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Storage dynamics and energy potential of lingo-cellulosic biomass from energy crops

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Abstract

Once harvested, the lingo-cellulosic biomass used for energy purposes is comminuted and stored in specific sites before conversion. Storage is a required process of the bioenergy chain since fresh biomass has characteristics such as moisture content that make inefficient the conversion.

However, the conservation of lingo-cellulosic biomass entails different problems such as the risk of self-ignition, the risks for human health during material handling, and the losses of dry matter due to microbial activity. The latter is due to the presence of fungi and bacteria that come in contact with the material during processing (i.e. immediately after chipping) as consequence of the presence of easily accessible carbohydrates. From an economic point of view, this phenomenon is considered a major problem since in a few months the amount of losses can be very significant, reducing dramatically the energy potential of the fuel delivered at the beginning to the storage station. The focus of the Ph.D. program has been the assessment of the phenomena related to the storage of lingo-cellulosic biomass and the evaluation of the respective fuel quality changes and energy losses occurred during the storage period. Indeed, the effects of storage on different types of lingo-cellulosic biomass and different storage forms (chips, chopped, logs) were investigated. In addition, the evaluation of the human exposure to biological risk of fungal spores was performed because such detailed experiences in a storage site were not identified.

The research has been designed in four activities, each one focused on different aspects of the storage process, which will be presented separately. The core of the results are summarized in the final chapter of the thesis; in general, they indicate that the factor affecting storage performance of the biomass can be partially controlled with minimal costs and that storage behavior differ according to storage form or plant part used.

Key words: bioenergy, storage, biomass, chips, microorganisms.

Abstract

Dopo la fase di raccolta, la biomassa ligno-cellulosica da destinare a scopo energetico viene generalmente cippata e stoccata in specifiche aree prima di essere convertita. Lo stoccaggio della biomassa è un processo necessario poiché il fuel allo stato fresco presenta caratteristiche, come l'alto livello di umidità, che rendono la conversione inefficiente. La conservazione delle biomasse ligno-cellulosiche presenta diversi problemi come ad esempio il rischio di autocombustione, il rischio biologico per gli operatori e le perdite di energia causate dalla degradazione della sostanza secca. Quest'ultima è provocata da funghi e batteri che, a seguito della cippatura, hanno facile accesso ai composti zuccherini più degradabili della biomassa.

Da un punto di vista economico, questo fenomeno è considerato il problema principale dato che in pochi mesi l'ammontare delle perdite può essere significativamente elevato, riducendo drammaticamente il potenziale energetico del fuel consegnato in centrale.

L'obiettivo del programma Ph.D. è stato quello di valutare le dinamiche relazionate allo stoccaggio di biomasse ligno-cellulosiche e delle relative perdite e cambiamenti prodotti sulla qualità del fuel. Gli effetti dello stoccaggio sono stati analizzati prendendo in considerazione diverse specie di colture energetiche e utilizzando diverse forme di conservazione. Inoltre, l'attività di ricerca ha previsto la valutazione dei potenziali rischi per la salute umana, causati dall'esposizione degli operatori alle spore fungine durante la fase di movimentazione del cippato; tale analisi è stata eseguita su base sia qualitativa che quantitativa. La ricerca è stata divisa in quattro casi di studio, presentati come il risultato di pubblicazioni scientifiche su riviste indicizzate. Gli aspetti più interessanti della ricerca sono stati riassunti nell'ultimo capitolo della tesi; questi mostrano che le performance di stoccaggio del fuel possono variare considerevolmente a seconda della specie, della forma del prodotto e della porzione di pianta utilizzata, ma che questi, in alcuni casi, possono essere controllati, anche con costi contenuti.

Key words: bioenergy, storage, biomass, chips, microorganisms.

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1. Introduction and objectives

1.1 Biomass as source of renewable energy: potential and policy in EU

The increasing security in energy supply is set as one the main objectives of the EU energy policy. By the year 2020, all members' states are called to produce at least the 20% of the total energy supply just from renewable energies. Today in Europe the average production from renewable is rather below this target, and only few countries have reached the objective. To achieve the European goal, research and development activities on renewable energies must be enforced in order to develop new supply systems as well as to maximize the efficiency of the existing ones (EU 2009).

Among the main renewable source of energy, the biomass is probably the most promising one. According to the EU perspectives, it is expected to cover by 2020 more than half of the 20% EU renewable energy target (Bentsen and Felby 2012) (Figure 1).

