3).

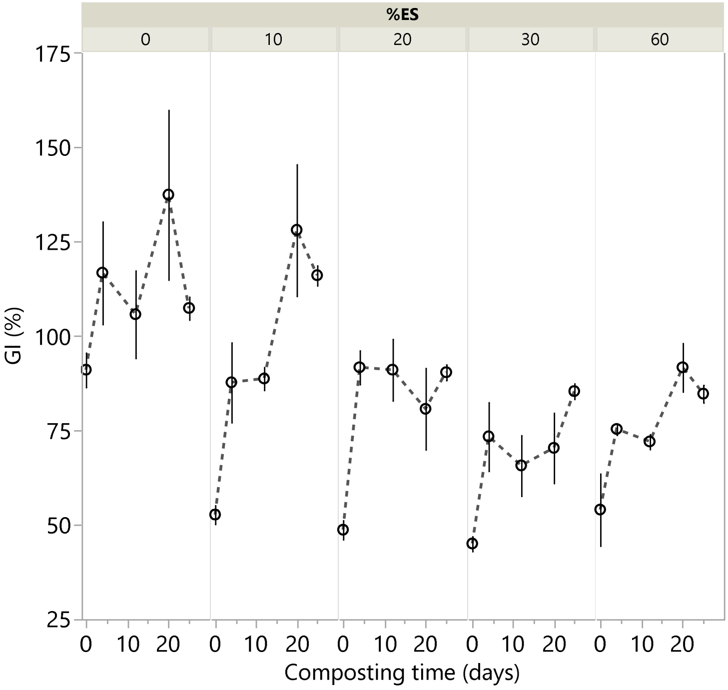


Figure 7.9: Effect of ES incorporation on germination index (mean±standard deviation, n=3).

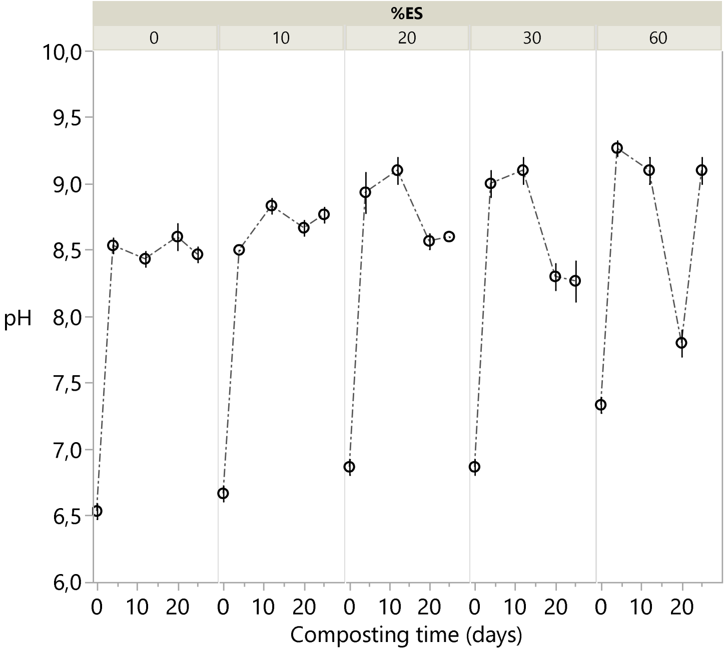


Figure 7.10: Effect of ES incorporation on pH during composting (mean±standard deviation, n=3).

As ES incorporation increases, the initial organic matter decay rate is more pronounced (Figure 7.6) indicating a higher microbial activity in comparison to M_2+0%ES. However, the amount of VS converted at the end of 25 days was similar in all cases.

Figure 7.7 depicts C:N evolution along time. Although the initial ratio decreases as the ES percentage increases, in all cases C:N tends to decline all along. However, M_2+60%ES has a low C:N ratio for effective retention of N released during microbial degradation (Brito et al., 2012). As a result, the risk of N volatilisation as NH_3 may be enhanced taking into consideration that this composting mixture is basified with ES presence (Figure 7.10).

Nitrification appears to be favored with ES. Figure 7.8 shows that independently of the ES content in the mixture, nitrification is inhibited during the thermophilic phase, as expected (Sanchez-Monedero et al., 2001). But as process evolves and mesophilic temperatures are established, a build-up of NO_3^- is observed.

Germination index tends to increase as composting process evolves (Figure 7.9), as expected. Though ES incorporation seems to be detrimental to the phytotoxin-free potential of the starting composting mixtures, by lowering GI to values near 50% (Huang et al., 2004).

7.3.2. Compost quality

The value of the final compost is of extreme importance to validate the composting feasibility for valorisation purposes. In this study, the final quality of products was investigated after 90 days of composting, by considering three levels (Komilis, 2015): i) physico-chemical (WHC, pH, Equivalent $CaCO_3$, OM, NH_4^+/NO_3^- ratio, heavy metals); ii) biological (respiration rate - CO_2 generation) and iii) plant growth related (seed germination).

All composts were alkaline (pH near 9), stable with moderately low respiration rate corresponding to a stable compost (Brinton et al., 1995) and its use as soil improvers was not conditioned by the presence of heavy metals. Nitrification ratio (NH_4^+/ NO_3^-) was less than 0.5 as expected for mature composts (Bernal et al., 2009) (data not shown). Table 7.4 features the quality properties that were the most distinct amongst mixtures. In particular, as ES incorporation increases WHC is reduced, neutralisation capacity enhanced and organic matter restricted. Despite M_2+60%ES presented a lower seed germination capacity, all end-products were phytotoxin-free, once germination indices were higher than 50%.

Table 7.4: Main properties of the final composts, after 90 days of composting (mean±standard deviation, n=3)

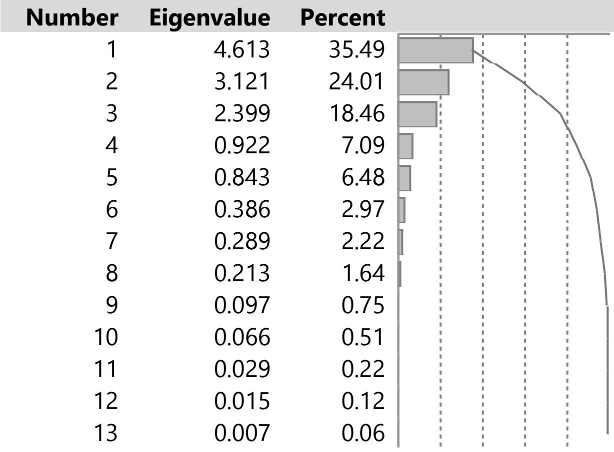
Property	M_2+ 0% ES	M_2+ 10% ES	M_2+ 20% ES	M_2+ 30%ES	M_2+ 60%ES
WHC (g $_{water}$ 100 g $_{dm}^{-1}$)	483±39	349±32	292±0.1	234±13	129±3
Equivalent $CaCO_3$ (g $CaCO_3$ 100g^{-1} $_{dm}$)	8.6±1.1	34.7±0.3	47.8±1.4	60.0±1.9	79.0±2.3
OM (g VS 100 g^{-1} $_{dm}$)	82.8±0.7	58.7±1.5	45.9±0.4	38.6±1.1	15.5±0.2
C_{org}/N	46.7	31.6	26.8	21.1	9.4
GI (%)	96±4	104±4	96±7	104±9	74±4

7.3.3. Multivariate exploratory data analysis

In this section, a general Multivariate Exploratory Data Analysis (MEDA) of all the parameters measured along the longitudinal studies, for different levels of incorporation eggshell waste (%ES) is performed. From this analysis, the main patterns of variation are extracted and commented for the first 25 days of composting. In particular, the dynamic of the observations are analysed with resort to the PCA scores and the main associations are identified with the loadings from this technique.

The collected data were arranged in a single data matrix $X_{n\times m}$ (with n=5 observations and m=13 variables) and pre-processed in order to centre all variables to zero mean and scaled them to unit variation. This transformation is called *autoscaling*, and is fundamental to avoid distortions in the analysis due to the use of variables with different units (alternatively, one can also base the PCA computations in the correlation matrix of X, instead of its covariance, which would lead to the same final PCA model). The %ES was just used as an indicator variable for stratifying the subsequent analysis, which was not incorporated in the PCA model.

Performing a PCA analysis over the experimental data obtained with all the ES mixtures, results in the following outputs. In Figure 7.11 the eigenvalues profile is presented, where one can see that 3 components are enough to explain approximately 78% of the variability of the entire data set, half of it being explained by just the first principal component that explains 35.5% of the total variation in all the experiments.



Number	Eigenvalue	Percent
1	4.613	35.49
2	3.121	24.01
3	2.399	18.46
4	0.922	7.09
5	0.843	6.48
6	0.386	2.97
7	0.289	2.22
8	0.213	1.64
9	0.097	0.75
10	0.066	0.51
11	0.029	0.22
12	0.015	0.12
13	0.007	0.06

Figure 7.11: Eigenvalues profile for the PCA analysis of all ES mixtures during composting (0-25 days).

The central importance of the first component justifies that some attention be devoted to its individual analysis, as it conveys a large portion of the total variability found in the data set. Figure 7.12 a) depicts the time-series plots of the scores of this component, after separating the observations according to the %ES. This plot clearly indicates two important aspects: i) the critical importance of the %ES in the variability of the scores (it basically sets the average level for the different profiles); ii) the existence of similar dynamic patterns in the different profiles, especially for %ES ≤ 30%.

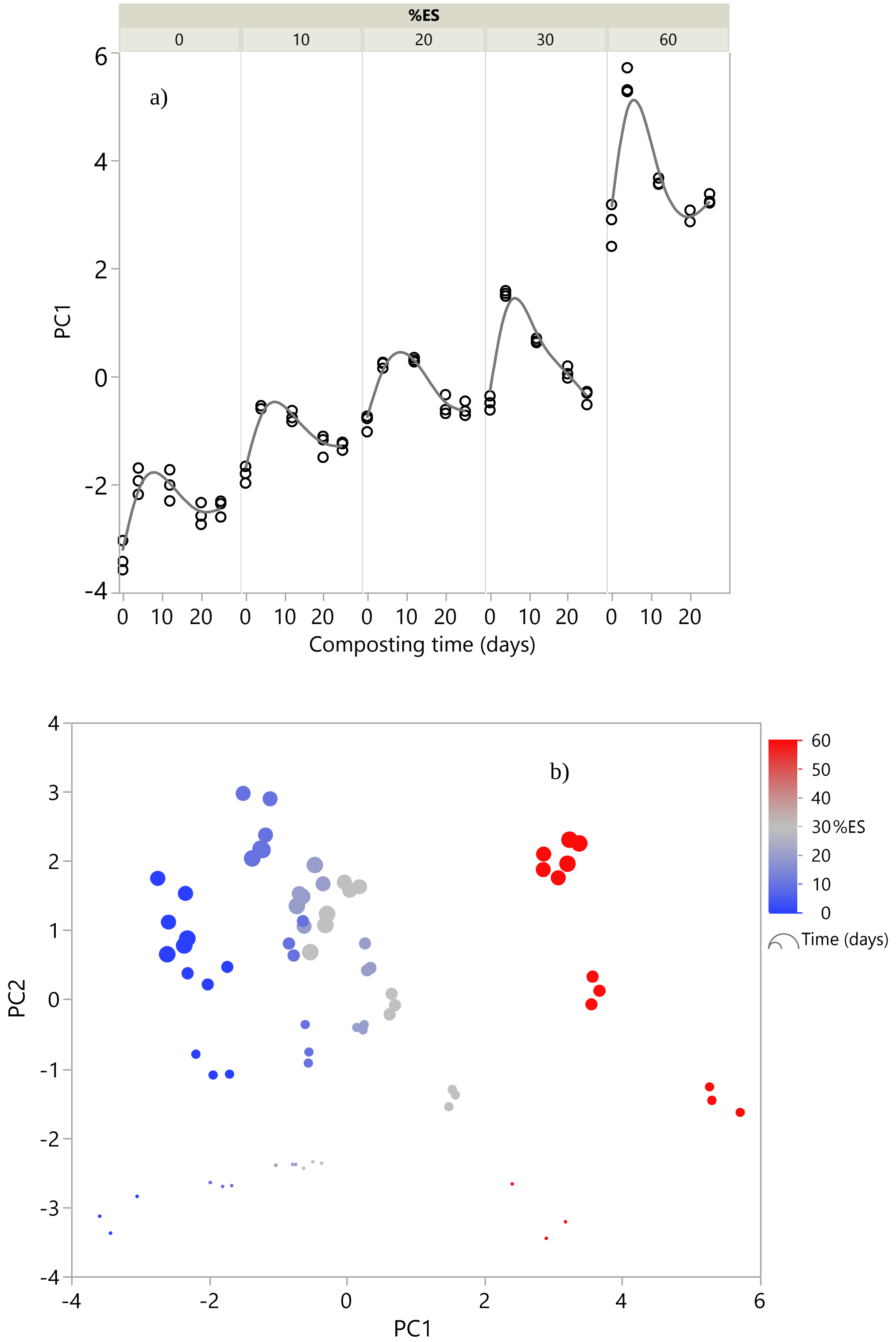


Figure 7.12: a) Time-series plots of the scores for the first principal component (PC1), stratified by %ES; b) Scatter plot for the first two scores (PC1 vs PC2), stratified by %ES (color of the bubbles) and time (size of the bubbles).

In fact, by analysing the first and the second principal components (Figure 7.12 b)), one can verify that PC1 is essentially capturing the variability originated by %ES (colour stratification in Figure 7.12 b)) whereas PC2 is explaining the time dynamics in each longitudinal experiment (size stratification in Figure 7.12 b)). As PC1 and PC2 are also the components explaining most of the variability of the experimental data (≈60%), one can also conclude that these are the two main structural factors contributing to the observed variability of the experimental results, with %ES having the primary role.

From these results, it would be suitable to find out which variables would be mostly involved in the definition of these dynamic patterns found for PC1 and PC2. This can be analysed through the loadings of the component (Figure 7.13 a) and b)). For PC1, one can find out that at least four variables are highly associated with each other: BD, C_{org}:N, FAS and H (Figure 7.13 a)).

Analysing the signals of the loadings, it is possible to infer that the former variable (BD) presents a negative association with the last three and that these (C_{org}:N, FAS and H) are all positively correlated with each other. This is indeed consistent with the mechanistic knowledge of the composting process, since higher BD values are associated to a decrease of air voids within the mixture, thus lowering the FAS. In addition, the relation between moisture content, water and porosity depends upon the ingredients used and their relative proportion within the blend (Ahn et al., 2008). Thus the positive relation between FAS and H found with PC1 may be a consequence of that. For PC2, VS/VS0 was the most relevant variable (Figure 7.13b)) with the higher loading value. In addition, four variables are highly interrelated, namely T, N-NO_3^-, pH and GI (Figure 7.13 b)). This relation might be associated with the nitrification phenomena typically occurring in composting process. When the thermophilic phase transits to mesophilic temperatures, conditions are met to promote NH_4^+ conversion to NO_3^- with a consequent increase in GI values, as previously reported.

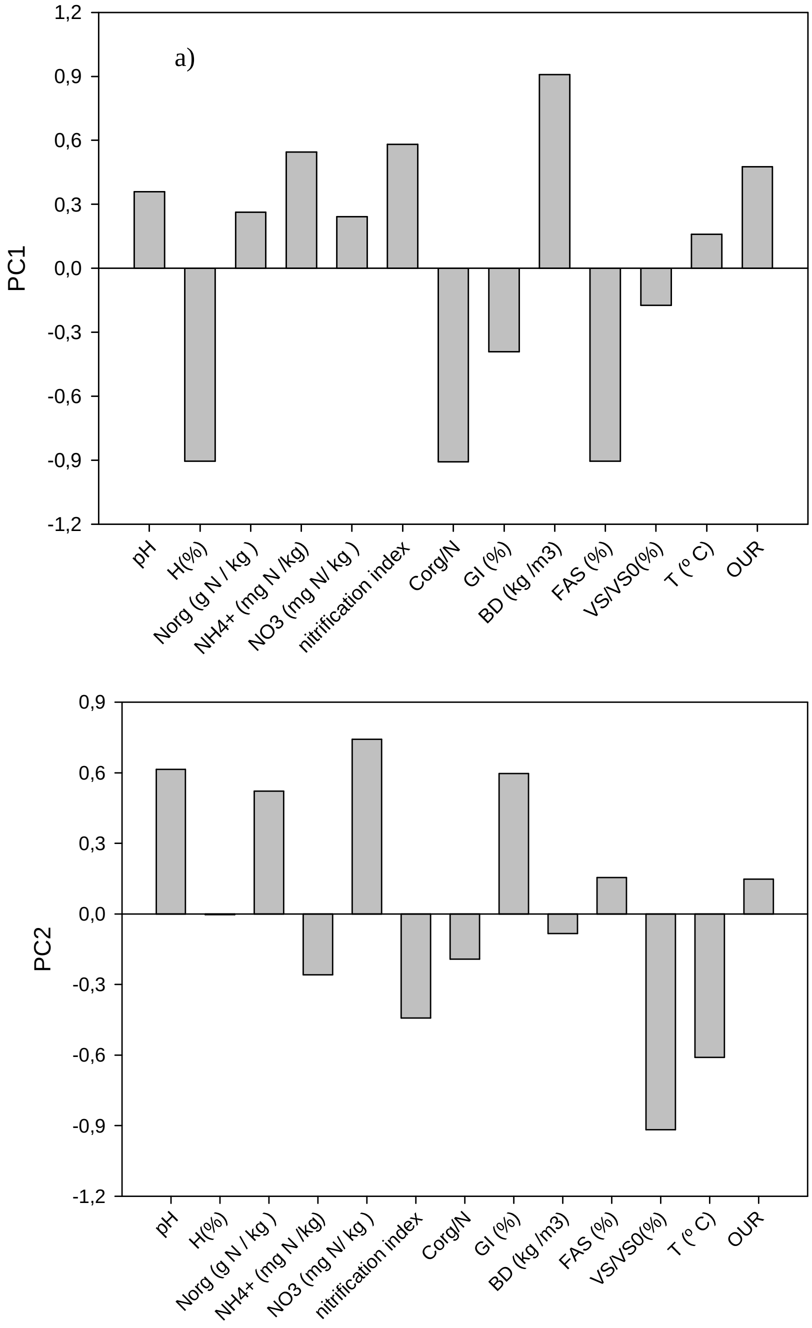


Figure 7.13: Loadings for the a) first principal component (PC1), b) second principal component (PC2).

7.4. Conclusions

The effect of incorporating high levels of industrial eggshell waste (until 60%ES) in an organic composting mixture (potato peel and rice husk) was evaluated, by using multivariate data analysis techniques and informative visualisation tools.

At the higher level of ES, the bulk density and lower water holding capacity of the starting mixture were drawbacks to the development of an aerobic biological activity. Still, pathogen-killing temperatures were attained, possibly due to the lower free air space of the matrix that retained convective heat losses.

Data visualisation allowed the recognition that ES co-composting was dependent on ES content and time. PCA showed that two principal components are enough to explain approximately 60% of the variability of the data set. PC1 captured the variability originated by %ES, while PC2 is related with the time dependency of the process. Physical properties like free air space, bulk density and moisture were indicated by the PCA as the process variables that capture the variability due to ES content. Also, C_{org}/N may play a role on PC1. The loadings for PC2 revealed that VS/VS0 is an important variable, as well as temperature, $N\text{-}NO_3^-$ and pH.

In conclusion, valorisation of eggshell by co-composting is prone to be mostly influenced by the physical properties of the mixtures, which has important implications on the design and operation of the process.

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Part D. Environmental applications for eggshell compost

The application of an eggshell rich-compost was envisaged for environmental applications, namely removal of lead and zinc from aqueous and solid matrices. In Chapter 8, compost derived from eggshell waste co-composting was evaluated as a biosorbent for Pb(II) uptake from aqueous medium and for comparison purposes, mature compost without eggshell and natural eggshell were tested as well.

This Parts ends up with the evaluation of using the eggshell-rich compost as a soil amendment for immobilisation of Pb and Zn in an acidic contaminated mining soil, by evaluating the environmental availability of metals, toxicity reduction and variation of CO_2 efflux from soil, due to the incorporation of a carbonate rich amendment.

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8. Biosorbent potential of an eggshell rich composting product for lead removal from aqueous solutions*

Compost derived from eggshell co-composting (CES) was evaluated as a biosorbent for Pb(II) uptake from aqueous medium. For comparison purposes, mature compost without eggshell (CWES) and natural eggshell (ES) were also tested. Batch experiments indicated that maximum sorption capacity of CES is 23 mg.g^{-1}.

Sorption kinetics and equilibria data were fitted to pseudo-second order and Langmuir model, respectively. From a kinetic point of view, lead sorption into CES was fast, attaining equilibrium within less than 180 min. The sorption capacity CES was not significantly altered when initial pH of aqueous medium ranged from 2-5.5. In comparison to ES, organic matter of CES provided supplementary sites for lead sorption and an increase of 43% in the sorption capacity was observed. Nevertheless, CWES was the biosorbent with higher sorption capacity.

Still, the study points out the potential of new use of CES as an effective biosorbent to lead removal from aqueous matrices.

Keywords: eggshell compost, biosorbent, lead sorption

*The information presented in this chapter was based upon:

Soares, M.A.R., Quina, M.J., Gando-Ferreira, L., Quinta-Ferreira, R.. Biosorbent potential of an eggshell rich composting product for lead removal from aqueous solutions. Submitted to Ecological Engineering, in March 2015.

Soares, M.A.R., Quina, M.J., Gando-Ferreira, L., Quinta-Ferreira, R., 2014. Removal of Pb (II) from aqueous solutions using eggshell composting products, presented at Athens'2014- 2nd International Conference on Sustainable Solid Waste Management, 12-14th June, In Proceedings, Athens, Greece. (*oral communication*).

8.1. Introduction

Industrial processes, mining exploration, manufacturing and disposal of some waste materials are examples of anthropogenic activities that foremost contribute to the build-up of heavy metals in the environment. In particular, the contamination of water bodies with those inorganic pollutants is becoming a severe public health issue and also presents toxic effects to the aquatic ecosystems (Ghaedi et al., 2013).

Amongst the technologies available for metal removal, sorption process has been recommended due to its effectiveness and easy operation (Anbia et al., 2015). However some sorbent materials like activated carbons, zeolites, polymers or silica may be expensive and present high regeneration costs, which constitutes a significant constraint. Thus the quest for low-cost alternative sorbents has received increasing attention (Shaheen et al., 2013).

In fact, feasibility studies addressing waste derived sorbents for heavy metal uptake from aqueous matrices have arisen during the last years. The use of waste materials for sorptive purposes is twofold attractive because they are inexpensive and contribute to environmental protection (Bhatnagar and Sillanpää, 2010). Among others, waste materials derived from non-living biomass like sawdust, crab-shell and citrus peels have been tested as biosorbents to sequester heavy metals from environmental systems (Fu and Wang, 2011; Paduraru et al., 2015).

Eggshell (ES) waste is the main by-product from the egg processing industry. Some studies addressing the use of ES as a biosorbent for heavy metals uptake from contaminated water are reported in the literature (De Paula et al., 2008; Park et al., 2007; Shaheen et al., 2013; Vijayaraghavan and Joshi, 2013). However, European legislation in force considers ES as an animal by-product not intended to human consumption, which requires pre-treatment to reduce pathogen spreading risk. Composting is one of the treatment alternatives foreseen. As a result of ES composting, enriched calcium compost can be obtained for value-added applications, namely for neutralising acidic soils with deficit in calcium, or to remediate metal-contaminated soils. In fact, the presence of carbonates and humic substances in a compost derived from eggshell composting could synergistically contribute to heavy-metal uptake, by pH-dependent mechanisms and due to the presence of negatively charged functional groups associated to humic-like substances (Paradelo and Barral, 2012). To our knowledge, the effect of calcium carbonate rich compost for lead removal from aqueous solution is scarcely addressed in the literature.

This study aims to evaluate the possibility of a mature compost (CES) obtained from industrial eggshell composting with other organic materials, to be further used as biosorbent, enabling an understanding of its relative capacity to sorb Pb(II) ions from aqueous solutions. For comparison, natural industrial eggshell (ES) and mature compost without eggshell (CWES) were also tested. Dynamic behavior of lead sorption onto CES was evaluated from experimental data by fitting kinetic models. In addition, the biosorbent behavior was predicted by using common isotherm models, and the effect of pH and metal concentration were also investigated.

8.2. Materials and methods

8.2.1. Biosorbents characterisation

The composts tested as biosorbents were obtained at laboratory scale by using self-heating reactors of 105 L with forced aeration, as described by Soares et al. (2013). After 25 days of in-vessel composting, the mixtures were allowed to conclude the maturation phase in small piles until 144 days. The starting materials of the composting mixture for CES were industrial potato peel, grass clippings, rice husk and industrial eggshell (ES) waste in the proportions of 35.4%, 22.7%, 11.9% and 30% (w/w), respectively. CWES compost was obtained in the same conditions as CES, but without eggshell in the initial mixture and keeping the relative proportions of the other ingredients. The eggshell was collected in an industry of pasteurized liquid eggs and boiled eggs and the source of the other starting ingredients is described elsewhere (Soares et al., 2013). For comparison with CES and CWES, industrial eggshell (ES) was also tested.

Each biosorbent was obtained by taking a random sample of 25 L from the composting piles (for CES and CWES) or from a 120 L industrial sample of ES. Then, each material was individually homogenized and divided into four parts, with one being eliminated. This procedure was repeated until samples of 250 g were obtained. Finally, biosorbents were air dried, grounded and sieved to a particle size between 25 to 500 µm, for further laboratorial studies.

8.2.1.1. Physical and chemical properties

The specific surface area (S_{BET}) and total pore volume of the biosorbents were quantified by applying the BET (Brunauer–Emmett–Teller) model to nitrogen adsorption data, obtained through the ASAP 2000 equipment (Micromeritics Co., USA). The moisture content was determined as the loss in weight, after samples were dried in an oven at 105ºC to constant weight. pH and electrical conductivity (EC) were measured in water extracts obtained with a solid to liquid ratio of 1:5 (v/v) for 1 h. pH was measured directly in suspensions, but in the case of EC, a filtration was performed and the contribution of water, glassware and filter paper (blank essay) was subtracted.

The calcium carbonate content, organic matter and nitrogen level were determined as detailed in section 6.2.3.

Metals (Cd, Cr, Cu, Pb, Zn) and Ca content was quantified by flame atomic absorption spectrometry (FAAS), Perkin Elmer – 3000, after *aqua regia* extraction, as detailed by Rauret et al. (2000).

The chemical functional groups of the tested biosorbents were characterized by Fourier transform infrared spectroscopy (FTIR) (Jasco FT/IR-4200). FTIR spectra were acquired by averaging 32 scans in the range of 400-4000 cm^{-1}, resolution of 4 cm^{-1} and scan speed of 2 mm s^{-1}. Dried samples (2 mg) were pressed into pellets together with KBr (200 mg). The biosorbent surface was analyzed before and after sorption by scanning electron microscopy (SEM, Phenom ProX) coupled with energy dispersive X-ray analysis (EDX).

8.2.1.2. pH at the point of zero charge and acid neutralisation capacity

The quantification of the pH at the point of zero charge (pH_{pzc}) associated to each biosorbent followed the immersion technique described by Fiol and Villaescusa (2009). This technique is based on the admission that surface groups of the biosorbent can dissociate or associate H^+ depending on the properties of the biosorbent and the pH of the aqueous solution. Briefly, each solid was placed in contact with several solutions of KNO_3 (0.03 M) with an initial pH ranging from 2.3 to 11. The pH values of KNO_3 solutions were adjusted using 0.1 M HNO_3 or 0.1 M NaOH. The suspensions with a liquid to solid ratio of (L/S) 100 L kg^{-1} were agitated during 24 h in an overhead shaker (16 rpm), at 25±2ºC and the final pH values of the supernatant liquid were measured. The pH_{pzc} corresponds to the plateau obtained in the plot of final pH against initial pH (Lambert et al., 2009).

The acid neutralisation capacity (ANC) of the tested biosorbents was determined by adapting the procedure described by Quina et al., (2009). Thus, at least ten acidic solutions (V=30 mL) with different volumes of 2 M HNO_3 and distilled water were added to the biosorbents, to form a suspension with L/S 10 L kg^{-1}. The suspensions were agitated during 24 h in an overhead shaker (16 rpm), at 25±2ºC and the final pH value of the supernatant liquid was measured. The ANC of each biosorbent was calculated according to the following equation:

$$ANC(meq\ g^{-1}) = \frac{C_{HNO_3} \times V_{HNO3}}{m} \tag{8.1}$$

where, C_{HNO_3} (eq-g L^{-1}) is the concentration of the used acid, V_{HNO_3} (mL) the acid volume used to prepared the acidic solution, and m the mass of the biosorbent (g).

8.2.2. Sorption studies

The affinity of the tested biosorbents towards Pb(II) was assessed by a set of experiments described in the following sections.

8.2.2.1. Kinetic studies

For establishing the solid-liquid contact period required to attain equilibrium conditions, kinetic sorption tests were performed. Experiments were carried out by putting the biosorbent in contact with 500 mg L^{-1} of Pb (L/S 100 L kg^{-1}). Suspensions were continuously agitated at 25±2ºC and the pH was controlled at 5±0.5 by addition of standard solutions (0.01, 0.2 and 0.5 M) of HNO_3 or NaOH as required. A set of samples were taken at predefined times and filtered with 1 µm pore size filter. The concentration of Pb was determined by flame atomic absorption spectrometry (Perkin Elmer – 3300). These experiments were performed at pH 5±0.5 to prevent lead hydroxide precipitation. According to data obtained through Visual Minteq 3.0 (represented in Figure S8.1 - Supplementary material), for high concentrations of Pb(II) (e.g. 2500 mg L^{-1}) and pH between 5.5 and 13, $Pb(OH)_2$ is a controlling solid phase.

Three kinetic models were considered to fit the experimental data. The pseudo-first order model is based on the Lagergren´s equation and it considers that the rate of occupation of binding sites is proportional to the number of unoccupied sites present in the biosorbent according to the following equation (Ho et al., 2000):

$$\frac{dq_t}{dt} = k_1(q_e - q_t) \tag{8.2}$$

where q_t (mg g^{-1}) and q_e (mg g^{-1}) are the sorption at any time t and equilibrium sorption capacity, respectively and k_1 (min^{-1}) is the sorption constant rate. Integrating Eq.(8.2) considering the lower and upper limits of t=0 to t=t and q_t=0 to q_t=q_t:

$$q_t = q_e(1 - e^{-k_1 t}) \tag{8.3}$$

The pseudo-second-order model takes into consideration that the sorption mechanism follows a second-order reaction, that depends upon the amount of sorbate on the solid surface and the amount of sorbate sorbed at equilibrium conditions (Ho and McKay, 2000) according to:

$$\frac{dq_t}{dt} = k_2(q_e - q_t)^2 \tag{8.4}$$

where k_2 (g mg^{-1} min^{-1}) is the rate constant of pseudo-second-order sorption. By integrating Eq. (4) for t=0 to t=t and q_t=0 to q_t=q_t, the Eq. (8.5) is obtained:

$$q = \frac{q_e^2 k_2 t}{1+k_2 q_e t} \tag{8.5}$$

A kinetic model that considers intraparticle diffusion during sorption may be as follows (Ho et al., 2000):

$$q = q_e \times \left[1 - \frac{6}{\pi^2}\sum_{n=1}^{\infty}\frac{1}{n^2} exp(-n^2 B.t)\right] \tag{8.6}$$

Where n is the integer used to define the infinite series, B (min^{-1}) is the model parameter defined as $B = \frac{D_i \pi^2}{r^2}$, D_i is the diffusion coefficient of sorbate within the biosorbent (cm^2 min^{-1}) and r the radius of solid particle (cm).

8.2.2.2. Equilibrium studies

Biosorption equilibrium experiments were carried out in batch and agitated mode. The initial Pb concentrations ranged from 100 to1500 mg L^{-1} for CES and ES biosorbents and from 300 to 2500 mg L^{-1} for CWES. All solutions were prepared from a stock solution of 3000 mg Pb L^{-1}, by performing the adequate dilutions. Operating conditions for equilibrium experiments were equal to the ones imposed in the kinetic studies: L/S 100 L kg^{-1}, 25 ± 2ºC and pH 5±0.5.

For describing the equilibrium data three models were tested. The Freundlich equation is an empirical model originally proposed for heterogeneous sorbents:

$$q_e = K_F C_e^{1/n} \tag{8.7}$$

where K_F (mg$^{1-1/n)}$ g^{-1} L$^{1/n}$) is the Freundlich isotherm constant, C_e (mg L^{-1}) the equilibrium concentration of the sorbate, q_e (mg g^{-1}) the amount of sorbate sorbed per mass of biosorbent at equilibrium conditions, and n is a measure of the sorption intensity. This model considers the possibility of multilayer sorption and each sorption site presents a specific binding energy (Foo and Hameed, 2010).

The Langmuir isotherm is defined by (Foo and Hameed, 2010):

$$q_e = \frac{q_m K_L C_e}{1+K_L C_e} \tag{8.8}$$

where K_L (L mg^{-1}) is the Langmuir constant and q_m (mg g^{-1}) is the theoretical maximum adsorption capacity. This model assumes that sorption is monolayer and takes place at specific homogeneous sites, all of them identical and energetically equivalent.

The Langmuir-Freundlich isotherm combines in one equation the individual features of Langmuir isotherm and Freundlich model (Foo and Hameed, 2010):

$$q_e = \frac{q_m K_{LF} C_e^{1/n_F}}{1 + K_{LF} C_e^{1/n_F}} \tag{8.9}$$

where q_m (mg/g) is the theoretical maximum adsorption capacity, as K_{LF} (L mg^{-1})$^{1/nF}$ and n_F are the model parameters. At high sorbate concentrations the model predicts a monolayer sorption capacity as considered by the Langmuir model, while at low concentrations the Langmuir-Freundlich isotherm is reduced to the Freundlich equation (Foo and Hameed, 2010).

8.2.2.3. Effect of pH on metal sorption

To evaluate the influence of the initial pH on the distribution of Pb (II) between the aqueous phase and the biosorbent, equilibrium tests were performed at a constant Pb (II) concentration of 500 mg L^{-1}, within an initial pH range of 2 and 5.5. The initial pH of each solution was adjusted by adding 0.5 M HNO_3 or 0.5 N NaOH, as required. The biosorbents were tested at L/S 100 L kg^{-1}, 25 ± 2ºC, under agitation, but without pH control. In equilibrium condition, pH of the suspensions was measured and Pb(II) concentration in filtered extracts was also assessed by FAAS.

8.2.3. Calculation of lead distribution between aqueous phase and biosorbent

The amount of Pb sorbed by unit mass of biosorbent (q, in mg g^{-1}) was calculated according to equation:

$$q = \frac{(C_0 - C) \times V}{m} \tag{8.10}$$

where C_0 (mg L^{-1}) is the initial aqueous concentration of Pb, m (g) the amount of biosorbent used in each test, V (L) the volume of aqueous phase in contact with each biosorbent, and C (mg L^{-1}) the aqueous phase concentration of Pb at any time (kinetic studies) or at equilibrium conditions. The percentage of lead sorbed by each biosorbent (%Ads) was calculated according to Eq.(8.11):

$$\% Ads = \frac{(C_0 - C)}{C_0} \times 100 \tag{8.11}$$

The distribution coefficient (L kg^{-1}) was calculated as followed (Shaheen et al., 2013):

$$K_d = \frac{q_e}{C_e} \times 1000 \tag{8.12}$$

8.2.4. Statistical analysis

The parameters of the models used in this study were calculated by non-linear regression, by using Sigma-plot 10 and Matlab softwares. To evaluate the fitting of the sorption models to the experimental data, two commonly used error functions were considered:

Residual root mean square error: $$RMSE = \sqrt{\frac{1}{n-2}\sum_{i=1}^{n}\left(q_{exp} - q_{model}\right)_i^2} \tag{8.13}$$

R^2 adjusted: $$R^2_{Adj} = 1 - \frac{\sum_{i=1}^{n}\left(q_{model} - q_{exp}\right)_i^2 / n-p}{\sum_{i=1}^{n}\left(q_{exp} - \overline{q_{exp}}\right)_i^2 / n-1} \tag{8.14}$$

where q_{model}, q_{exp} and $\overline{q_{exp}}$ (mg g^{-1}) represent the model prediction, experimental value and mean of all experimental values, respectively; n is the number of observations during experiments and p the number of parameters of the model. The smaller value of RMSE and the higher values and R^2_{adj} indicate the best model fitting.

8.3. Results and discussion

8.3.1. Biosorbent properties

Table 8.1 addresses the main properties of the sorbents tested. The biosorbents obtained through composting are characterized by alkaline pH, phytotoxin-free (germination indices close to 80%) and stable (moderately slow respiration rates between 2-8 mg CO_2 g^{-1} C d^{-1}, (Gómez et al., 2006). Industrial ES is also an alkaline material, with high content of $CaCO_3$ and low percentage of organic matter (close to 6% (w/w)). The total heavy metals concentration in CWES, CES and ES is low in comparison to the levels present in other biosorbents obtained by composting of source separated municipal solid waste mixed with green waste (50 mg kg^{-1}, 62 mg kg^{-1} and 200 mg kg^{-1} for Cu, Pb and Zn, respectively) (Paradelo and Barral, 2012).