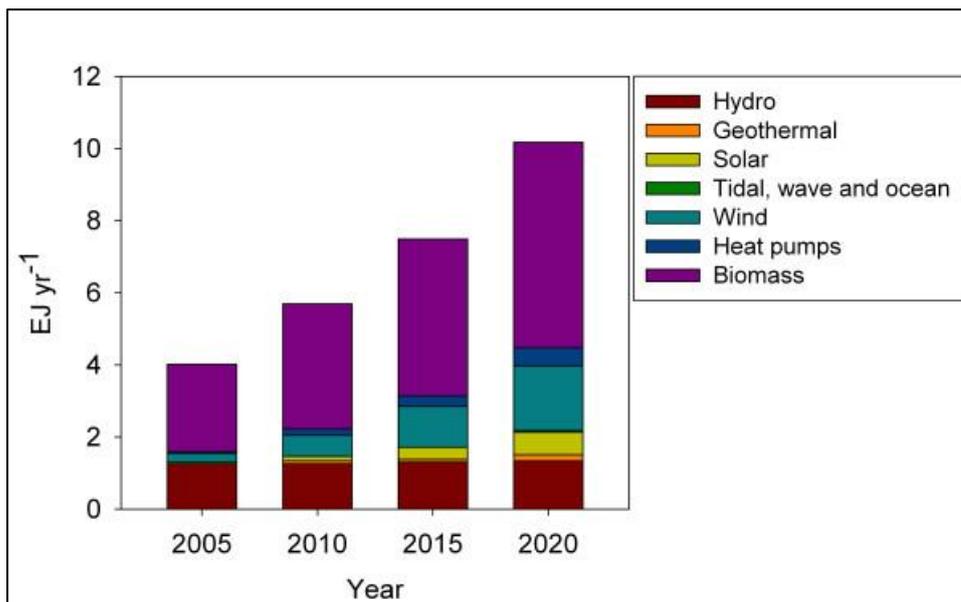


Figure 1: Projections on the stipulated production of energy from renewable resources in the EU27 countries based on national renewable energy action plans.

According to Italian regulations, Biomass for energy is defined as any agricultural and forestry product, any residue deriving from the wood and paper industry, and any organic product deriving from the biological activity of animals and humans such as those coming from urban waste.

Biomass is therefore defined as any organic matter with animal or plant origin intended for energy purposes (DLgs 28/2011). It is possible to classify the biomass in three chains: the wood chain, the agricultural chain deriving from dedicated cultivation (rapeseed, soy, etc.), chain of residues and waste.

Focusing the attention on ligno-cellulosic (LC) biomass coming from arboreal and herbaceous crops, it must be pointed out that for practical reasons these types of feedstock cannot be used in their natural form, but they must be mechanically processed in order to get suitable energetic material.

The basic forms of LC biomass to be used in the large scale energy industry consist of wood chips, hog fuels and pellets. However, depending on geographic location and season, the availability of the feedstock do not always meet the demand for energy production, so there could be periods of the year in which the demand for energy cannot be satisfied (Jirjis 2004). In this regard, identifying efficient biomass conservation methods would guarantee a safe and continuous supply, as required by the EU. However, during storage, the preservation of the energy characteristics of the ligno-cellulosic biomass is usually difficult for different reasons.

1.2 The problem of storage

Beyond particular locations and climatic conditions, the production of biomass takes place all over the year. This almost constant production does not always meet the demand for energy production, which varies country by country according to the geographic location and season. For instance, the Northern European countries that suffer hard climatic conditions concentrate the energy demand in specific periods of the year, which generally correspond to the coldest months.

This unbalance implies the need of energy storage to guarantee constant production also during the period of highest demand. However, unlike other forms of energy, the electricity cannot be accumulated, so the energy stored in form of biomass represents a way to meet the demand of the peak periods. In figure 2 are shown the data concerning the electricity supply in 2015 the principal European countries.

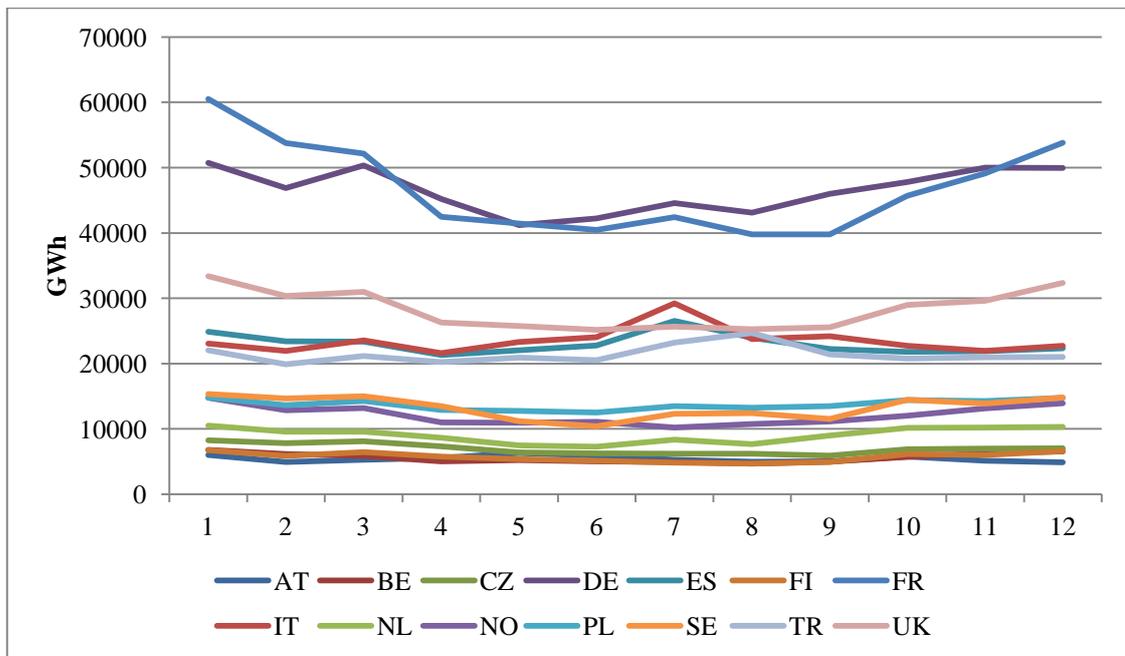


Figure 2 - Monthly electricity supply in the principal European countries in 2015.

Another reason that implies the need of biomass storage is the initial moisture content of the fuel delivered to the power plant. Usually, the moisture content of fresh biomass is about 40-50%, but to obtain an efficient conversion the level of moisture content should be below 30% (Kofman, 2006). The storage process allows the material drying because wood chips exposed to wind and sun tends to dehydrate.

Finally, a short term period of storage at the storage site is physiologic because biomass power plant can process only certain amount of fuel per day and delivery just in time is almost impossible. Indeed, power plants have always a buffer zone for the storage of fuel in order to be less vulnerable for fluctuations and delays in the delivery chain. (Thörnqvist and Jirjis, 1990).

The fuel can be stored at the heating plant in many different ways depending on the type of fuel and the local conditions. They could be stored uncomminuted or comminuted. They can be stored outdoors or inside in barns, silos or container. Wood powder and pellets can be stored in silos. Large scale wood chip or hog fuel storage are in almost all cases stored outdoors in piles. There are other storages methods such as in piles under roofs, but these methods are for technical and economical reasons not used for large quantities.

There are several problems related to the storage of wood chips and hog fuels in piles: among the most significant there are the risk of self-ignition, the losses of dry matter and the risks for human health during material handling.

1.2.1 Risk of self ignition and thermogenesis

The thermogenesis in stored biomass is linked to the development of heat started by the respiration of the cells of the wood structure still alive and continued by the biological activity of microorganisms, chemical oxidation and acid hydrolysis of the cellulosic components of the wood.

All these processes release heat, especially in the deeper parts of the pile, which are more isolated from ambient conditions and, for that reason, more convenient for the first development of microorganisms (Ericson 2011). The heat produced creates a difference in temperature between the inside and the outside and so a gradient of pressure, provoking the ascension of warm moist air current to the higher part of the pile and the entrance of external air to the lowest part of the pile (chimney effect).

The temperature acquired during thermogenesis and the time it is maintained during the storage period depends on the characteristics of the biomass itself (composition, moisture content and particle size distribution) and the ones belonging to the pile (shape, dimension) (Thörnqvist 1985). However, when the thermal balance between heat generated and heat being dissipated is not offset, problems of self-ignition may take place (Armstrong 1973).

1.2.2 Energetic losses due to microbial degradation

The presence of microorganisms (mainly aerobic) is developed in the first step of the material processing, i.e. immediately after chipping as consequence of the presence of easily accessible carbohydrates (Ericson 2011). Moisture content and nitrogen amount are both critical factors affecting the entity of colonization degree. Microorganisms are fed by vegetable material, need oxygen and as a consequence of respiration process generate heat, carbon dioxide and water vapor.

Apart from the release of heat by microorganism, thermogenesis also includes the development of chemical reactions (oxidation and acid hydrolysis). In general, most

chemical reactions increase in importance if a previous initial degradation by fungi has occurred (Armstrong 1973).

These modifications in the composition of the biofuel in most cases mean a degradation of their energetic and physical characteristics such as a decrease of the heating value and an increase of the ash content and fine particles.

1.2.3 Health risks related to storage of biofuel

Large-scale biomass storage sites may represent a “reservoir” of fungal spores, similarly to composting and recycling plants. Moving large quantities of wood materials leads to the release into the air of a high concentration of spores that may then be inhaled and ingested by workers and/or deposited onto their skin and eyes.

Since airborne mold spores have a diameter of only 2-10 µm and are ubiquitous, they can easily penetrate into the lower airways of the human respiratory tract, causing severe diseases like invasive pulmonary infection, or contributing to allergic sinusitis and allergic broncho-pulmonary diseases (O’Gorman 2011).

1.3 The Characteristics of lingo-cellulosic biomass and their relation with the storage performance

All woody material consists of water, burnable substance and ash. Basically dry material has about 52 % carbon, about 42 % oxygen, 6 % hydrogen and a few percent of minerals.

In general, the LC biomasses have similar major components but in different proportions. Some differences can be identified between fuels according to the age or part of the plant that is studied. The principal components in LC biomasses are carbohydrates (cellulose and hemicelluloses), lignin and extractives.

The cellulose is composed approximately by 10.000 glucose units, while hemicellulose includes in its composition also xylose, mannose, galactose, and arabinose. The lignin is formed by several units of phenolpropane, which furnish to the material its mechanical strength due to its gluing effect to the cellulose.

The extractives mainly include terpene, phenol and different types of fats. The amount of the singular component varies in the woody cells according to age, species and plant part, but in general the cellulose represents about 40% of the dry weight, hemicellulose about 30%, lignin about 20-30% and the extractives 2-4%.

The heating value of the biomass varies according to the amount of these components. For instance, cellulose has an energy content of 17-18 MJ/kg, hemicellulose around 16-17 MJ/kg, lignin around 25-26 MJ/kg DM, while the extractives around 33-38 MJ/kg DM (Strömberg, 2005).

1.3.1 Fungal activity

According to Noll and Jirjis 2012, the establishment of microbial communities in wood chip piles and wood logs are due to the following aspects:

- surface area of wood particles (e.g. microorganisms present on and in bark, needles or leaves);
- previous microbial colonization of inner parts of wood (e.g. tunneling bacteria and wood-rotting fungi transported by arthropods or other vertebrates);
- atmospheric deposition by rain;
- transportation into the wood chip piles and wood logs by wind;
- active microbial immigration from nearby environments such as the belowground.