Regarding the specific surface area (S_{BET}), there is a significant difference between the tested biosorbents. CWES has the highest S_{BET} (0.74 m^2 g^{-1}) and total pore volume (4.67 µL g^{-1}), while ES is the material with lower surface area (0.12 m^2 g^{-1}) and less pore volume (0.98 µL g^{-1}). Both S_{BET} and total pore volume recorded for CWES are similar to other reported values for garden organic composts (S_{BET}=0.66 to 1.31 m^2 g^{-1} and pore volume of 2.6 to 8 µL g^{-1}) (Al-

Mashaqbeh and McLaughlan, 2014, 2012) and composted cotton burr (S_{BET}=0.86 m^2 g^{-1}) (Su and Puls, 2007).

Table 8.1: Main properties of the biosorbents tested (mean±standard deviation).

	Biosorbent		
Parameters	CWES	CES	ES
S_{BET} (m^2 g^{-1})	0.74	0.42	0.12
Total pore volume (µL g^{-1})	4.67	3.00	0.98
pH	9.3	8.9	8.3
Equivalent $CaCO_3$ (g $CaCO_3$ 100 g $^{-1}$)	5.0±0.1	61.4±1.7	89.4±0.2
Organic matter (%)	79.4±0.5	28.5± 0.3	6.3± 0.1
TOC/TN	21	11.9	2.1
Respiration rate (mg C-CO_2 g^{-1}C d^{-1})	5.31±1.1	3.55±0.2	-
Pb *aqua regia* (mg kg^{-1})	12.7±0.01	7.3±0.02	3.55±0.02
Cu *aqua regia* (mg kg^{-1})	8.2±0.2	5.2±0.2	-
Zn *aqua regia* (mg kg^{-1})	47±0.3	11.9±0.1	4.95±0.1

CES: compost with industrial eggshell; CWES: compost without industrial eggshell; ES: industrial eggshell waste; S_{BET}: surface area; TN: total nitrogen; TOC: total organic carbon; concentration units are presented in a dry weight basis.

On the other hand, the values of the ES physical properties found in this study are clearly lower that the ones reported in the literature (S_{BET}=0.49 to 21.2 m^2 g^{-1} and pore volume of 4 to 86 µL g^{-1}) (Tsai et al., 2008). Nevertheless, those literature values are referred to eggshell stripped from its membrane and washed, which was not the preparation method followed for ES in our study. Concerning CES physical properties, clearly this composting end-product presented intermediate values for S_{BET} and pore volume in comparison to CWES and ES.

The major functional groups of the biosorbents were identified using FTIR spectroscopy. FTIR spectrum of the tested biosorbents are represented in Figure S8.2 - Supplementary material. FTIR spectrum of CES is characterized by peaks at 2515, 1800, 1420 and 875 cm^{-1}, which are ascribed to carbonates (Smidt and Meissl, 2007) conveyed to CES by eggshell. A broad and intense absorption peak around 3400 cm^{-1} is indicative of the existence of bounded hydroxyl groups. The peak near 1080 cm^{-1} can be attributed to Si-O-Si stretching vibrations in silica (Grube et al., 2006) which is typically present in rice husk (Yalçin and Sevinç, 2001) that were used for obtaining CES. Nevertheless, peaks in the range of 1030-1080 cm^{-1}, may also be attributed to C–O stretching of polysaccharides or polysaccharide-like substances (Smidt and Meissl, 2007). In addition, low intensity peaks at 1740 $cm^{-1}$and 2930-2850 cm^{-1} are characteristic of C=O vibrations (aldehydes, carboxylic acids or esters) and aliphatic compounds, respectively (Smidt and Meissl, 2007).

Since CES was derived from composting processes, FTIR spectrum should present a band at 1650 cm^{-1} and a weak band at 1595 cm^{-1} as result of humic like substances. However in this case, the strong and broad peak at 1420 cm^{-1} (due to carbonates) alters the spectrum resolution in that region. As a result, it is only visible a shoulder between 1650-1600 cm^{-1}, which may still be related to C=C stretching vibrations of aromatic structures in humic substances and/or amide groups. FTIR spectrum of ES shows the typical peak attributed to carbonates, as found is CES and a slight shoulder near 1650-1600 cm^{-1} that might be associated to C=O stretching in amides groups that constitute the organic membrane typically present in eggshell. Regarding CWES, the identification of functional groups is more complex. Significant peaks are found at 3400 cm^{-1} (associated to -OH groups), 2920 cm^{-1} (associated to aliphatic methylene groups), 1650 cm^{-1} (C=C stretching that can be ascribed to aromatic C–C bond), 1515 cm^{-1} (C=C stretching of aromatic rings in lignin), 1080 cm^{-1} (silica or polysaccharide-like substances). In addition, the shoulder at 1595 cm^{-1} reveals the presence of humic like substances due to C=C bond in the aromatic skeleton of that substances (Smidt and Meissl, 2007). Peaks at 1220 and 1420 cm^{-1} indicate phenolic groups, while the band at 1380 cm^{-1} is due to COO^{-} antisymmetric stretching.

8.3.2. pH$_{pzc}$ and acid neutralisation capacity

The point of zero charge corresponds to the pH of the aqueous solution at which the particle surface charge is neutral (Lambert et al., 2009). This means that if the aqueous pH is higher than pH$_{pzc}$, the surface charge is negative and the biosorbent has enhanced affinity for cations. By contrast, for aqueous solutions at pH less than pH$_{pzc}$, the solid has higher affinity towards anions. Figure 8.1 shows the point of zero charge (pH$_{pzc}$) of the studied biosorbents, at L/S 100 L kg^{-1}.

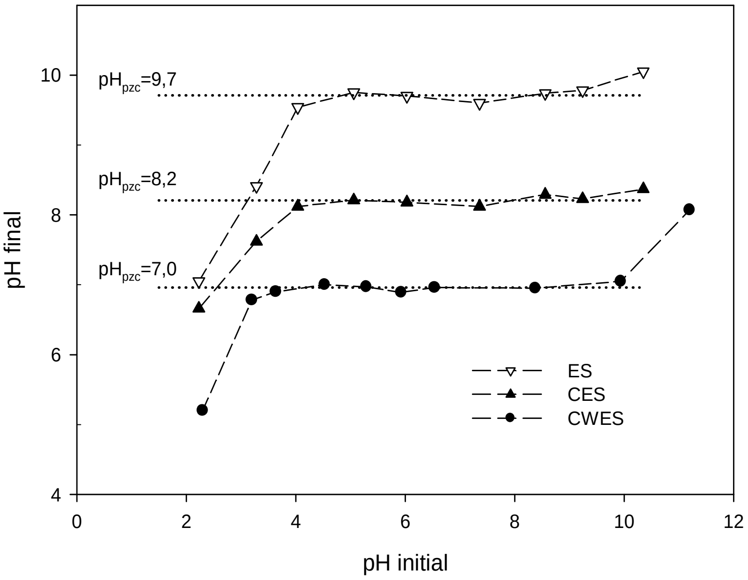

Figure 8.1: Determination of pH$_{pzc}$ at L/S 100 L kg^{-1} for tested biosorbents (ES, CES and CWES).

The point of zero charge of CES is observed to pH 8.2 (Figure 8.1), and it is strongly influenced by the presence of eggshell in this material, which explains the elevated value in comparison to CWES (pH_{pzc}=7). Above pH=8.2, CES exhibits a net negative surface charge that enhances electrostatic attractions between Pb^{2+} and CES surface. ES is also characterized by a high pH_{pzc} (equal to 9.7) which is within the values reported in the literature for commercial $CaCO_3$ and calcite (8.9 and 10.1, respectively) (Kosmulski, 2011, 2009).

The quantification of ANC for the biosorbents is shown in Figure 8.2. This property corresponds to the ability of a material to neutralise acids, due to any species that can accept and neutralise protons (Quina et al., 2009) and reflects the overall buffering capacity of the material against acidification.

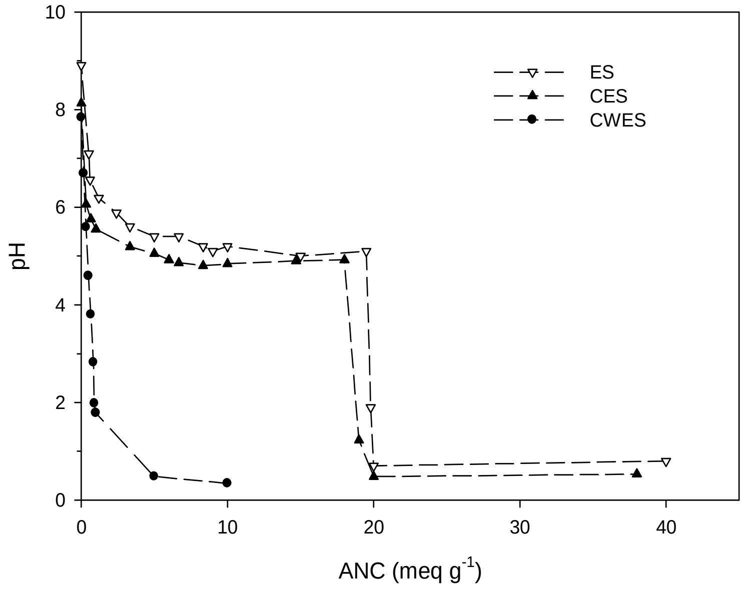

Figure 8.2: Determination of ANC for tested biosorbents (ES, CES and CWES).

ANC curves for CES and ES are very similar, presenting a steep initial decline to pH near 6 followed by a plateau at pH=5-5.5. This plateau may be associated to the buffering effect induced by the calcium carbonate in the biosorbents (Chen et al., 2009). For CWES, the ANC profile shows a sharper negative slope from pH≈8 until pH≈2, which is related to the weak ability of the biosorbent to resist to pH changes. In quantitative terms, the ANC was measured in miliequivalents of acid required to reduce pH until a reference value (4 and/or 7). For the biosorbents under analysis, ANC_{pH7} is very similar for CWES and CES (0.16 meq g^{-1}) and slightly higher for ES (0.52 meq g^{-1}). In the case of ANC_{pH4}, ES still shows the more pronounced value (19.7 meq g^{-1}) followed by CES and CWES with 18.2 and 0.64 meq g^{-1}, respectively. To the best of our knowledge, there are no studies addressing the ANC for biosorbents. Therefore, for the sake of comparison, air pollution control residues from municipal solid waste incineration were taken into consideration. These residues are considered to have a considerable ANC (ANC_{pH4} ≈11 meq g^{-1} (Quina et al., 2009)), which strengthen the idea that CES and ES present a high ANC.

8.3.3. Kinetic studies

Figure 8.3 depicts the kinetic behavior of Pb uptake by the biosorbents tested, when the initial concentration was 500 mg L^{-1}. The kinetic experimental data followed a biphasic evolution (Zhang, 2011), including a fast initial stage followed by a slower equilibrium period. For CES, 80% of the maximum lead uptake capacity was attained in the first 30 min. In opposition, ES accounted only for 61%, while CWES achieved 94%. The equilibrium state was considered to be reached when changes in q are less than 5% during more than 5 h. Thus, in the present study, for all tested biosorbents 180 min were enough to reach equilibrium conditions. Therefore, further studies for the establishment of sorption lead uptake behavior were conducted for 180 min of equilibrium time.

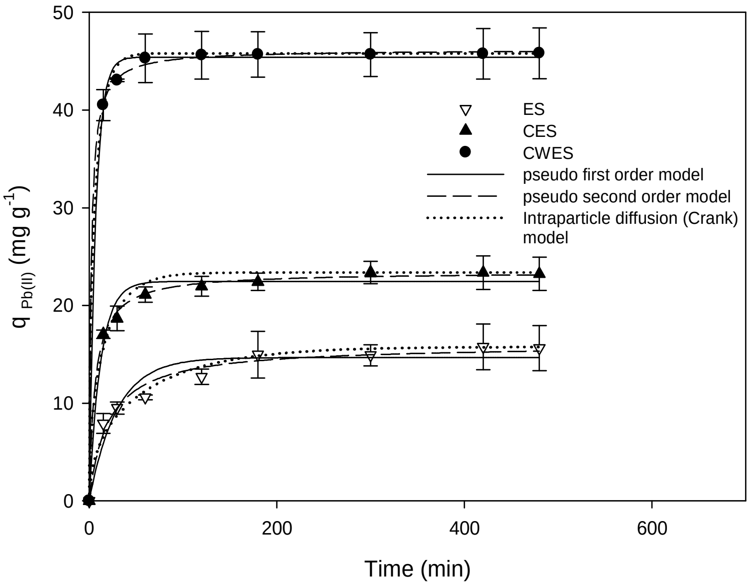

Figure 8.3: Kinetic behavior of Pb(II) uptake by CES, ES and CWES (L/S 100 L kg^{-1}, $[Pb(II)]_{initial}$ = 500 mg L^{-1}, 25°C, pH=5.0).

In previous works, other authors assumed similar equilibration period for Pb uptake by eggshell (Vijayaraghavan and Joshi, 2013), but longer contact time was considered by Zhang (2011) for sorption studies using manure compost (4 h) and by Paradelo and Barral (2012) in the case of municipal solid waste compost (16 h).

Three different kinetic models (pseudo-first, pseudo-second and intraparticle diffusion models) were used to describe the experimental data of Pb uptake by CES, ES and CWES and to assess the kinetic mechanism. According to a literature review conducted by Liu and Liu (2008) both pseudo-first and pseudo-second order models have been widely used to describe kinetic biosorption data by considering sorption mechanism controlled by surface reactions. On the other hand, the intraparticle diffusion model accounts for diffusion controlled mechanisms during sorption (Cheung et al., 2007). The fitting of the models to experimental data is shown in Figure 8.3 and Table 8.2.

Table 8.2: Kinetic model parameters for Pb(II) sorption onto CES, ES and CWES.

Biosorbent	Model type	Model parameters	Error functions
CES	Pseudo-first order Eq (8.3)	q_e=22.46 mg g^{-1} k_1=0.082 min^{-1}	RMSE= 1.118 R_{adj}^2=0.978
	Pseudo-second order Eq (8.5)	q_e=23.43 mg g^{-1} k_2= 0.678 x 10^{-2} g mg^{-1} min^{-1}	RMSE= 0.418 R_{adj}^2=0.997
	Intraparticle diffusion E q(8.6)	B=0.043 min^{-1}	RMSE= 0.980 R_{adj}^2=0.985
ES	Pseudo-first order Eq (8.3)	q_e=14.66 mg g^{-1} k_1=0.034 R_{adj}^2=0.923min^{-1}	RMSE= 0.932 R_{adj}^2=0.923
	Pseudo-second order Eq (8.5)	q_e=15.97 mg g^{-1} k_2= 0.307 x 10^{-2} g mg^{-1} min^{-1}	RMSE= 0.823 R_{adj}^2=0.977
	Intraparticle diffusion Eq (8.6)	B=0.013 min^{-1}	RMSE= 0.796 R_{adj}^2=0.979
CWES	Pseudo-first order Eq (8.3)	q_e=45.42 mg g^{-1} k_1=0.143 min^{-1}	RMSE= 0.729 R_{adj}^2=0.998
	Pseudo-second order Eq (8.5)	q_e=46.19 mg g^{-1} k_2= 1.050 x 10^{-2} g mg^{-1} min^{-1}	RMSE= 0.295 R_{adj}^2=0.999
	Intraparticle diffusion Eq (8.6)	B=0.130 min^{-1}	RMSE= 0.651 R_{adj}^2=0.998

Lead uptake by CES was adequately described by the pseudo-second order model (higher $R_{adj}\ ^2$, lower RMSE, Table 8.2), though the other considered kinetic models also presented good fittings parameters. This model is based on the premise that the rate limiting step is chemisorption (chemical interactions between superficial functional groups of biosorbent and metal ion), that includes valence forces with the exchange of ions or the formation of covalent bonds (Ho and McKay, 2000). CWES kinetic data also showed a very good agreement with the pseudo-second order model (Table 8.2). However, CWES has higher initial sorption rate ($k_2q_e^2$) in comparison to CES (22.39 and 3.72 mg g^{-1} min^{-1}, respectively) which might be related to the higher S_{BET} of CWES (Table 8.1) and the greater diversity of functional groups in the biosorbent surface. The suitability of the pseudo-second order model for CES and CWES biosorbents is in agreement with the results of other studies for compost-like biosorbents (Al-Mashaqbeh and McLaughlan, 2014; Zhang, 2011).

Regarding ES, an acceptable fitting was provided by both pseudo-second order and intraparticle diffusion models (Table 8.2). Nevertheless, other studies indicated pseudo-first order model as adequate to describe the kinetic data of lead uptake by eggshell (Vijayaraghavan and Joshi, 2013).

In spite of that, one must have in mind that different mathematical models can fit a limited number of experimental data with a high degree of accuracy (Ho et al., 2000), and so the fitting goodness may not reveal the nature of the sorption mechanisms involved. In addition, different sorption mechanisms may occur concurrently due to the complexity of the biosorbents, namely chemical functional groups, surface area, etc. Thus, further studies are necessary to evaluate other sorption variables such as initial concentration, particle size, temperature, to provide additional information regarding i) confirmation of the sorption mechanism for CES and CWES and ii) sorption mechanism identification for ES.

8.3.4. Sorption studies

Sorption isotherms are models that describe the equilibrium between a solute sorbed in a solid phase and the soluble concentration of that solute in an aqueous medium, for a specific temperature. These equations contain parameters that express the surface properties and affinity of the biosorbent at given operating conditions. Several types of isotherm have been applied to experimental data of metals into biosorbents, namely Langmuir and Freundlich models have been widely used (Rangabhashiyam, 2014). A number of approaches have been suggested to facilitate the choice of a model to describe the experimental data. Specifically, Hinz (2001) presented a set of guidelines to identify the class and subgroup of isotherms (according to Giles classification) to select the most appropriate model.

Figure 8.4 depicts the results from equilibrium sorption experiments for CES, ES and CWES (symbols in Figure 8.4a), b) and c), respectively). By following the guidelines of Hinz (2001), the data in Figure 8.4 follow a high affinity sorption model, classified as H2-class isotherm (class H, subgroup 2), which is characterised by high uptake at low metal concentrations and by a relevant steep slope that suddenly reaches a plateau. In this study, three isotherms were fitted to sorption data: Langmuir, Freundlich and Langmuir-Freundlich (Eq. 8.7, 8.8 and 8.9). The first two models (with two parameters) are the simplest and the most widely used, while the three parameter Langmuir-Freundlich isotherm is specific for H2 isotherms (Hinz, 2001). Table 8.3 summarises isotherm parameters obtained for non-linear fitting and the respective error functions as well.