The particle size of the stored biomass, as well as particle size distribution, plays a major role in the microbial establishment and growth. Chipping or crushing forest residues or other woody biomass, especially freshly harvested material, damage plant cells and release its soluble contents.

The enhanced accessibility of substrates in comminuted wood chips compared to wood logs may explain shifts in microbial community composition. Moreover, the comminution process greatly increases the surface area of the biomass which facilitates extensive colonization.

Storage of woody biomass is a multi factorial environment that determines the presence and activity of the microbial community. For this reason, a universal decomposition patterns in wood chips and logs are unlikely. However the decomposers of the main wood

compounds are white and brown-rot fungi (Rajala et al. 2010; Noll et al. 2010). White-rot fungi degrade mainly lignin, and at a lower rate also hemicellulose and cellulose, while brown and soft-rot fungi remove cellulose and leave lignin essentially unchanged (Schwarze 2007).

1.3.2 Particle size

According to the machine used for harvesting, the comminuted LC biomass will take the form of wood chip or hog fuel. Basically, wood chips consist of chipped woody biomass in the form of pieces with a defined particle size produced by mechanical treatments with sharp tools such as knives. They have a sub-rectangular shape with typical length 5 to 50 mm and low thickness compared to other dimensions. Hog fuel is wood fuel in the form of pieces of varying size and shape, produced by crushing with blunt tools such as rollers, hammers, or fails (Alakangas, 2014) (Figure 3).



Figure 3 - Visual differences between wood chip (left) and hog fuel (right).

To determine the particle size distribution, the biomass is sieved through a range of sieve sizes. The amount retained on each screen is divided by the total weight of the sample to give a percentage. The new criteria of classification is based on EN ISO 17225 standard. Table 1 shows the dimensional classes that have been defined in the new standards.

Table 1 - Dimensional classes according the new classification (Alakangas, 2014).

Class	Main fraction (at least 60%) mm	Course fraction (mm)	Maximum length for oversized particle, mm	Cross sectional area of course fraction m ² Only for EN ISO 17225-4 standard
P16S	3,15 <P ≤ 16	≤6% >31,5 mm	≤45 mm	≤2
P16	3,15 <P ≤ 16	≤6% >31,5 mm	≤150 mm	
P31S	3,15 <P ≤ 31,5	≤6% >45 mm	≤150 mm	≤4
P31	3,15 <P ≤ 31,5	≤6% > 45 mm	≤200 mm	
P45S	3,15 <P ≤ 45	≤10% >63 mm	≤200 mm	≤6
P45	3,15 < P ≤ 45	≤10% >63 mm	≤350 mm	
P63	3,15 <P ≤ 63	≤10% >100 mm	≤350 mm	
P100	3,15 <P ≤ 100	≤10% >150 mm	≤350 mm	
P200	3,15 <P ≤ 200	≤10% >200 mm	≤400 mm	
P300	3,15 <P ≤ 300	To be stated		

Analysis according to EN ISO 17827-1/EN ISO 17827-1
Classes marked by S are for EN ISO 17225-4 standard (for small plants)

As for the previous classification system, the letter P before the number indicates the Particle size. On the other hand, the introduction of the letter S after the number reflects a new criterion that has been introduced for small size plants. The variation has been applied because small size plants do not tolerate large size material, so the main requirement in P16S and in P31S is the absence of fractions larger than 45 and 150 mm respectively. On the contrary, in P16 and P31 toleration until 150 and 200 mm is included. In table 2 are shown the criteria of material classification also for moisture content (M) and ash (A).

Table 2 - Material classification according to moisture content and ash content (Alakangas, 2014).

Class, M	w-%	Class, A	w-% dry basis
M10	≤ 10	A0.5	≤ 0.5
M15	≤ 15	A0.7	≤ 0.1
M20	≤ 20	A1.0	≤ 1.0
M25	≤ 25	A1.5	≤ 1.5
M30	≤ 30	A2.0	≤ 2.0
M35	≤ 35	A3.0	≤ 3.0
M40	≤ 40	A5.0	≤ 5.0
M45	≤ 45	A7.0	≤ 7.0
M50	≤ 50	A10.0	≤ 10.0
M55	≤ 55	A10+	> 10.0*
M55+	> 55*		

* Maximum value to be stated.

Generally, each biomass power plant requires specific nominal size of the fuel. For instance, small boilers (<250 kW) requires a nominal size of 8-15 mm. The amount of oversize particles should be restricted, which means avoiding lumpy chips or overlong particles (longer than 10 cm). Oversize or overlong particles can clog the auger feeding the boiler.

For medium boilers (250 kW<X<1 MW) a nominal size of 8 to 25 mm can be used. The demands on the amount of oversize and overlong chips are not as stringent as for the small boilers. The augers feeding this type of boiler are much larger and more robust than for the small boilers. Too many overlong particles will, however, increase the tendency of the fuel to bridge over openings, which might cause the boiler to stop because of a lack of fuel.

For large boilers (>1 MW) a large nominal size of 25 to 35 mm is usually required. There are hardly any restrictions on oversize or overlong particles, because this type of boiler is usually fed by a crane into a hopper and from the hopper by hydraulic ram into the boiler. Even though the requirements are low, too many overlong particles might get the fuel to bridge over the infeed hopper and thus cause the boiler to stop because of a lack of fuel (Kofman, 2006).