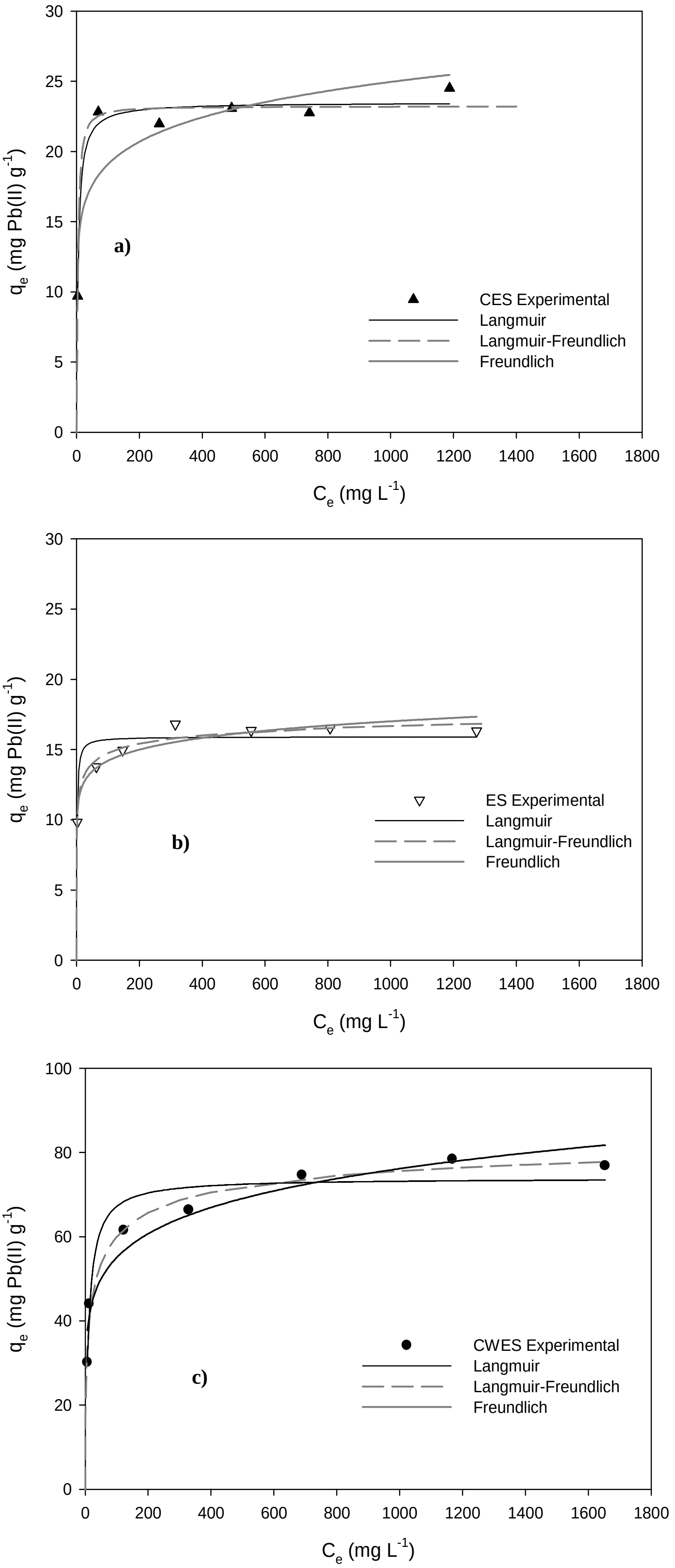

Figure 8.4: Equilibrium isotherms sorption of lead onto a) CES; b) ES and c) CWES (L/S 100 L kg^{-1}, 25ºC, pH 5.0, contact time 180 min).

CES and ES revealed q_m of 24 mg Pb(II) g^{-1} and 16 mg Pb(II) g^{-1}, and if expressed in terms of $CaCO_3$ equivalent mass, the capacities are 39 mg Pb(II) $g^{-1}{}_{CaCO3}$ and 18 mg Pb(II) $g^{-1}{}_{CaCO3}$, respectively (Figure 8.4 a) and b)). Such difference can only be attributed to the presence of the organic matter, which contributed to a higher surface area of CES (Table 8.1) and to oxygenated functional groups commonly found in humified compost, that increase the active sites due to negative charges density of the biosorbent. In comparison to other biosorbents derived from composting, Table 8.4 shows that CES is less efficient but comparable with other organic biosorbents like peat or marine green macroalgae. CWES is the biosorbent with higher organic content, featuring a maximum capacity, q_m, near to 80 mg Pb(II) g^{-1} (Figure 8.4 c)), as a consequence of the negative charged functional groups associated to humic substances and of the higher S_{BET}. The sorption capacity of CWES is comparable to other composts with different origin and composition (Table 8.4).

Table 8.3: Parameters of lead sorption isotherm models onto CES, ES and CWES biosorbents.

Biosorbent	Model type	Model parameters	Error functions
CES	Langmuir Eq. (8.8)	q_m=23.50 mg g^{-1}	RMSE= 0.942
		K_L=0.207 L mg^{-1}	R_{adj}^2=0.97
	Freundlich Eq.(8.7)	K_F=11.17 L mg^{-1}	RMSE= 2.911
		n_F=8.6	R_{adj}^2=0.72
	Langmuir-Freundlich Eq. (8.9)	q_m=23.21 mg g^{-1}	RMSE= 1.036
		K_{LF}=0.145 L mg^{-1}	R_{adj}^2=0.97
		n_F=0.78	
ES	Langmuir Eq. (8.8)	q_m=15.91 mg g^{-1}	RMSE= 1.078
		K_L=0.801 L mg^{-1}	R_{adj}^2=0.81
	Freundlich Eq.(8.7)	K_F=9.899 L mg^{-1}	RMSE= 0.798
		n_F=12.76	R_{adj}^2=0.90
	Langmuir-Freundlich Eq. (8.9)	q_m=19.23 mg g^{-1}	RMSE= 0.623
		K_{LF}=0.845 L mg^{-1}	R_{adj}^2=0.94
		n_F= 3.38	
CWES	Langmuir Eq. (8.8)	q_m= 73.94 mg g^{-1}	RMSE= 4.492
		K_L= 0.098 L mg^{-}	R_{adj}^2=0.98
	Freundlich Eq.(8.7)	K_F= 28.49 L mg^{-1}	RMSE= 4.675
		n_F= 7.02	R_{adj}^2=0.97
	Langmuir-Freundlich Eq. (8.9)	q_m= 86.81 mg g^{-1}	RMSE= 3.101
		K_{LF}= 0.244 L mg^{-1}	R_{adj}^2=0.99
		n_F= 2.08	

Langmuir isotherm best describes the Pb(II) sorption behavior on CES (lower RMSE, Table 8.3) while Langmuir-Freundlich model is more suitable for ES and CWES (lower RMSE and R^2_{adj} –Table 8.3). According to the literature, the Langmuir equation best fits the sorption of Pb(II) onto calcium carbonate like materials (Guru and Dash, 2014), which presumes that interactions between Ca(II) and Pb(II) may occur at the sorbent surface (monolayer sorption), but the Toth model (three parameters model) is more adequate to eggshell (Vijayaraghavan and Joshi, 2013).

By using scanning electron microscopy (SEM) coupled with energy dispersive X-ray spectra (EDX) the chemical composition of the biosorbents surface was analysed, before and after sorption (Figure S8.3 to S8.5 – Supplementary data).

Table 8.4: Sorption capacity of several biosorbents for Pb(II).

Biosorbent	Batch operating conditions				Maximum Pb uptake (mg g^{-1})	Reference
	pH	T(ºC)	L/S (L kg $^{-1}$)	Pb(II) (mg L^{-1})		
Manure Compost	4	25	66.7	N_{ind}	95	(Zhang, 2011)
Green waste compost	N_c	N_{ind}	500	310-1036	85	(Nwachukwu and Pulford, 2008)
Compost from recycled materials and organic fertilizers	N_c	N_{ind}	500	310-1036	59	(Nwachukwu and Pulford, 2008)
Nopal cladodes	5	N_{ind}	400	10-50	13	(Miretzky et al., 2008)
Peat	6	20	400	20-120	28.5	(Ho, 2006)
Marine green macroalgae (*Caulerpa lentillifera*)	5	21	60	N_{ind}	29	(Pavasant et al., 2006)
Marula seed husk	5	20	62.5	10-80	20	(Moyo et al., 2015)
CES	5	25	100	100-1500	23	This study
CWES	5	25	100	300-2500	77	This study
ES	5	25	100	100-1500	16.5	This study

N_c- Not controlled; N_{ind}- Not indicated

The EDX spectrum taken in spot profile for CES, confirmed as expected the presence of Ca before sorption due to calcium carbonate., A strong peak of Pb and a minor peak intensity of Ca (in comparison to the one before sorption) were observed after sorption. That might be a result of i) occupation of Ca-sites by Pb ions at the surface of CES, which has been indicated for calcite (Sturchio et al., 1997), ii) the acidic conditions (pH 5) imposed during sorption experiment may lead to Ca leaching from the surface and incorporation of lead in carbonate-based microprecipitates, as suggested by Vijayaraghavan and Joshi (2013). In addition, Pb(II) may interact with negatively charged functional groups like carboxylic acids, hydroxyl existing on CES, which might be reflected by the small reduction of oxygen content in CES surface. The complexation of Pb with carbonyl groups has been reported in the literature (Ahmad et al., 2012; Guru and Dash, 2014) for $CaCO_3$ like materials and could occurred in CES. Indeed, due to the complexity of CES in terms of organic and inorganic constituents, the above sorption mechanisms may act simultaneously. EDX spectra for ES before and after sorption display a behavior similar to CES. The same sorption mechanisms considered for CES may be associated to ES, by considering that ES contains an organic membrane with oxygenate groups with affinity towards Pb ions.

Regarding CWES, EDX spectrum showed a high diversity of elements at the surface, but oxygen appears in a high percentage (60%). After sorption, the percentage of oxygen was halved and an increase of Pb in CWES surface was detected. This might indicate interactions between lead and oxygen-based functional groups, like reported in other studies with composting derived materials (Carrasquero-Durán and Flores, 2009). Furthermore, there is a significant reduction of potassium in CWES surface, which may be indicative of an ion-exchange mechanism as reported by Zhang (2011) for manure compost.

8.3.4.1. pH influence

The pH is an important parameter in sorption processes, not only due to the ability to alter the surface properties (surface charge, degree of ionization and speciation of the surface functional groups), but also because it dictates the speciation and availability of metals in solution (Ozer, 2007). In this study, the effect of pH on the sorption of Pb (II) by CES, ES and CWES was evaluated by varying the initial pH of a 500 mg Pb(II) L^{-1} solution from 2 to 5.5, and keeping constant the other operating conditions. Figure 8.5 and Figure 8.6 illustrates the effect of the initial pH on the equilibrium pH attained for the aqueous solution, and on the Pb (II) removal.

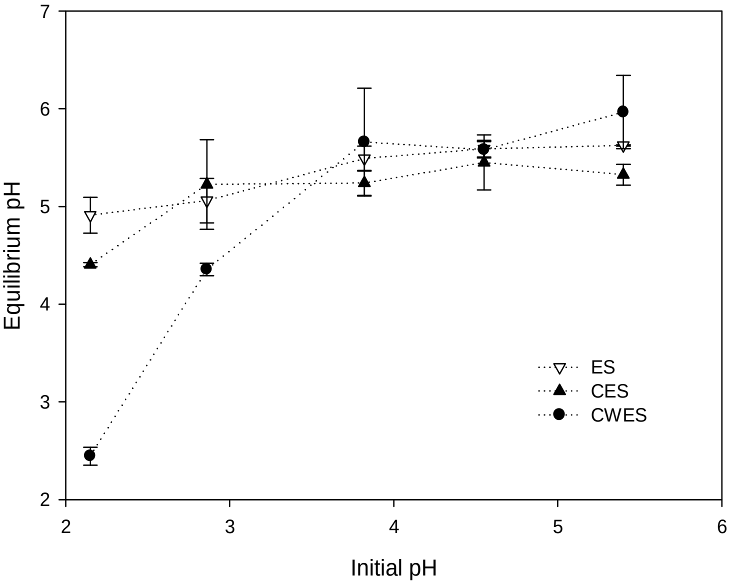

Figure 8.5: Effect of initial pH on equilibrium pH (initial concentration 500 mg Pb(II) L^{-1}, L/S 100 L kg^{-1}, contact time 180 min, 25ºC).

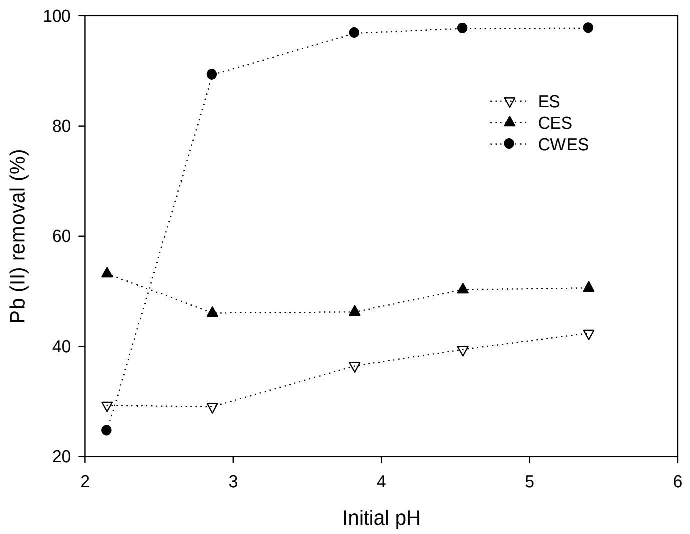

Figure 8.6: Effect of initial pH on lead removal (initial concentration 500 mg Pb(II) L^{-1}, L/S 100 L kg^{-1}, contact time 180 min, 25ºC).

The equilibrium pH attained at the end of 180 min is dependent on the type of biosorbent tested (Figure 8.5). For CES and ES, regardless the initial pH, the equilibrium pH was relatively constant (4.5-5.5), which may be attributed to the high ANC of the biosorbents (Figure 8.2)). In the case of CWES, the equilibrium pH is near 5.5 if initial pH is 4 to 5.5, while more acidic conditions (between 2.5 and 4.3) are reached to the initial pH 2 and 3. These results are in agreement with the low ANC observed to CWES.

The percentage of Pb (II) removed by the biosorbents (Figure 8.6) reveals a similar behavior to the equilibrium pH. In fact, CES and ES showed a removal yield of lead almost independent from pH, though the average removal attained were 49% for CES and 35% for ES. These results are related to physical properties of the biosorbents, namely smaller S_{BET} for ES and higher content of organic matter for CES (Table 8.1). Indeed, CES shows an intrinsic ability to naturally enforce a proper pH to the aqueous solution without favoring precipitation of lead hydroxides. Nevertheless, the precipitation mechanism could benefit the overall removal of lead, if the sorptive process was conduct at pH>6, and it could be very interesting in a real application point of view. In addition, higher pH would favor the presence of negative sites in the surface, decreasing repulsions between the biosorbent and Pb(II).

Considering CWES removal efficiency towards Pb(II) (Figure 8.6), the smaller value attained (25%) to pH 2 is a consequence of: i) the low ANC of CWES to overcome acidic conditions (ANC_{pH4}=0.64 meq g^{-1}) and ii) the positive sites that dominate on the surface of the biosorbent which enhances electrostatic repulsion between the surface and Pb(II) ($pH < pH_{pzc}$; pH_{pzc}=7). In addition, the competition effect between H_3O^+ and Pb(II) promoted by the low equilibrium pH (pH 2.5) also has an effect on the relative low removal yield. However, as the equilibrium pH increases, that competition effect for biosorbent active sites is hindered and

Pb(II) removal is increased (Wang et al., 2010). As a result, for CWES the metal removal yield reached 97% for equilibrium pH 5.5.This behavior may suggest that an ion-exchange mechanism may be part of the overall sorption mechanism of Pb(II) by CWES (Wang et al., 2010). Furthermore, higher pH values favor deprotonation of some chemical functional groups (like hydroxyl, phenolic, carboxylic acids) increasing the negative charges of biosorbent (Anastopoulos et al., 2013). Thus, the electrostatic attractions between Pb (II) and CWES may also play a role, when the initial pH led to equilibrium pH near 5.5.

8.3.4.2. Concentration influence

Figure 8.7 illustrates the influence of the initial concentration from 100 to 2500 mg Pb(II) L^{-1} on lead(II) removal for each biosorbent.

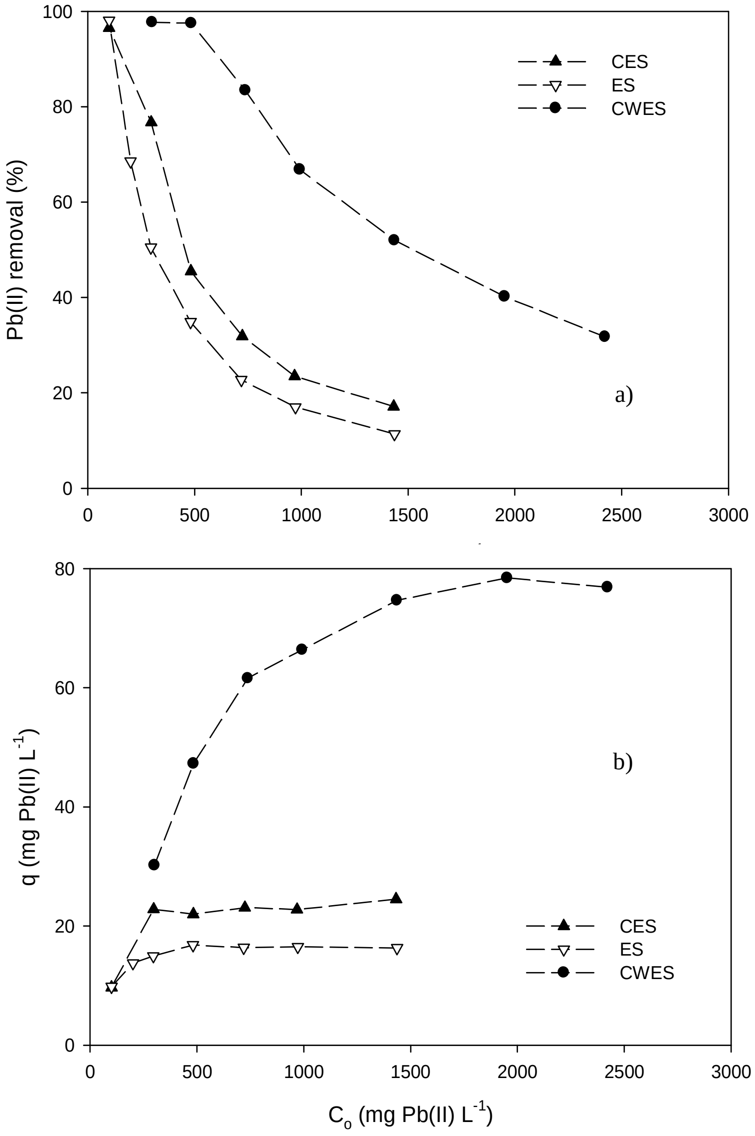

Figure 8.7: Influence of initial concentration of Pb(II) on a) metal removal and b) uptake from aqueous solution (L/S 100 L kg^{-1}, 25°C, pH 5.0, contact time 180 min).