The particle size is an important parameter that can significantly affect the storage performance of the biomass. Firstly, the chip size influences the exposed surface area of the material to air influencing the heat release occurring immediately after chipping through the respiration of the crop. Secondly it can influence the degree of microbial colonization and consequently further heat release.

In general, the production of small size chips creates many fine fractions, limiting the air penetration and the heat dissipation in the piles. The air penetration is responsible of the chip drying process which in turn determines the entity of the microbial attack.

Jirjis in 2005 tested the storage of comminuted *Salix viminalis* creating chips (particle size between 7 and 16 mm) and chunks (particle size between 22-45 mm). The study showed that microbial growth during the first two months of storage was clearly higher in the chips compared to the chunk wood.

1.3.3 Moisture content

LC biomass is not typically found in the oven-dry state, but it has a moisture which may vary from 60 to 15% depending on the duration of open-air seasoning. In arboreal plants, the wood behaves as a hygroscopic material and, due to its chemico-histological structure, it has two different types of porosity:

- the macroporosity created by the cavities of the conductive vessels and by parenchymal cells containing free (or imbibition) water.
- the microporosity of the actual wood substance (mainly cellulose, hemicellulose and lignin), which always contains a certain amount of bound (or saturation) water.

Wood begins to lose water from the moment the tree is cut down. First, imbibition water evaporates from the outermost (sapwood) and, later, innermost (*duramen*) parts of the trunk. At a certain point in time, all free water in seasoned wood evaporates, while saturation water reaches a dynamic balance with the outward moisture, reaching a value below 20%.

Wood moisture is expressed as a percent and is calculated using these two formulas:

Moisture on dry basis is indicated as u (%) and represents the mass of water present in relation to the mass of oven-dry wood. The formula for its calculation is the following:

$$u = \frac{W_w - W_0}{W_0} \times 100$$

W_w = wet weight of wood

W_0 = oven-dry weight of wood

On the other hand, moisture content on wet basis is indicated as M (%) and represents the mass of water present in relation to the mass of fresh wood. This measure is used in the marketing of wood fuels. The formula used for its determination is the following:

$$M = \frac{W_w - W_0}{W_w} \times 100$$

W_w = wet weight of wood

W_0 = oven-dry weight of wood

Assuming that the mass of newly-chopped fresh wood is made up half by water and half by wood substance, wood has a moisture on w.b. (M) of 50% and a moisture on d.b. (u) of 100%.

1.3.4 Heating value

The heating value or calorific value of a fuel represents the amount of energy released during the complete combustion of a mass unit. Regarding LC biomasses, the heating value depends by several factors such as biomass type (species, plant parts), moisture content, bulk density, storage before conversion.

The biomass type affects heating value because the distribution of the components such as lignin, cellulose and hemicelluloses varies according to species and plant parts. Lignin is the component having higher energy potential (27 MJ/kg), while cellulose and hemicelluloses have respectively 17.2-17.5 MJ/kg and 16 MJ/kg. Therefore, a biomass type having high lignin content in proportion respect the other components, will have more energy potential respect a species that in proportion has more cellulose and hemicelluloses.

Another important parameter affecting the heating value of the LC biomasses is the moisture content, because part of the energy released during the combustion process is spent for the evaporation of water. Water evaporation involves the 'consumption' of 2.44 MJ per kilo of H₂O. It is thereby possible to distinguish between the following:

- **Higher heating value (HHV):** in this case the energy deriving from the combustion process includes the energy spent for water evaporation
- **Lower heating value (LHV):** in this case the energy obtained from the combustion of the biomass does not include the energy spent for the evaporation of water.

It widely accepted the LHV is the main reference parameter used to describe the fuel quality. In order to calculate the LHV (MJ/kg) of wood with given moisture content (M) the following formula is used:

$$LHV_m = \frac{LHV_0 \times (100 - M) - 2,44 \times M}{100}$$

LHV_m= Lower heating value of the wet biomass

LHV0= Lower heating value of the dry biomass

M= Moisture content of the biomass

In figure 4 it is shown the heating value as a function of the moisture content.

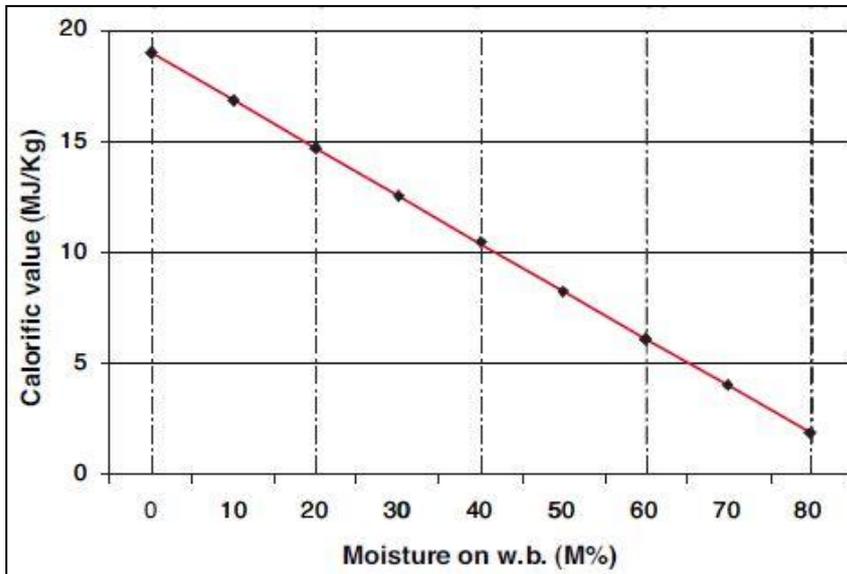


Figure 4 - Heating value as function of the moisture content.

The third parameter affecting the heating value is the bulk density of the fuel. The bulk density is expressed as kg of biomass/m³; obviously, as the bulk density is higher, the energy produced per kg of biomass will be higher.

Finally, the storage is the last parameter that can affect the heating value of the fuel. In fact, during storage, the microorganisms that colonize the biomass can be specialized for the degradation of one component than another. For instance, as described in paragraph 1.3.1, the white-rot fungi degrade mainly lignin, while brown and soft-rot fungi remove cellulose and leave lignin essentially unchanged. Indeed, the attack of a species respect another determines the reduction of a specific component respect another influencing the final heating value of the fuel.

1.3.5 Ash content and ash melting point

Ash is the general term used to describe the inorganic matter in a fuel. In biomass fuels, the ash content may originate from the biomass itself, e.g. materials that the plant absorbed from the water or the soil during its growth, or from the supply chain, e.g. soil collected along with biomass. In any case, after the collection of a sample the ash content is typically

measured by combusting the biomass at a laboratory furnace under controlled conditions, taking into account the relevant standard EN 14775.

It is important to notice that the ashing temperature for biomass fuels is 550 °C, lower than the typical ashing temperature for coals, which is 780 °C. Generally, the ash content of herbaceous biomass is higher than that of woody biomass. Among solid biofuels, wood without bark is the one with the lowest ash content, whereas agricultural biofuels typically have high ash content. While ash weight content (in dry basis) values of less than 1% are expected for wood, different herbaceous biomass types have reported values ranging from less than 2% up to 8 – 10 % or even up to 25% for rice husks.

During combustion, some physical modifications in the ashes may occur. In fact, with the rise in temperature, they soften until the complete fusion of the particles is reached. Using fuels with low ash fusion temperatures increases the risk of ash slagging being formed on the grate. Fusion slags disturb the combustion process by altering primary air flows and favoring the overheating of the grate as well as corrosive phenomena.

Wood and bark have a relatively high melting point (1,300-1,400°C) and thus do not have any criticalities. On the contrary, the melting point of herbaceous plants is below 1,000°C and, consequently, slags can easily be created during combustion. In the case of cereal (grains), the melting point is lower than 750°C and is, thus, particularly critical.

For the reasons listed above, agricultural biofuels have higher criticalities as compared to wood, and are only to be used in specific combustion devices. Ash can be divided into two categories:

- 1) Bottom ash - it is a considerable portion of the ash that gathers under the boiler grate and it is channelled into a storage tank. It has a mass density of 1.3 t/m³.
- 2) Fly ash - it is the ash that derives from flue gas cleaning and can further be divided into cyclone light ash and fine particles from electrostatic and bag filters. It has a mass density of 0.8-0.9 t/m³.

Table 3 shows the heating value, the ash content and the ash melting point of different biomasses.

Table 3 - Heating value, ash content and ash melting point of different biomass sources (Strömberg B., 2005)

	NCV ₀ MJ/kg	Ash (wt% d.b.)	Ash-melting point (°C)
Typical values for virgin wood materials Coniferous wood	19.2 (18.8-19.8)	0.3 (0.2-0.5)	
Typical values for virgin wood materials Deciduous wood	19 (18.5-19.2)	0.3 (0.2-0.5)	
Typical values for virgin bark materials	20 (19-21)	4-5 (2-10)	
Typical values for virgin wood materials Logging residues	19-20	1.5-2	
Typical values for virgin wood materials Short rotation coppice (SRC) (Willow and Poplar)	18.6-19.2	2	
Spruce (with bark)	18.8	0.6	1,426
Beech (with bark)	18.4	0.5	1,340
Poplar (SRC)	18.5	1.8	1,335
Willow (SRC)	18.4	2.0	1,283
Bark (coniferous trees)	19.2	3.8	1,440
Vine wood (chips)	19.8	3.4	1,450
Miscanthus	17.6	3.9	973
Wheat straw	17.2	5.7	998
Triticale (grains)	16.9	2.1	730
Rape cake	21.2	6.2	-

1.4 Purpose of the research work

The research program has been focused on the assessment of the phenomena related to the storage of LC-biomass and on the evaluation of the respective fuel quality changes and energy losses occurred during storage. For such a purpose, three case studies were performed as follows:

- 1) Storage test last six months carried out with poplar wood chips piles deriving from different tree parts (stem and crown). The effects of storage were evaluated with respect to fuel quality and dry matter losses due to microbial degradation.
- 2) Storage test last six months carried out using whole poplar stems. The reduction of moisture content and dry matter losses were evaluated as for the previous study to understand differences in storage dynamics of different products (chips vs whole stems).

- 3) Storage test last three months using comminuted biomass of the perennial grass *Arundo donax L. L.*. The aim of the study was to investigate, in small-scale, different storage systems assessing the dynamics of storage and their effects on energy losses and fuel quality.

Another important objective of the Ph.D. has been the study of the occupational exposure of workers during the handling of the wood chip in the storage site. In fact, when lingo-cellulosic biomasses are stored, they may become a reservoir of microorganisms. The contact with these biologic entities can represent a “biologic risk” for workers. For this reason, another activity has been performed and it is indicated as follows:

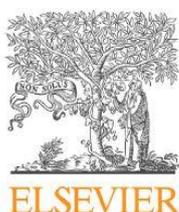
- 4) Analysis of a wood chip storage station as “working environment”, identifying and quantifying the microorganisms present in bio-aereosol surrounding the storage site.