The removal and uptake behavior of Pb(II) for the biosorbents that contain eggshell (CES and ES) is very similar (Figure 8.7 a) and b)). For the lowest initial concentration of 100 mg L^{-1} there is a removal capacity of near 100%, which is sharply inhibited as the initial concentration is enlarged up to 1500 mg L^{-1}. Beyond 500 mg L^{-1}, the sorption capacity of CES and ES attains a plateau (Figure 8.7 b)), which indicates that biosorbent active sites are all occupied. Nevertheless, the CES capacity is in average 1.4 times higher than for ES when metal concentration is greater than 500 mg L^{-1}, which might be a result of the organic matter conveyed by CES.

To the best of our knowledge, the literature is lacking of lead sorption studies in aqueous media by using a biosorbent derived from composting with an equivalent level of $CaCO_3$, as in the case of CES. Nonetheless, some studies have addressed the ES sorption properties towards lead. Namely, Vijayaraghavan and Joshi (2013) studied the sorption of lead by chicken eggshell waste and achieved a removal efficiency of 30.7% (at 25ºC, pH 5 and L/S 500 Lkg^{-1}, C_0=1045 mg L^{-1}) which is almost twice the removal recorded in this study (16%), for the same concentration conditions. In addition, Shaheen et al. (2013) observed a removal efficiency of 99% (at ambient temperature, pH not controlled, L/S 20 L kg^{-1} and C_0=700 mg L^{-1}) while in this study only 22% of Pb(II) was removed from aqueous solution with ES. This difference can be attributed to the lack of pH control during the sorption tests performed by Shaheen et al. (2013), occurring an increase of the pH to alkaline levels due to partial ES dissolution. Accordingly, suitable conditions for the precipitation of Pb(II) hydroxides may lead to a removal enhancement, which masks the quantitative sorptive ability of the biosorbent. At lower lead concentration (100 mg Pb(II) L^{-1}) (Ahmad et al., 2012) found a removal of 89.7% (at 25ºC, pH 5.5 and L/S 40 Lkg^{-1}) which is slightly lower than the removal efficiency measured in this study for the same lead concentration (98%).

With respect to CWES biosorbent the removal observed was close to 100% for a broader range of initial concentrations (300 to 500 mg L^{-1}) (Figure 8.7 a)). Then, a smoother decrease of lead removal is verified when concentration is increased. However, at the highest concentration tested (2500 mg Pb(II) L^{-1}) is still possible to attain a removal efficiency near 30%. The saturation of the active site of CWES occurs when Pb(II) concentration is 2000 mg L^{-1}, which is significantly higher than CES.

To compare the sorptive properties of the biosorbents towards lead ions, the distribution coefficient K_d (L kg^{-1}) was calculated. This parameter relates the metal concentration in the solid phase to its concentration in solution, at equilibrium conditions (Ahmad et al., 2012; Shaheen et al., 2013). Low values of K_d indicate that high amount of metal remains in solution, while high

K_d means that the solid phase has high affinity to the metal. Table 8.5 summarises the K_d for CES, ES and CWES according to the initial lead concentration.

Table 8.5: Distribution coefficient (L kg^{-1}) for CES, ES and CWES biosorbents to different initial Pb concentrations.

Initial Pb (II) concentration (mg L^{-1})	CES	ES	CWES
100	2796	5065	-
200	-	218	-
298	330	102	4360
483	83.5	53.6	3904
727	46.8	29.4	503
977	30.7	20.5	201
1435	20.6	12.8	108
1952	-	-	67.1
2422	-	-	46.5

At the lowest metal concentration, ES is the biosorbent that has the higher distribution coefficient. These results indicate that ES might have a higher capacity to sorb Pb(II) than CES, at concentration levels lower than 100 mg L^{-1}. Though, when Pb(II) concentration is higher than 100 mg L^{-1}, CES enhances the retention affinity towards lead in comparison to ES, while CWES shows the highest affinity to retain lead.

8.4. Conclusions

The potential use of a compost derived from industrial eggshell co-composting (CES) as a biosorbent for Pb(II) was evaluated. The sorption capacity was dependent on the initial metal concentration, but almost independent on initial pH within 2-5.5. The sorption isotherms followed the Langmuir model suggesting a monolayer sorption feature. EDX spectra reveal the importance of Ca^{2+} in the sorption mechanism, but a better understanding of other functional groups involvement is still need. This study illustrated that CES in comparison to ES is a better alternative for lead removal in aqueous solutions, though optimisation of some operational conditions (higher pH or L/S) should be addressed in further studies.

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8.6. Supplementary material

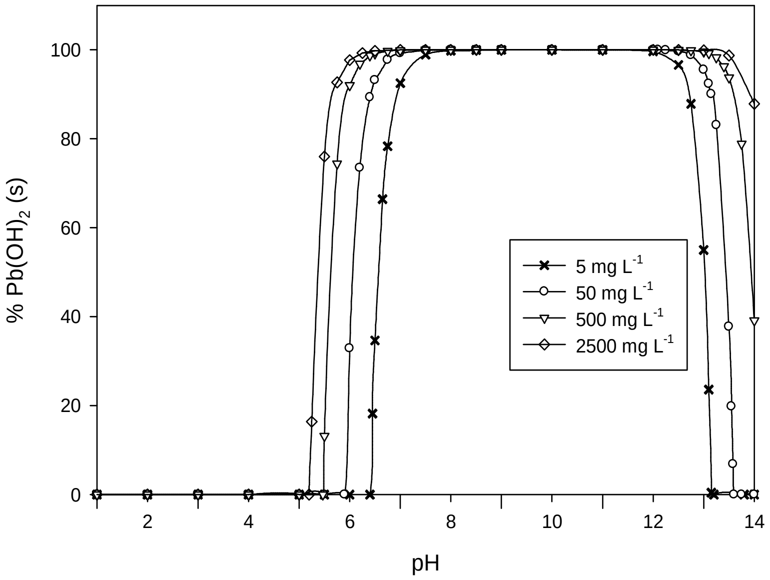

Figure S8.1: Percentage of $Pb(OH)_2$ for different Pb(II) concentrations, considering an aqueous solution prepared by dissolving lead nitrate salt (simulations from Visual Minteq v3.0).

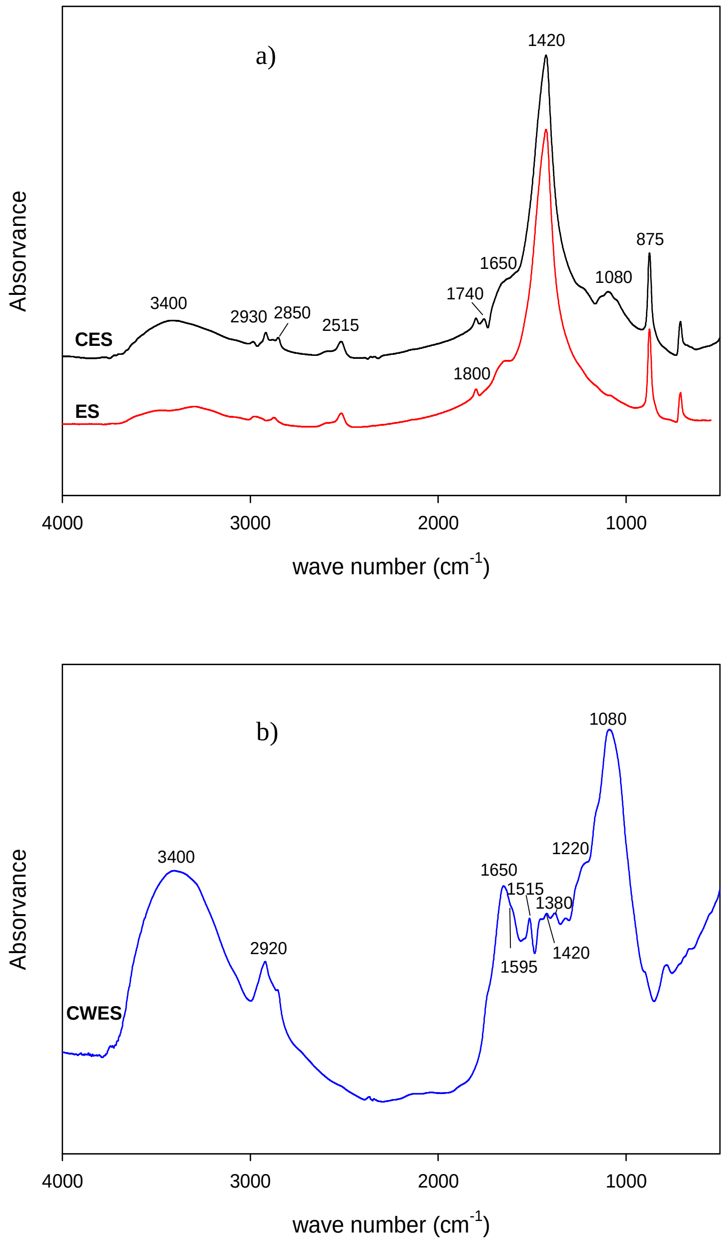

Figure S8.2: FTIR spectra of the tested biosorbents: a) CES and ES, b) CWES.

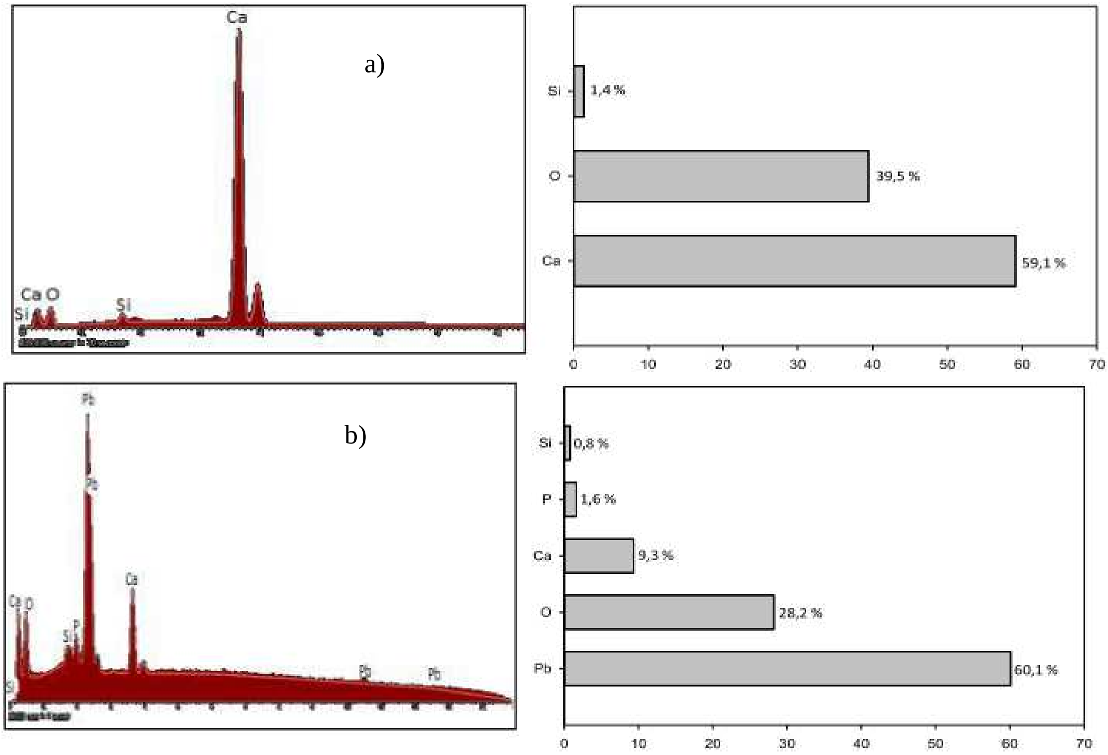

Figure S8.3: EDX spectra and elemental composition of CES surface: a) before, b) after sorption.

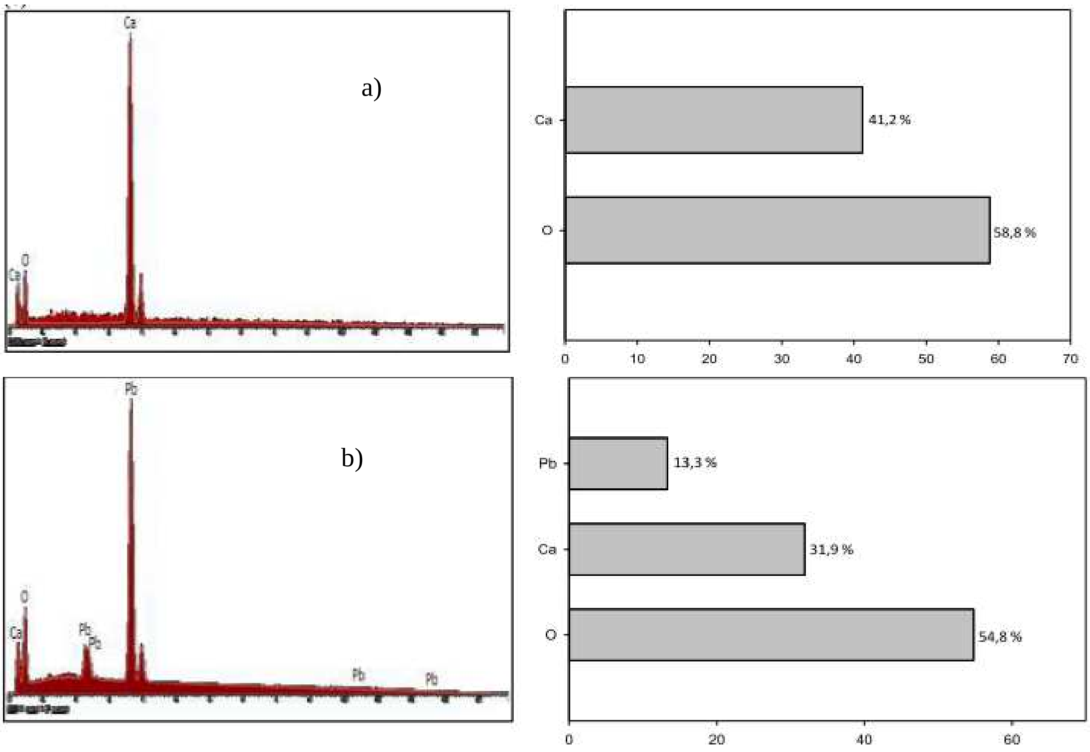

Figure S8.4: EDX spectra and elemental composition of ES surface: a) before, b) after sorption.

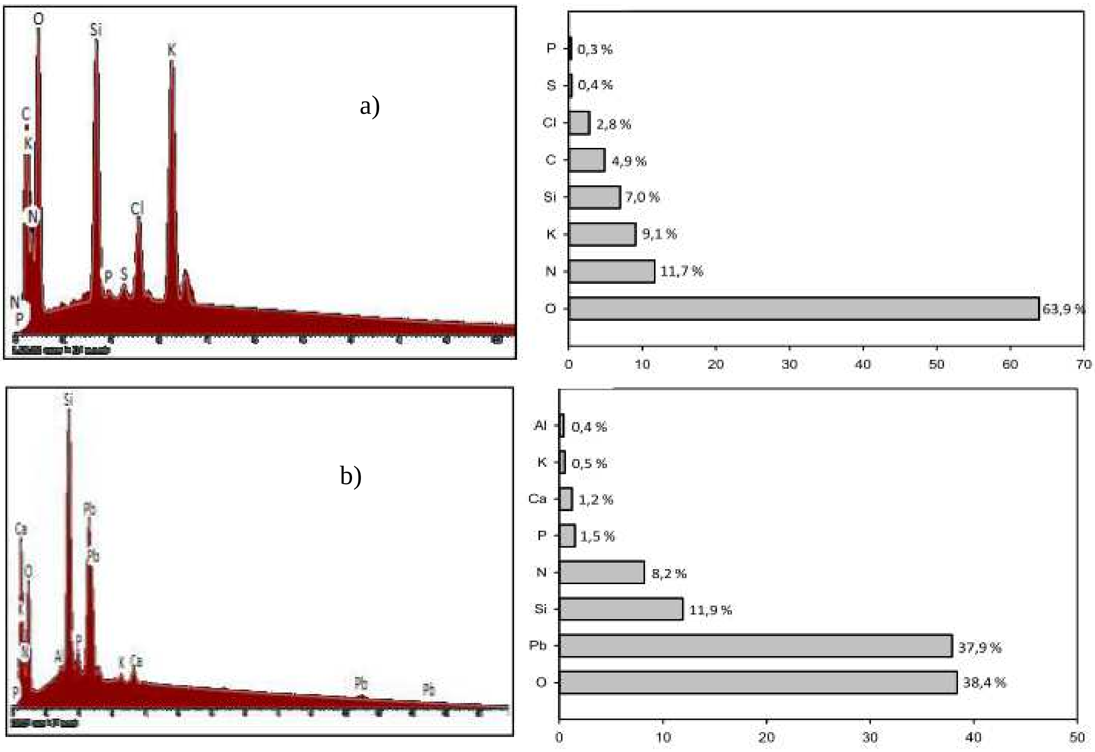

Figure S8.5: EDX spectra and elemental composition of CWES surface: a) before, b) after sorption.

9. Immobilisation of lead and zinc in contaminated soil using compost derived from industrial eggshell*

This study aims to evaluate the capacity of a compost obtained by co-composting of industrial eggshell (CES) to immobilise lead (Pb) and zinc (Zn) in an acidic soil contaminated by mining activities. Mature compost without eggshell (CWES) and natural eggshell (ES) were also tested as soil amendments for comparison purposes. Three different application rates were used for each material, ensuring the same quantity in terms of neutralising capacity. Incubation experiments were conducted under controlled conditions and CO_2 emissions monitored for 94 days. The environmental availability of Pb and Zn in the amended soil was assessed and bioassays were performed at the end of the incubation period.

When eggshells were present, the CES compost raised the soil pH to values higher than 6 and reduced the soil mobile fraction for both Pb and Zn, in more than 95%. Soil toxicity towards *Vibrio fischeri* was also suppressed and environmental risk decreased to “low level”. However, the immobilisation in the acid insoluble soil component was significantly achieved only for Zn. In addition, regarding soil carbon dynamics the CO_2-C emissions were enhanced, mainly in the case of the highest rate of amendment. Both first order-E and parallel first order models may adequately describe the kinetic data of CO_2-C cumulative release. Without eggshells, the CWES compost revealed limited effect on heavy metals immobilisation, likely due to its small capacity to correct soil acidity, at lower application rates. Using solely eggshells, the ES waste had similar outcomes when compared with CES, but at the higher application rate, CO_2 emissions were enhanced with the eggshell compost due to the contribution of biotic carbon present therein. Therefore, this study points out that CES is an effective liming material and may be used for *in situ* remediation of contaminated soil with Pb and Zn.

Keywords
Eggshell compost; Soil remediation; Heavy metal; Environmental availability.

*This chapter is based upon the publications:

Soares, M.A.R., Quina, M.J., Quinta-Ferreira, R., 2015. Immobilisation of lead and zinc in contaminated soil by using compost derived from industrial eggshell. J. Environ. Manage. 164, 137–145.

Soares, M.A.R.; Quina, M.J.; Quinta-Ferreira, R, 2013. Use of mature organic composts as immobilising agents of Pb and Zn in a mine soil, presented at Proc. WASTES’2013- 2nd International Conference Wastes: Solutions, Treatments and Opportunities, 11-13th September, In Proceedings pg.267-268, Braga, Portugal. (*oral communication*).

9.1. Introduction

Anthropogenic activities are considered to be the foremost responsible for soil enrichment in toxic heavy metals, even though these pollutants may also be natural constituents of the soil. Industrial processes, mining exploration, manufacturing and disposal of some waste materials are examples of mankind actions that can introduce in the soil non-essential metals with phytotoxic and zootoxic properties (Park et al., 2011; Wuana and Okieimen, 2011). The risk that these contaminants pose to the environment and human health is directly related to their bioavailability in soil.

Several studies have tested different type of materials as amendments for promoting metals immobilisation in soil (Kumpiene et al., 2008). Both organic and inorganic materials such as mature composts, biochar and phosphates, as well as calcium sources including lime, mineral apatite and carbonates have been used (Janoš et al., 2010; Wuana and Okieimen, 2011). In addition, composts enriched with minerals like zeolites, clays, oxides or carbonates have also been addressed with the purpose of reducing the bioavailability of heavy metals in contaminated soils (Gadepalle et al., 2009, 2008; Lagomarsino et al., 2011; Siebielec and Chaney, 2012; Van Herwijnen et al., 2007). Furthermore, low-cost industrial residues have also been exploited for heavy metals retention in soil (Garrido et al., 2005). This approach has additional environmental benefits besides reducing waste disposal costs in landfill sites, is simultaneously able to tackle soil remediation.

Efficiency of amendments on the rehabilitation of soil contaminated with heavy metals may be addressed at chemical and ecotoxicological levels (Pardo et al., 2014). In this regard, chemical extraction methods have been commonly used to assess their environmental availability providing relevant information about the corresponding mobility (Pauget et al., 2011). On the other hand, addition of amendments may affect the soil inorganic and organic carbon dynamics. This influence can be observed through the rates of CO_2 production due to organic carbon mineralisation (biotic CO_2) and/or resulting from correction of soil acidification with liming materials (abiotic CO_2) (Bertrand et al., 2007). Bioassays have been used to evaluate if toxicity in the original soil was reduced after soil treatment in order to estimate if there is no longer risk to humans, plants or animals (Park et al., 2011). Bioassays using luminescent bacteria *Vibrio fischeri* allow the assessment of the eco-toxicity impact of soil composition on surface water and groundwater (i.e. soil retention function) (Alvarenga et al., 2009).

Industrial eggshell (ES) is the main waste stream from egg processing industry and its European production is about 350 thousand tonnes per year (Soares et al., 2013).

Lee et al. (2013) evaluated the effect of eggshell waste, combined with a nitrogen, phosphorous and potassium fertilizer, on Cd and Pb immobilisation in a neutral agricultural soil nearby abandoned mines. Their study concluded that 80 days after incorporating 5% (w/w) eggshell into soil, Cd and Pb concentrations extracted with acetic acid were reduced up to 68 and 93%, respectively. In addition, Ahmad et al. (2012) found that by adding 5% (w/w) of eggshell to a neutral military shooting range soil, the Pb concentration extracted with acetic acid was reduced by 68%, mainly due to pH increase with further lead immobilisation via formation of Pb-hydroxide or lanarkite. On the other hand, Ok et al. (2011) reported a reduction of only 24.6% for Pb extracted with acetic acid, by also using 5% (w/w) of eggshell, in a moderately acid soil. Nevertheless, according to the European legal framework, ES is classified as an animal by-product not intended for human consumption (Regulation (EC) Nº 1069/2009 of the European Parliament and the Council). Thus, the application of ES to soil may require a prior thermal treatment to reduce the spreading risk of pathogens into the environment and human health.

Co-composting is one of the ES treatment options that besides promoting eggshell waste sanitation, can also deliver a tailored compost rich in calcium carbonate. This product can enhance soil remediation due to the presence of: i) stabilized/humified organic matter that may promote soil metal immobilisation mainly through adsorption reactions (Park et al., 2011) and ii) $CaCO_3$ that may precipitate heavy metals from soil solution. But to the best of the authors' knowledge, the effect of eggshell rich organic compost on heavy metal immobilisation in soil has not been addressed.

Accordingly, this study aims to evaluate the feasibility of using a compost (CES) obtained from composting industrial eggshell with other organic wastes, as a soil amendment for immobilisation of Pb and Zn in acidic contaminated mining soil. For comparison, a mature compost without eggshell (CWES) and natural eggshell (ES) were also tested. The effects of such amendments on soil properties were investigated at three levels: i) environmental availability and speciation of Pb and Zn in soil, by using chemical extraction procedures, ii) toxicity reduction of amended soil by performing bioassays and iii) evaluation of CO_2 emission from soil, after the addition of such a carbonate rich amendment.

9.2. Materials and methods

9.2.1. Soil and amendments

About 5 kg of soil sample was collected at 5-20 cm depth from a Portuguese abandoned mining complex. The collection site was constituted by a set of mines, where hydrothermal veins rich in Pb and Zn were explored during the 19th and 20th centuries. The soil sample was air dried and sieved through 2 mm mesh to remove large particles.

The compost soil amendments that were tested were obtained at laboratory scale by using self-heating reactors of 105 L with forced aeration. CES compost was derived from industrial potato peel, grass clippings, rice husk and industrial eggshell (ES) wastes in the proportions of 35.4%, 22.7%, 11.9% and 30% (w/w), respectively. CWES compost was prepared in the same conditions as CES, without eggshell in the initial mixture while keeping the relative proportions of the other ingredients.

9.2.2. Experimental design

Laboratory scale incubation studies were conducted for 94 days on soil conditioned with three different treatments (T_1 to T_3), for each amendment tested. Representative soil samples (about 50 g) were mixed with each compost (CES and CWES) at three rates: T_1=2.5 g, T_2=5 g and T_3=15 g (air dried basis). In the case of ES amendment, equivalent rates to CES_T_1 to T_3 in terms of neutralising capacity (equivalent $CaCO_3$) were tested. Thus, the application rate to soil was ES_T_1=1.6 g, ES_T_2=3.2 g and ES_T_3=9.5 g (air dried basis).

For each treatment and control samples, three replicates were performed (i.e. twelve experiments were followed during the incubation period). The moisture of all mixtures was adjusted to 65% of their water holding capacity (WHC) and maintained during each experiment by adding distilled water. Incubation tests were carried out in 1 L closed mason jars, in dark conditions, while keeping the temperature at 25 ºC.

9.2.3. Physico-chemical analysis

9.2.3.1. pH and electrical conductivity

pH and electrical conductivity (EC) were measured in 1:5 (w/v) solid:water extracts obtained at the beginning and at the end of the incubation period, which lasted for 94 days.

9.2.3.2. Single chemical extractions

The mobile fraction associated with very active and available metal forms was extracted with 0.01 M $CaCl_2$, unbuffered (Gupta et al., 1996; Pueyo et al., 2004). The mobilisable fraction that includes potentially available, leachable and partly active compounds was extracted with a solution of 0.5 M CH_3COOH, 0.5 M NH_4CH_3COO and 0.02 M EDTA at pH 4.7 (Gupta et al., 1996; Hammer and Keller, 2002). The extractions were performed for 2 h at room temperature, in an overhead shaker (16 rpm), using a soil:solution ratio of 1:10 (w/v). Blank samples without soil were used as control. Lead and zinc were determined by flame atomic absorption spectrometry (Perkin Elmer – 3000) and standard solutions were prepared with the appropriate extracting reagent, to reduce matrix interferences.

9.2.3.3. Sequential chemical extractions

Heavy metals in soil were determined by a three-step sequential extraction method based on the BCR (Community Bureau of Reference), which is summarised in Table 9.1 according to the procedure presented by Rauret et al. (2000).

Table 9.1: Main features of BCR three-step sequential extraction method.

Extraction Step	Soil fraction evaluated	Experimental procedure
1- Acid extractable	F1 - exchangeable, water and acid soluble species	extraction with 0.11 mol L^{-1} acetic acid (v:w ratio of 40:1, 16 h);
2- Reducible	F2 - metals associated with the reducible phases, namely bound to Fe/Mn oxyhydroxides	extraction with 0.5 mol L^{-1} $NH_2OH.HCl$ in 0.05 mol L^{-1} HNO_3 (v:w ratio of 40:1, 16 h)
3- Oxidisable	F3 - oxidisable metal fraction, e.g. bound to organic matter or sulphides	digestion with conc. H_2O_2 at 85ºC followed by extraction with 1 mol L^{-1} ammonium acetate adjusted to pH 2 by conc. HNO_3 (v:w ratio of 50:1, 16 h)

A Heidolph Reax 20 overhead shaker, at 16 rpm and room temperature, was used to obtain each extract. Between each step, separation of the extract from the solid was undertaken by centrifugation at 3000 rpm for 20 min followed by decantation of the supernatant liquid. According to Sulkowski and Hirner (2006), incomplete carbonate dissolution may occur in step 1, which in turn may overestimate metal content associated with the following extraction steps. These authors showed that if the extract from Step 1 presents pH lower than 4, the carbonate dissolution of a soil containing 27% of dolomite was nearly complete. Thus, in the present study, whenever pH was above 4, a second extraction with 0.11 mol L^{-1} acetic acid was repeated (v:w ratio of 40:1, 16 h). After each extraction step, the residue was washed with distilled water (v:w ratio of 20:1), shaked for 15 min, centrifuged for 20 min at 3000 rpm and the supernatant discarded.

As a checking procedure, residue from Step 3 of two random soil mixtures was digested in *aqua regia*, according to the method described by Rauret et al. (2000). The total amount of metal extracted (sum of Step 1 + Step 2 + Step 3 + residue) was compared to the pseudo-total amount of metal obtained by *aqua regia* digestion. A maximum relative error of 8.2% for Pb and

5.1% for Zn was observed, which is within the errors found by Rauret et al. (2000). Blank sample extractions (without soil) were carried out throughout the complete procedure.

A residual fraction (F4) was calculated as the difference between the pseudo-total metal concentration and the sum of the extracted metals from the three BCR steps, which provides information about the fraction that is resistant to extracting processes (Hass and Fine, 2010).

9.2.4. Environmental risk of metals in soil

The risk assessment code (RAC) usually employed for soils and/or sediments (Guillén et al., 2012). The RAC index was calculated by the following equation:

$$RAC(\%) = \frac{C_m}{C_t} \times 100 \qquad (9.1)$$

where C_m is the metal concentration in the exchangeable and carbonate fractions (equivalent to the fraction obtained in step 1 (F1) of the BCR procedure) and C_t is the total metal concentration. The RAC index is stratified into five risk categories (Gusiatin and Kulikowska, 2014): no risk (<1%), low risk (1-10%), medium risk (11-30%), high risk (31-50%) and very high risk (> 50%). This index is based on individual metal mobility and it does not account for the metal toxicity.

9.2.5. Bioassays

Germination tests using *Lepidium sativum* seeds were conducted on extracts obtained from soil after treatment (CES_T1 to T3, CWES_T1 to T3 and ES_T1 to T3). The germination index (GI) was calculated following the usual procedure as described in Chapter 6.2.3 (Pardo et al., 2011). Inhibitory effects of the soil mixture extracts on the light emission of *Vibrio fischeri* were measured using a Lumistox 300 equipment. Soil was extracted with aqueous solution of NaCl (2% v/v) (Mantis et al., 2005). The decrease of luminescence of the bacteria before and after being incubated (15 ºC, 30 min) with 9 dilutions of soil extract (from 6.25% to 100% v/v) was assessed in duplicate. The effective concentration (%v/v) with a toxic effect on 50% of the population of the marine microorganisms (EC_{50}) was calculated.

9.2.6. Monitoring of CO_2 and kinetic modelling

During the incubation tests, CO_2 production from untreated and treated soil with CES, CWES and ES was quantified. A trap with 20 mL of 0.5 M NaOH was placed in each mason jar to capture CO_2 flux during incubation tests. Until the 60th day of incubation, the jars were opened every two days to maintain aerobic conditions by exposing them for 5-10 min to ambient atmosphere. From there on, flasks were opened once a week. Whenever jars were opened: i) the CO_2 trap was removed and the residual NaOH therein was back-titrated with 0.5 M HCl, using phenolphthalein as indicator ($BaCl_2$ in excess was added for precipitation of carbonates); ii) moisture of soil samples was maintained at 65% of WHC by gravimetric adjustment with deionized water; iii) before closing each incubation flask, a trap with fresh NaOH was inserted. Consumption of NaOH in each monitoring interval was stoichiometrically converted into CO_2 (Pardo et al., 2011). CO_2 emission was expressed in terms of mg CO_2-C 100 $g^{-1}{}_{\text{soil air dried}}$ d^{-1}.

Kinetic parameters describing CO_2 release during incubation were calculated by fitting the cumulative CO_2-C profile to two kinetic models (Bernal et al., 1998; Fernández et al., 2007): first order-E model (Eq. 9.2) and parallel first order model (Eq. 9.3).

$$CO_2 - C\ emission\ (t) = C_0(1 - e^{-kt}) + C_f \qquad (9.2)$$

$$CO_2 - C\ emission\ (t) = C_f\left(1 - e^{-k_f t}\right) + C_s(1 - e^{-k_s t}) \qquad (9.3)$$

where CO_2-C emission (t) is the cumulative amount of C emitted in the form of CO_2 at time t, C_0, C_f and C_s are the CO_2-C pools with potential, fast and slow turnover time, respectively; k, k_f and k_s are the emission constants of potential, fast and slow carbon pools. In addition, half-life time, $t_{1/2}$, (corresponding to the 50% of the concentration of CO_2-C in each pool) was estimated, as well as the ultimate CO_2-C (C_∞) corresponding to the sum of C_s and C_f.

9.2.7. Statistical analysis

Differences between soil treatments were estimated with one-way ANOVA and *post hoc* Tukey test, at a *p*-value (significance level) of 5%, after checking the normality of data according to the Shapiro-Wilk test. Selection of the best-fitting kinetic model for the C-CO_2 cumulative release was based on the higher R^2_{adj}. Pearson correlations coefficients (r) were calculated between soil chemical parameters at a significance level of 5% ($P<0.05$). All statistical analyses were performed using the software Statistica 7 (StatSoft, Inc) and Sigmaplot 10 (Systat Software, Inc.).

9.3. Results and discussion

9.3.1. Properties of soil and amendments

Table 9.2 addresses the main properties of the soil and amendments used in this study. The soil has a sandy loam texture, acidic pH and low content in essential nutrients (namely P and K).

Table 9.2: Main properties of the soil and amendments used in the experiment (mean±standard deviation).

			Soil amendments			
Parameters	Soil	Limiting values$^{a)}$	CWES	CES	ES	Limiting values$^{b)}$
Moisture [%]	n.a.	-	7.5	3.7	1.0	-
pH	4.5±0.2	-	9.3±0.1	8.9±0.1	8.3±0.1	-
EC (dS m^{-1})	0.29	-	0.85	1.10	0.45	-
Equivalent CaCO$_3$ (g CaCO$_3$ 100 g$_{\text{air dried}}$$^{-1}$)	n.a.	-	4.5±0.1	56±2	88±0	-
Organic matter (%)	8.59	-	79.4±0.5	28.5± 0.3	6.3± 0.1	-
Texture (%)						
Sand	66.1	-	n.a.	n.a.	n.a.	-
Silt	27.0	-	n.a.	n.a.	n.a.	-
Clay	6.9	-	n.a.	n.a.	n.a.	-
TOC/TN	n.a.	-	21	11.9	2.1	-
Available P (mg kg^{-1})	n.d.	-	n.a.	n.a.	n.a.	-
Available K (mg kg^{-1})	32.3	-	n.a.	n.a.	n.a.	-
GI (in aqueous extract with *Lepidium sativum*) (%)	37±9.0	-	75.5±9.1	76.4±10.7	53.6±3.3	-
Respiration rate (mg C-CO$_2$ g^{-1}C d^{-1})	n.a.	-	5.3±1.1	3.6±0.2	24±1	-
Pb$_{\text{mobile}}$ (mg kg^{-1})	121±3	-	n.a	n.a	n.a	-
Pb$_{\text{mobilisable}}$ (mg kg^{-1})	7697±531	-	n.a	n.a	n.a	-
Zn$_{\text{mobile}}$ (mg kg^{-1})	32±0	-	n.a	n.a	n.a	-
Zn$_{\text{mobilisable}}$ (mg kg^{-1})	52±5	-	n.a	n.a	n.a	-
Cd$_{\textit{aqua regia}}$ (mg kg^{-1})	0.75 ± 0.21	1	0.70±0.01	0.40±0.01	n.a	1.5
Cr$_{\textit{aqua regia}}$ (mg kg^{-1})	26.6 ± 1.1	50	12.4±1.0	4.2±0.0	n.a	100
Pb$_{\textit{aqua regia}}$ (mg kg^{-1})	10 008±80	50	12.7±0.0	7.3±0.0	3.6±0.0	120
Cu$_{\textit{aqua regia}}$ (mg kg^{-1})	0.12± 0.21	50	8.2±0.2	5.2±0.2	n.a	200
Zn$_{\textit{aqua regia}}$ (mg kg^{-1})	315±5	150	47±0	11.9±0.1	5.0±0.1	600
Ca$_{\textit{aqua regia}}$ (mg kg^{-1})	n.a.	-	4008±20	222500±3230	n.a.	-

CES: compost with industrial eggshell; CWES: compost without industrial eggshell; ES: industrial eggshell waste; EC: electrical conductivity; TN: total nitrogen; TOC: total organic carbon; GI: germination index; n.d.: not detected; n.a.: not analysed; -: not applicable. Concentration units are presented in a dry weight basis. a) According to limiting values in agriculture soils for sewage sludge application imposed by Portuguese legislation (Decreto-Lei no276/2009). b) Based on End-of-waste criteria for biodegradable waste subjected to biological treatment (draft final report) (JRC - European Commission, 2013).

The electrical conductivity is low, as expected for mining soils that are exposed to weathering conditions (Alvarenga et al., 2009). Heavy metal pseudo-total concentration (associated with the soil content extracted with *aqua regia*) is of special concern for Pb and Zn, exceeding by far the legal limits set by Portuguese legislation (Decreto-Lei n^{o}276/2009) for sewage sludge applications in agriculture soils. In particular, the potentially bioavailable fraction of Pb, assessed by the mobilisable content, represents about 77% of the total content.

Composts CES and CWES were collected after 144 days of composting. Both materials were phytotoxin-free (germination indices close to 80%) and may be considered stable with moderately slow respiration rates between 2-8 mg CO$_2$ g^{-1} C d^{-1} (Gómez et al., 2006). Whereas ES showed a lower GI (nearly 54%) but higher respiration rate (25 mg CO$_2$ g^{-1} C d^{-1}). Heavy metals content in the tested soil amendments (CWES, CES and ES) was low in comparison to the limits set by the end-of-waste criteria for biodegradable waste submitted to biological treatment (draft final report) (JRC - European Commission, 2013).