The four research activities that have been indicated will be described separately as chapters of this Ph.D. thesis.

Chapter 2: Storage dynamics and fuel quality of poplar chips

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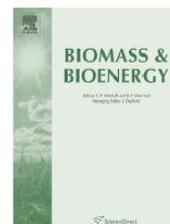
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Storage dynamics and fuel quality of poplar chips



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ABSTRACT

Poplar cultivation for wood/timber production has a growth production cycle of about 10–15 years. Usually the stem is separated from the crown and used to produce material of different kind such as veneer, pallets, panels, etc. For wood industries, crowns generally represent waste material to be disposed of, causing economic and time losses. It is generally believed that the costs of managing crown biomass are higher than the potential incomes obtainable. Nonetheless, it is worthwhile investigating the possibility of using these byproducts as energy source and evaluating their value as a fuel. However, storing such residues presents several problems connected with spontaneous microbial degradation.

The aim of this work was to evaluate the storage effects on chipped biomass deriving from the crown and stem wood of poplar and how they affect fuel quality and dry matter losses.

A storage trial was carried out with three piles of stem wood chips and three of crown chips coming from a 15 year old poplar plantation. The piles were stored outdoors for six months under the same climatic conditions.

The effect of storage on fuel quality was evaluated with respect to moisture content, gross and net calorific values, chemical composition, ash content, and bulk density.

The variation of temperatures inside each pile due to heat development was continuously monitored and showed different trends between piles depending on source material. Results showed that chips from crown material had better storage properties and exhibited lower decay than chips from stem wood.

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2.1 Introduction

Italian poplar plantations cover about 120.000 hectares and represent 1.3 % of the national forest surface (Spinelli et al., 2011). Even if they cover a very small surface, poplar crops play a major role in the domestic raw material supply for the national wood industries. Due to their intense photosynthetic activity, poplar plantations also play an important role in CO₂ sequestration and climate change mitigation (Zenone et al., 2007).

The strategic importance of poplar for industrial wood supply is recognized in many other countries, such as China, France, India, and Turkey – each producing more than 1 million m³ of poplar wood per year from specialized plantations (Spinelli et al., 2008).

Poplar wood has many potential uses, and even the longest rotation cycles are relatively short compared to those of most other trees; this can favour their integration with agricultural systems (Ulrich, 2004). Managed on 10 to 30 years rotations, selected poplar clones can be used for different purposes. The tree portion utilized is the stem, which is separated from the crown, and prepared for production of veneer-grade logs, boards, boxwood and pulp material.

In contrast, the physical characteristics of tree branches make it unsuitable for industrial use, resulting in a low economic value. In many cases, branches and tops represent a waste, and its disposal incurs additional costs (Manfredi, 2011). Studies have shown that branch wood may account for up to 40 % of the total biomass available on the plantation (Federlegno, 2011). Disposing of such a quantity of wood could be costly and time-consuming, if profitable end-uses are not identified.

A possible outlet for branch wood is offered by the particle board industry, where poplar residues are chipped into very fine particles and used to produce boards for various uses (Jahan-Latibari and Roohnia, 2010). However, studies have confirmed that delivery to the particle board industry offers very little benefit to plantation owners, due to the high costs of transportation and handling (Ondro, 1989).

Despite the widespread presence of poplar plantation across the world, it is clear that the identification of a viable use for harvesting residues is crucial to make poplar plantations a more attractive investment (Vietto et al., 2011).

In this regard, the increasing demand for renewable energy across Europe may offer new perspectives to this product. The Renewable Energy Directive 2009/28/EC sets new and ambitious targets for all member states, with the goal of increasing the share of renewable energy to 20% of the total by the year 2020 (European Parliament, 2009).

Within this context, the identification of new renewable energy sources is considered a priority. There is now much interest for harvesting residues (pruning, crowns, and stumps), because they are available in large quantities and their recovery can give a dramatic boost to the production of renewable energy (FAO, 1990).

However, forest operations are often seasonal, which entails the need for storage. In turn, wood storage is a complex business, whose economical efficiency is affected by many factors, and especially handling cost and product susceptibility to decay.

Wood fuels can be stored before or after comminution. Storage before comminution helps in minimizing decay by reducing the surface area exposed to potential microbial degradation (Afzal et al. 2010). However, handling cost is higher for uncomminuted wood than it is for chips (Manfredi et al., 2011). It is therefore necessary to find ways to store comminuted wood, such as chips, without causing biomass loss or a quality decline.

Unfortunately, wood chips are extremely vulnerable to microbial degradation during storage, which often leads to high dry matter losses, reduction of energy value, risk of self-ignition, and potential human health risk due to exposure to airborne microspores.

Several investigations have been carried out to evaluate the changes in fuel quality and dry matter losses which occur during storage of comminuted wood (De Toro et al., 1994; EU, 2009; Ericson, 2011; Ilder et al., 2005; Jirjis et al., 2005; Jirjis et al., 2008; Jirjis, 2001; Jirjis and Theander, 1990; Samuelsson et al., 2006; Thörnqvist, 1990, Nellist et al., 1993). However, many of these studies have focused on storage of whole tree wood chips, and on species other than poplar. Moreover, there is little information available concerning the conservation capacity of comminuted poplar crown wood and its susceptibility to microbial degradation.