9.3.2. Effect of amendments on soil pH and electrical conductivity

The response of contaminated soil treated with amendments CES, CWES and ES regarding pH is shown in Figure 9.1. Bars denoted with the same letter are not significantly different according to Tukey´s test at P<0.05. pH classes included in this figure are according to US Department of Agriculture Natural Resources Conservation Service (2013).

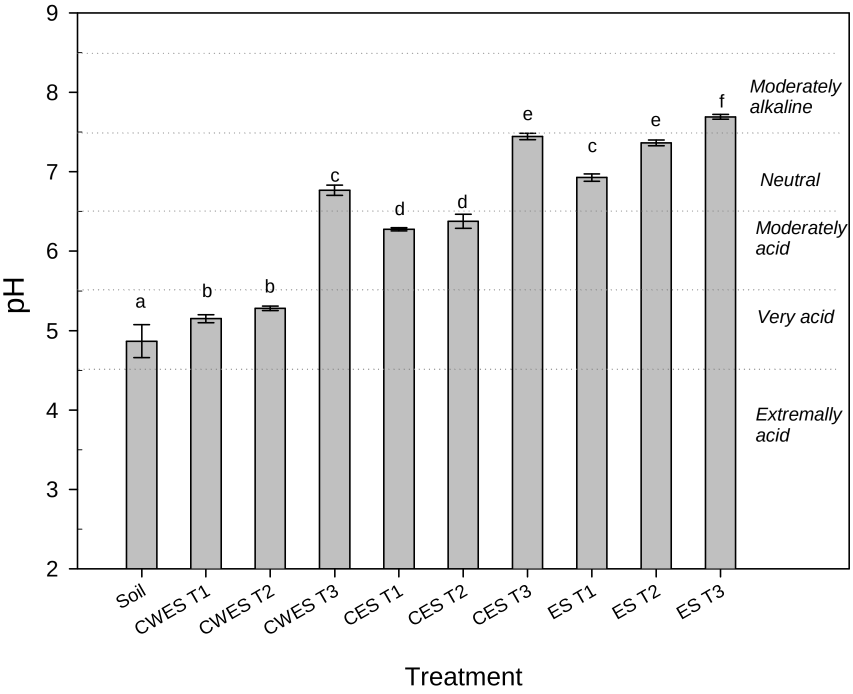

Figure 9.1: Soil pH at the end of 94 days of incubation after treatment with CWES, CES and ES.

All amendments ameliorated soil acidity (P<0.05), but the liming effect (pH>6) was more relevant with CES and ES application. Although CES and ES application rates to soil were identical in terms of neutralising capacity (equivalent $CaCO_3$), only ES presented a dose-response effect on the soil pH. In all cases, increasing the incorporation rate of the amendments induced a salt level upsurge (P<0.05) (Figure S9.1 - supplementary data) and EC was positively correlated with pH (Pearsons's correlation coefficient of 0.912, 0.956, and 0.842 for CES, CWES and ES, respectively, P<0.05).

9.3.3. Influence of soil treatment on lead and zinc environmental availability

9.3.3.1. Mobile and mobilisable fraction

The influence of the tested amendments in the mobile and mobilisable fractions of Pb and Zn in the soil after 94 days of incubation is shown in Figure 9.2.

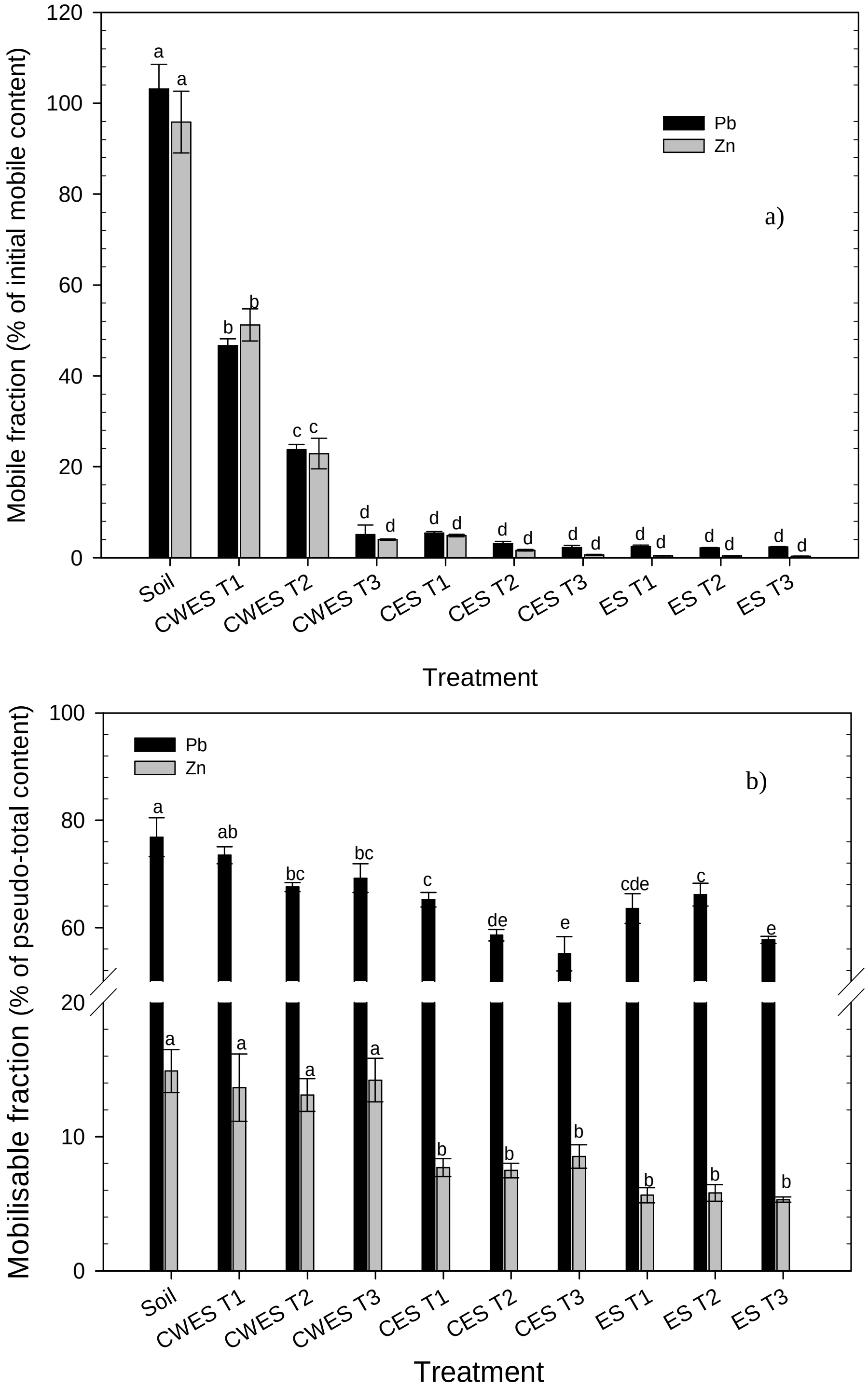

Figure 9.2: Influence of amendments on heavy metal availability (Pb and Zn) in soil, after 94 days of incubation (mean±standard deviation): a) mobile fraction; b) mobilisable fraction. For each series, bars marked with the same letter are not statistically different according to Tukey´s test at $P<0.05$.

The mobile fraction achieved on each test is represented in Figure 9.2 a) and was expressed as the percentage of the metal that was present in the corresponding mobile fraction measured at the start of incubation (Table 9.2), since that fraction was less than 2% of the pseudo-total metal content.

Figure 9.2 a) shows that as the rate of the CES application to soil increases a significant reduction ($P<0.05$) on the mobile fractions of Pb and Zn was observed. This behaviour may be due to the pH increase caused by CES treatment in the soil, as previously mentioned. Soil pH is negatively correlated with the mobile fraction for both Pb ($r=-0.749$, $P<0.05$) and Zn ($r=-0.740$, $P<0.05$). In general, pH is considered the main variable controlling solubility and sorption mechanisms of heavy metals in soils (Alloway, 1995; Alvarenga et al., 2009; Fleming et al., 2013). Thus, pH is likely to have a major influence on mobility reduction of metals depicted in Figure 9.2 a).

Regarding the mobilisable fraction, the CES treatment fostered a reduction ($P<0.05$), but the effect was more pronounced for Zn (Figure 9.2 b)). However, results may be overestimated by the extracting solution used to quantify the mobilisable fraction (0.5 M CH_3COOH, 0.5 M NH_4CH_3COO and 0.02 M EDTA at pH 4.7). Indeed, the EDTA extractant may stimulate the release of metals to solution not only from carbonates or Fe and Mn oxides, but also through the formation of stable chelates (Hammer and Keller, 2002; Santos et al., 2010). Nevertheless, CES was able to immobilise near 50% of Zn (Figure 9.2 b)). Thus, it can be concluded that the mobile form of Zn can be transformed into less available/soluble compounds by treating the soil with CES. The ES treatment leads to similar effects on the mobile and mobilisable soil fractions when compared with the ones observed for the CES experiments. Since ES and CES application rates were identical in terms of liming capacity, it appears that the organic matter provided by CES had no visible effect on restricting neither mobile nor mobilisable contents of these elements. Hence, the correction of soil acidity above the upper limit of ”moderately acid” (Figure 9.1) seems enough to foster the reduction of the mobile fraction.

With respect to CWES, the reduction of the soil mobile fraction showed a dose-response behaviour (Figure 9.2 a)). In the case of CWES_T1 and T2, this can be entirely attributed to the amount of organic matter added, since the pH remained close to 5 after the addition of this amendment (Figure 9.1) and the mobility reduction is double for CWES_T2 in comparison to CWES_T1. The significant mobility reduction observed for CWES_T3, could also be a result of the neutral pH that was achieved, which affects the distribution of Pb and Zn between water-soluble, exchangeable and non-exchangeable forms. Nevertheless, the mobile fraction of Pb in soil revealed a very high negative correlation with pH ($r=-0.867$, $P<0.05$). Regarding the Zn mobilisable fraction, the addition of CWES to soil had a negligible effect ($P<0.05$) (Figure 9.2 b)) while CWES_T2 and CWES_T3 led to a statistically relevant Pb reduction. These results show that CWES has low capability to reduce the potentially bioavailable, leachable and partly active metal fraction. This low ability to alter the Pb and Zn mobilisable fraction was also verified by Alvarenga et al. (2009) when treating highly-acidic contaminated soil with garden waste compost in a greenhouse experiment of two months.

9.3.3.2. Distribution in soil phases and risk assessment

Contaminated sites may pose a significant risk to human health and to other environmental receptors. In this study, the environmental risk associated to the mobility of Pb and Zn in untreated soil was of “medium level” as calculated by Eq. (9.1), (Table S9.1 – supplementary data). Figure 9.3 shows Pb and Zn fractions in soil before and after amendment addition and incubation for 94 days, determined by a sequential extraction test that allows evaluation of four different fractions: F1, acid soluble; F2, reducible; F3, oxidisable; F4, residual. Initially, Pb was mostly associated with the reducible phase (58% in F2) that accounts for soil Fe/Mn oxyhydroxides (Figure 9.3 a)), while Zn was predominantly connected to the residual fraction (64% in F4; Figure 9.3 b)). Although some changes were observed, none of the treatments was able to reverse this situation.

In the case of Pb CES_T3 treatment reduced F1 by 23%, and thus the environmental risk category was lowered to the “low risk” class. However, CWES was more effective ($P<0.05$) at reducing this metal in F1 (Table S9.1 – supplementary data). The reduction of Pb associated with F1 was generally associated to an increase in the residual fraction (F4), thus leading to the immobilisation of that pollutant. This probably means that favourable conditions (such as soil pH and redox potential) were created to enhance Pb occlusion into minerals like well-crystallised oxides inaccessible to chemical extraction (Hass and Fine, 2010). Siebielec and Chaney (2012) also showed that a neutral soil amended with a $CaCO_3$-rich compost increased lead adsorption in Fe oxides.

The CES treatment caused not only a slightly different distribution among the soil fractions, but also a mitigation of the environmental risk of Zn pushing it to the “low level” classification (Table S9.1 – supplementary data). Increasing the application rate of CES, the decrease in the acid soluble (F1) and oxidisable (F3) fractions were also increased, while an increase in the reducible (F2) and residual (F4) fractions was observed. When compared to untreated soil, CES_T3 reduced by nearly 50% F1 and F3 fractions, while F2 was doubled and the F4 fraction increased by 10%. The pH that was achieved (7.4) with CES_T3 has probably driven the formation of Al, Fe and/or Mn oxides, which precipitate at neutral pH conditions (Fan et al., 2011). In addition, since the surface charge of Mn oxides is negative at pH 7.4, they provided extra sites for sorption of Zn cations (Alloway, 1995). F4 fraction could also account for some Zn co-precipitated as Fe and Mn oxides (Alloway, 1995), which may become attached to crystallised secondary minerals (Hass and Fine, 2010; Ma et al., 2006). Comparison of ES and CES treatments showed that: i) ES was effective at reducing the environmental risk (Table S9.1 – supplementary data); ii) at the lower application rate (T1), Zn bound to the oxidisable fraction is favoured with CES (P<0.05), probably as a result of the organic matter conveyed by the amendment; iii) at the higher rate (T3) ES was more effective than CES to allocate Zn in F4 (P<0.05), probably due to the higher soil pH attained with ES, which may favour oxides formation. Finally, the environmental risk of Zn was not reduced with CWES treatment (Table S9.1 – supplementary data). The higher amount of organic matter conveyed by CWES provoked a relocation of Zn to F2 and F3. The same behavior has been found in other studies (Fleming et al., 2013). However, further investigation should be performed to conclude about the mechanism involved or the lack of selectivity of the BCR method (Vodyanitskii, 2006).

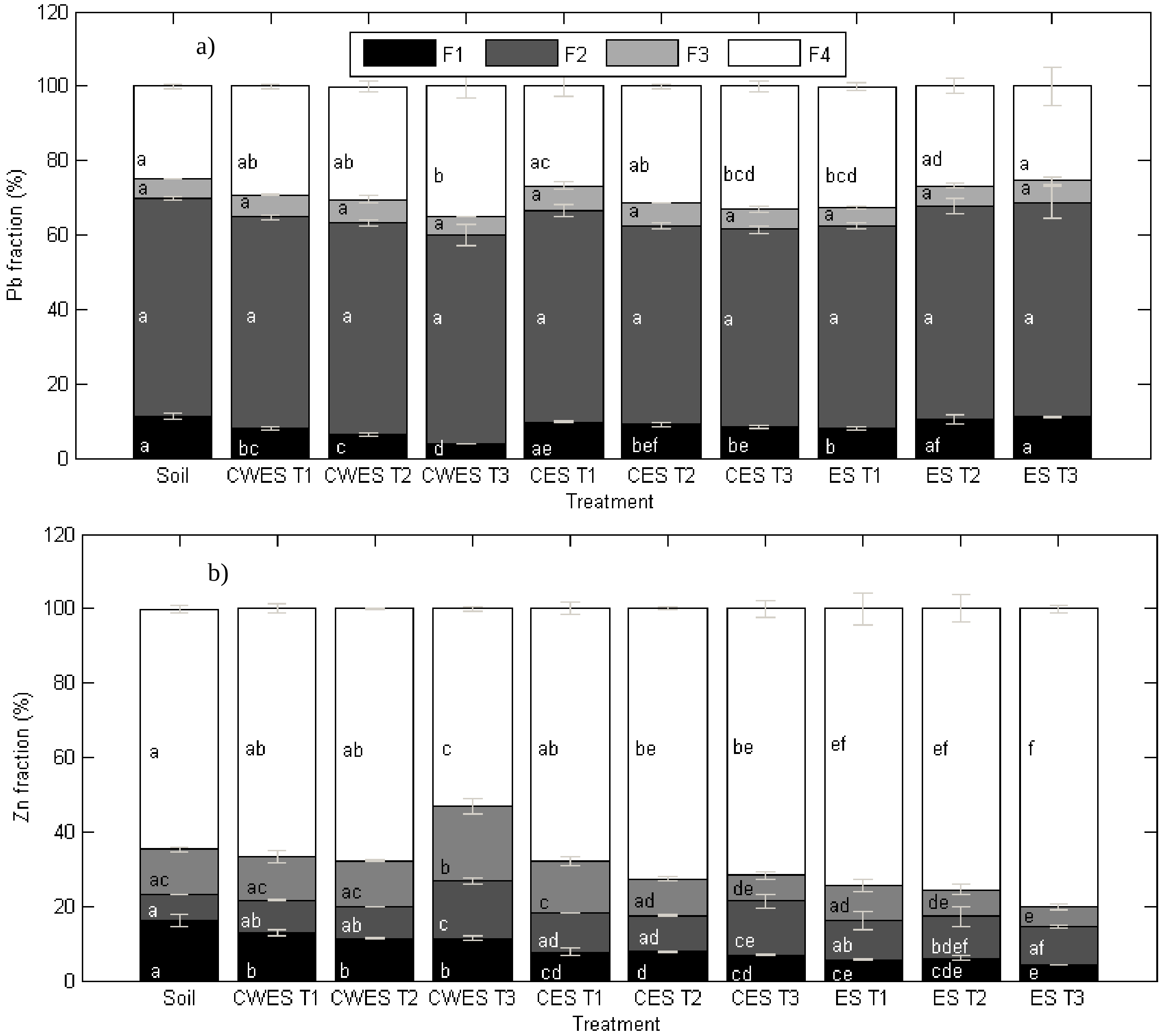

Figure 9.3: Lead (a) and zinc (b) fractions of untreated and treated soil after 94 days of incubation, according to BCR procedure (mean±standard deviation, n=3) expressed in % of pseudo total content: F1 (exchangeable, water and acid soluble fraction), F2 (reducible fraction), F3 (oxidisable), F4 (residual fraction). For each metal fraction, bars denoted with the same letter are not statistically different according to Tukey´s test at P<0.05.

9.3.4. Toxicity evaluation

Table 9.3 shows the results of GI and EC50 measured in the extracts of soil treated with the three different amendments. For CES treatment, GI based on *Lepidium sativum* germination showed that the initial soil toxicity was partially overcome (GI>50%). This effect suggests the presence of toxic soluble elements in very low concentrations (Pardo et al., 2011). In addition, the high toxicity of untreated soil towards *Vibrio fischeri* was effectively suppressed with CES, regardless of the application rate. These results confirm the reduction of the environmental risk prompted by CES (Table S9.1 – supplementary data) in terms of Pb and Zn mobility. The ES treatment yielded similar results to CES with respect to soil toxicity. Concerning the CWES treatment, the *Vibrio fischeri* inhibition was gradually reduced from CWES_T1 to CWES_T3, but only the higher application rate (T3) was non-toxic to 50% of the bacteria population. This smaller capacity of CWES to inhibit soil ecotoxicity may be associated with its low ability to reduce the mobile fractions of the metals, as pointed out in Figure 9.2a) and discussed previously in section 9.3.3.1.

Table 9.3: Results from bioassays in extracts from untreated and treated soil (mean±standard deviation).

		Treatments								
	Control Soil	CWEST$_1$	CWEST$_2$	CWEST$_3$	CEST$_1$	CEST$_2$	CEST$_3$	EST$_1$	EST$_2$	EST$_3$
GI (%)	37±9	92±16	128±13	133±10	103±14	112±7	93±13	95±5	93±18	79±4
EC$_{50}$ (30 min) (%v/v)	7.84	26.4	33.14	nt	nt	nt	nt	nt	nt	nt

nt: means that EC$_{50}$ value is out of the equipment's range, and thus undiluted soil extract cannot induce inhibition of 50% of the *Vibrio fischeri* population.

9.3.5. CO$_2$ efflux and kinetic modelling

Over the entire incubation period of 94 days, the respiration rate of the untreated and treated soil was quantified as CO$_2$-C production. The total CO$_2$-C released is shown in Figure 9.4 a). All amendments tested enhanced the CO$_2$ emission from soil, but in the case of eggshell derived materials (CES and ES) the increase was more pronounced compared to the initial soil sample (about 2 to 3 fold for T2 and T3 application rates).

In fact, for acidic soils exposed to liming treatment with carbonate based materials, the CO2 emissions may increase due to (i) dissolution of carbonates and/or (ii) improvement of biological processes with the consequent release of organically derived CO$_2$ (Ahmad et al., 2013; Biasi et al., 2008). Nevertheless, in our study the discrimination between these two contributions was not assessed. However, there is a high positive correlation between total cumulative CO$_2$-C production (Figure 9.4a)) and pH (Figure 9.1) ($r=0.881$, $P<0.05$) which is indicative of the liming capacity of the amendments.

The rate of CO_2-C accumulation from soil treatments fitted the kinetic models described by Eq. (9.2) and (9.3). The fitting parameters can be found in Table 9.4 for each kinetic model. Overall, the parallel first order model was found to describe data better (higher R^2 adj) than first order E-model. For CES_T1 and CES_T2 both models seem adequate, since the contribution of Cf (carbon fraction with fast turnover to CO_2-C) in both cases is very low and the kf is high.

Despite acidic soil conditions, the carbon release rate was significant (high kf, low t1/2,f and high Cfkf) what is in agreement with other similar studies (Turrión et al., 2012). Figure 9.4b) compares, as an example, the kinetic data of CES_T3 and ES_T3, based on the parallel first order equation. In the two cases, this kinetic model perfectly represents the experimental data. As previously mentioned, these two treatments have the same liming potential (equivalent $CaCO_3$) and thus their abiotic CO_2 release capacity is similar. However, important differences were detected in the kinetic behaviour, as well as in the ultimate CO_2-C fraction ($C\propto$). In fact, for CES_T3 not only the $C\propto$ is higher, but also the rate of CO_2-C flux of both fast and slow fractions is lower. These results can be confirmed through $C\propto$, Cfkf, Csks, t1/2,f and t1/2,s parameters reported in Table 9.4. Such behaviour can be attributed to the biotic carbon present in CES, which may extend the CO_2-C emission for longer periods, while ES had a faster effect on soil.

Addition of CWES amendment to soil increased the carbon fraction that can be slowly decomposed and retracted the initial potential mineralisation of the fast C pool (Cfkf). This indicates that CWES is characterised by a stable fraction of organic matter that did not encourage labile carbon mineralisation, as expected from a mature compost. However, the complex organic compounds present in the amendment were less humified than the stable fraction of soil organic matter (increase of Csks), which may favour microbial conversion (Turrión et al., 2012).

Organic and liming amendments can enhance CO_2 emissions from soil (Paradelo et al., 2015; Thangarajan et al., 2013) with the consequent effects on greenhouse gas emissions. Thus, when selecting an appropriate amendment for soil remediation, the CO_2-C release should be taken in consideration for the sake of reducing GHC emissions from soil treatment. In this study, the application of CWES was beneficial for the labile C fraction of soil and presented lower CO_2-C emissions (Figure 9.4 a)). Nonetheless, this amendment was not effective to reduce the environmental risk of zinc in soil (Table S9.1 – supplementary data). Also the negative impact of soil on surface water and groundwater persisted after treatment, as indicated by the EC_{50} value

(Table 9.3). In contrast, CES and ES (with the exception of ES_T3) were able to reduce the risk of heavy metal mobility in soil, and revealed ability to suppress its toxicity as well.

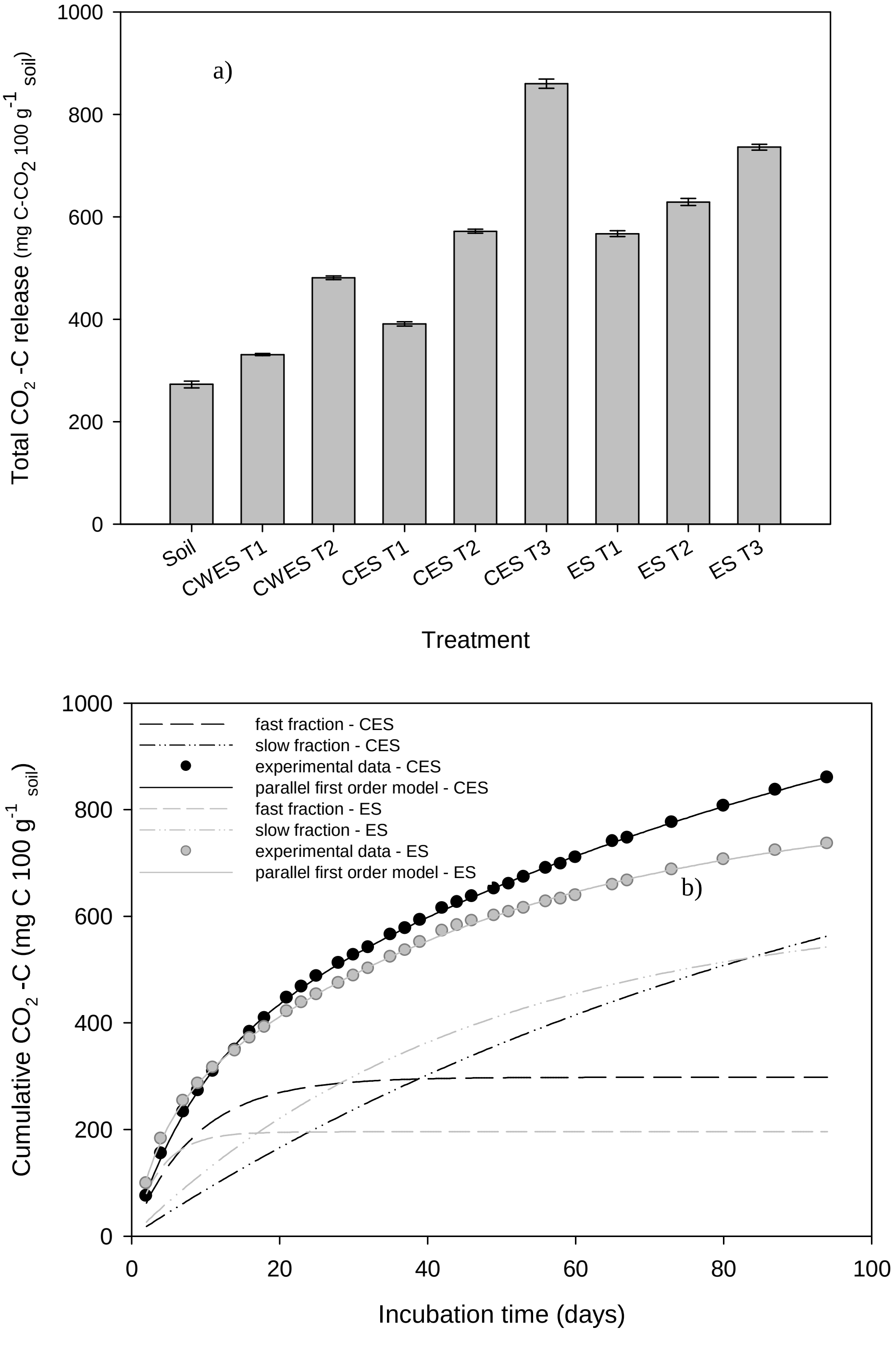

Figure 9.4: (a) Total CO_2 release at the end of incubation (mean±standard deviation, n=3); (b) Cumulative CO_2-C release during incubation tests of soil treated with CES_T3 and ES_T3.

Table 9.4: Kinetic parameters of the studied models, half-life time and initial potential emission for each CO_2-C pool.

	First order-E model						Parallel first order model									
	C_f	C_o	k	$t_{1/2,\,0}$	C_o k	R^2 adj	C_f	C_s	k_f	k_s	$t_{1/2,\,f}$	$t_{1/2,\,s}$	C_f k_f	C_s k_s	C_∞	R^2 adj
Treatment	(mg CO_2-C 100 g^{-1} $_{soil}$)		($days^{-1}$)	(days)	(mg CO_2-C 100 g^{-1} $_{soil}$ $days^{-1}$)		(mg CO_2-C 100 g^{-1} $_{soil}$)		($days^{-1}$)		(days)		(mg CO_2-C 100 g^{-1} $_{soil}$ $days^{-1}$)		(mg CO_2-C 100 g^{-1} $_{soil}$)	
Soil control	39.2	376	0.010	69.3	3.8	0.996	52.1	492	0.318	0.006	2.2	109.7	16.6	3.1	544	0.999
CES_T1	6.8	521	0.014	49.5	7.3	0.999	6.9	521	7.3×10^4	0.014	9.5×10^{-6}	49.5	5.0×10^5	7.3	528	0.998
CES_T2	6.3	710	0.017	41.0	12.0	0.999	16.3	710	119	0.017	5.8×10^{-3}	40.8	750	12.1	716	0.999
CES_T3	91.8	807	0.026	26.7	21.0	0.991	298	941	0.117	0.010	5.9	69.3	34.9	9.1	1239	0.999
ES_T1	59.0	566	0.022	31.5	12.4	0.997	91.7	575	0.275	0.018	2.5	38.5	25.2	10.4	667	0.999
ES_T2	88.3	565	0.027	25.7	15.3	0.994	149	559	0.252	0.020	2.8	34.6	37.5	11.2	708	0.999
ES_T3	115	641	0.030	23.1	19.2	0.992	196	621	0.262	0.022	2.6	31.5	51.4	13.7	817	0.999
CWES_T1	11.3	472	0.012	57.8	5.6	0.999	19.4	499	0.194	0.010	3.5	67.2	3.8	5.0	518	0.999
CWES_T2	17.2	671	0.012	57.8	8.1	0.998	79.2	938	0.074	0.006	9.0	117.5	5.8	5.6	1017	0.999

C_o, C_f and C_s are the CO_2-C pools with potential, fast and slow turnover time, respectively; C_∞ is the ultimate CO_2-C released; k, k_f and k_s are the emission constants of these pools and $t_{1/2}$, $t_{1/2,\,f}$ and $t_{1/2,\,s}$ represent the half-life times for the potential, fast and slow CO_2-C fractions, respectively.

9.4. Conclusions

CES presented a liming capacity able to shift soil pH to values higher than 6. The environmental availability of Pb and Zn in the contaminated soil was ameliorated, when considering the metals mobile and mobilisable fractions. However, in acid conditions CES effectiveness was only clearly proven for Zn immobilisation. Reducible and residual soil fractions were the major sinks for zinc retention in soil amended with CES at higher application rates (CES_T3), but in this case the CO_2 emissions were about 6-fold higher than in untreated soil. It is important to strengthen that the environmental risk decreased from “medium” to “low level” for both heavy metals at any application rate of CES.

In comparison to CWES, CES showed more effective characteristics to promote soil acidity correction and metals immobilisation. Nevertheless, the ability of ES to promote remediation of the tested soil was not effectively overcome by CES.

In summary, CES was found to be an effective liming material and revealed intrinsic properties for *in situ* remediation of soil contaminated with Pb and Zn. Nevertheless, due to the stable organic matter present within CES, its use could be more beneficial to metal-contaminated soils that also require restoration of organic matter levels, thus contributing to a long term soil quality and fertility.

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9.6. Supplementary material

Table S9.1: Environmental risk of lead and zinc according to risk assessment code (mean±standard deviation).

	Pb		Zn	
Treatment	RAC value	Environmental risk	RAC value	Environmental risk
Soil	11.3±0.7	Medium	16.2±1.6	Medium
CWES_T1	8.0±0.3	Low	12.9±0.6	Medium
CWES_T2	6.5±0.5	Low	11.4±0.2	Medium
CWES_T3	3.9±0.1	Low	11.4±0.7	Medium
CES_T1	9.8±0.3	Low	7.7±1.1	Low
CES_T2	9.1±0.7	Low	7.9±0.3	Low
CES_T3	8.6±0.4	Low	6.9±0.3	Low
ES_T1	8.1±0.3	Low	5.7±0.2	Low
ES_T2	10.4±1.1	Low	6.1±0.6	Low
ES_T3	11.1±0.3	Medium	4.2±0.1	Low

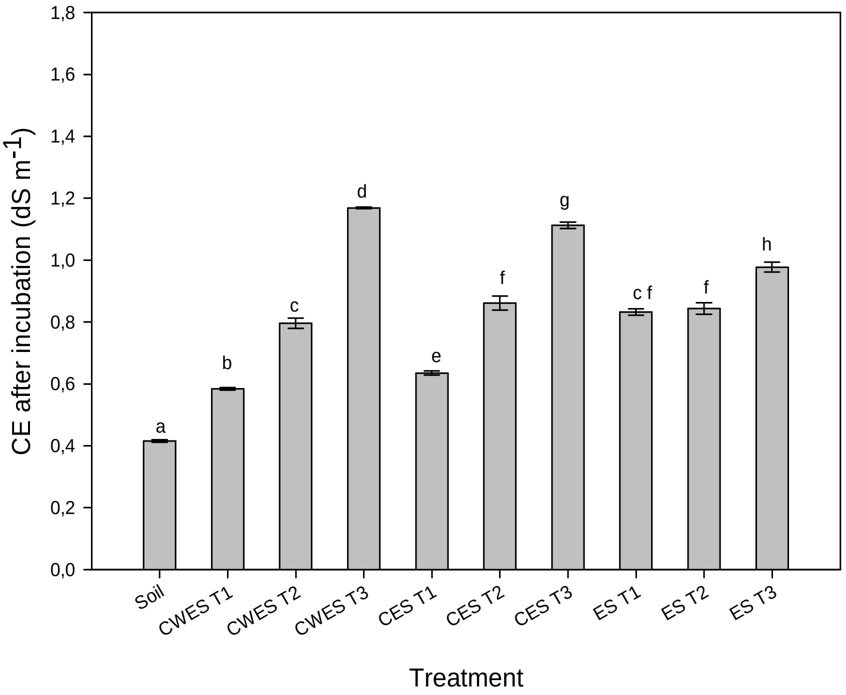

Figure S9.1: Soil characteristics after 94 days of incubation, with tested amendments: electrical conductivity. Bars denoted with the same letter are not statistically different according to Tukey´s test at $P<0.05$.

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Part E. Conclusions and forthcoming work

A general overview of the most relevant conclusions of the work is presented in this part of the thesis. Moreover, some proposals for future work are suggested.

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10. Main conclusions and future work

The production of eggshell waste imposes the need to move towards waste management solutions that boost the retrieval of ES as a resource. In this scope, this study addressed the application of co-composting as a technology capable of promoting ES sanitation and its application as operating supply for environmental remediation.

On the basis of the results of the experiments performed in this study, the following conclusions can be outlined:

- The presence of grass clippings (an N-rich source) in the composting mixture containing ES was determinant to attain the pathogen-killing temperature (>70ºC for 1h) required by regulations in force. However, by increasing incorporation of ES up to 30% (w/w, wet basis) the capacity of the N-rich composting mixture to accomplish that sanitising criteria was not affected.

- In the absence of an N-rich source, ES proved to be as an ingredient that can change the ammonification ability of the mixture, thus increasing the potential for N-losses as ES incorporation increases until 30%ES.

- At a higher level of ES (60% w/w, wet basis), the upper bulk density and lower water holding capacity of the starting mixture were the main drawbacks to the development of an aerobic biological activity, still pathogen-killing temperatures were attained.

- Principal component analysis suggested that physical properties of the mixture are the most relevant for explaining the variability of the process data due to ES content, namely bulk density and moisture content.

- In spite of the starting composting mixture, after more than 90 days of composting all ES composts obtained in the composting tests showed to be stable and without properties that can cause plant injury.

- Organic matter of the eggshell composting product (CES) provided supplementary sites for lead sorption in aqueous media in comparison to untreated ES.

- CES was found to be an effective liming material and revealed intrinsic properties for *in situ* remediation of soil contaminated with Pb and Zn. Despite ES ability to promote remediation of the tested soil was not clearly overcome by CES, its stable organic matter can be beneficial to metal contaminated soils that require organic matter restoration.

This study constitutes a first insight into the feasibility of co-composting industrial eggshell waste (ES) to attain pathogen-killing temperatures in accordance to the legal requirement. The results obtained were promising and envisage that co-composting process can be applicable for high levels of ES. Nevertheless, there are still some issues to be further evaluated, aiming to completely assess the merits of co-composting for managing industrial eggshell waste.

The use of intermittent aeration strategies should be tested in the case of starting mixtures with a lower self-heating potential. These experiments would allow a broader understanding of how the sanitising conditions can be attained, by lowering convective heat losses in comparison to the biological reaction heat. Furthermore, the analysis of the influence of high ES content on air permeability would be important for design and operation of the process.

Industrial eggshell waste has a low C:N ratio and such peculiarity may have a significant impact in the gaseous emission of N_2O, which is a relevant greenhouse gas in the composting process. Therefore a deeper understanding of the nitrogen dynamics during ES co-composting should be tested, by using additional automatic gas analysers. In this case, also the use of intermittent aeration strategies should be addressed as a strategy to promote N conservation during co-composting of ES.

The microbiological analysis (pathogens presence) of the untreated ES and ES rich compost also needs to be performed, to completely evaluate the pathogen-killing effectiveness of the co-composting process. The eradication of plant pathogens should be covered, due to the origin of the organic materials used in this study.

A better understanding of the sorption capacities of eggshell-rich products for lead uptake, should be broaden to other operational conditions, namely pH and liquid to solid ratios. Additionally, other metals and multielement systems can also be addressed.

Moreover, it would be interesting to assess the influence of the liming capacity of eggshell-rich products on the microbial biomass and nitrification of metal polluted soils.

Finally, given that composting is a biological process, the study of ES co-composting is not exhausted within the scope addressed and suggested in this thesis. In fact, the characterisation of the microbiota involved during the all composting phases is essential to better understand the entire process, as well as the properties of the final product. Thus, it should not be set aside from the operational design of such process.