Nowadays, the international literature on wood chips degradation suggests that the material should be stored in conical piles, under aerated sheds (roof); eventually the material can be covered with transpiring sheets such as Gore-Tex or Top-Tex (Barontini et al., 2013). However, these measures are often very costly and the advantages gained do not always

justify the expense (Bonari et al 2009). Therefore, further studies are still needed to define cost-effective systems for fuel chip storage. This will be crucial for the future exploitation of poplar residues.

This study aims at determining the storage dynamics of poplar chips from two tree parts; stem and crown, with respect to internal heat development in the storage piles, changes in moisture content, dry matter losses and fuel quality. It is particularly important to know if, under the conditions typical of Southern Europe, chips obtained from poplar tree crowns are more difficult to store compared to that of poplar stems. Such information may help devising better storage techniques for chips obtained from energy plantation.

2.2 Material and methods

The storage trial was conducted in Central Italy at Cra-Ing Research Station in Monterotondo (42° 10' 19'' N latitude, 12° 62' 66'' E longitude). The study compared the storage behavior of wood chips produced from two different parts of poplar trees (*Populus x canadensis* M.): stems with a top diameter > 20 cm, and crown wood smaller than 20 cm in diameter. The threshold of 20 cm was chosen because it corresponds to the average branching point of the studied trees.

Stems and crowns, completely free of leaves, were chipped separately using the same machine, (Pezzolato), a commercial drum chipper. Details of the chipping operations have been published separately (Assirelli et al., 2013). The chips were used to build three large-scale piles for each tree part (stem and crown).

The experimentation ran between March and September 2012. Considering the importance of producing good quality fuel at a minimum cost for storage facility, 6 plastic sheets (1 per pile) were used as storage floor. Plastic sheets are less suitable than asphalt due to their low drainage properties, but they offer a much cheaper way to avoid direct microbial contamination from the soil.

Experimental piles

All piles were 10m long, 8m wide, and 4m high, with a mean volume of 117 m³ of loose chips (Figure 5). During the entire storage period, all piles were exposed to the same climatic conditions (Figure 6).



Figure 5- View at soil level of the wood chip piles.



Figure 6 - Aerial view of the wood chip piles.

Climatic data were recorded using a weather cab “SIAP-MICROS DA9000” certified by SIAN (National Informative System for Agriculture). This was located approximately 800 m from the storage site (Lat. 42° 05', Long. 12° 38', Alt. 51 m AMSL). The collected data including local microclimate, precipitation, external temperature, and air humidity were recorded during the entire storage period.

Each pile was divided into two equal semi-piles, using a plastic net. This was done to produce two semi-piles, which could be sampled at different times. Therefore, we opened and sampled 6 semi-piles after six months, leaving the remaining semi-piles for future studies. The piles were marked with sampling points at three levels: 1.0, 2.5 and 3.5 m from the ground and identified as L1, L2 and L3 respectively. The points within each layer are shown in figure 7.

Parameters studied

At each measurement point we placed a thermocouple (model PT 100) and 4 plastic net bags filled with fresh chips. Each bag was weighed and the chips moisture content was determined at the beginning and the end of the trial. In order to monitor heat development during storage, thermocouples were placed close to the sampling bags and the other end was connected to a computerized control unit that transmitted temperature data to the office computer in real-time (Figure 8).

Six thermocouples were placed in each semi-pile, with a total of 36 thermocouples in all piles. Around each thermocouple, we placed 4 net bags/ sampling point, for a total of 144 net bags (36 thermocouples x 4 net bags).

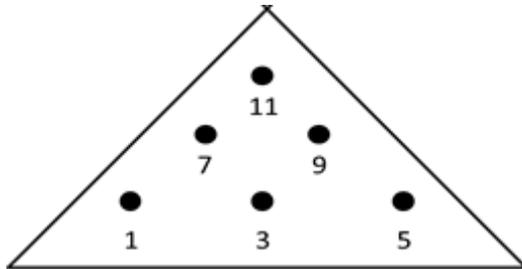


Figure 7 - Arrangement of measurement points in the three layers of the pile.



Figure 8 - Installation of samples and thermocouple in a measurement point.

Fuel quality is defined here by the parameters: moisture content, ash content, heating value (lower and higher) and chemical composition. These parameters were determined both before and after storage, according to the respective European standards UNI EN 14774-2, 14918, 14775, 15104, and 15289. Fuel quality at the beginning of the storage period was determined using 24 chip samples (12 for each treatment) with a weight of 500 g each. Samples were randomly selected and collected from the piles during their construction. Fuel quality after storage was determined using the chips stored in the 36 net bags, collected from the different sampling points.

Dry matter losses and moisture content after storage were determined on the remaining 3 chip bags present in each point of the 6 semi-piles (i.e. 108 net bags). Initial dry weight was calculated using the moisture content of parallel samples of fresh material. At the end of the trial, sample bags were collected and weighed before and after drying in a ventilated oven set at $105 \pm 2^\circ\text{C}$, until they reached constant weight. Dry matter losses were calculated using the following equation (equation 1) (Jirjis and Theander, 1990):

$$\text{DML} = [1 - (\frac{\text{DW}_2}{\text{DW}_1})] \times 100 \quad [1]$$

DML: Dry matter losses [%DM]

DW1: Dry weight before storage [kg]

DW2: Dry weight after storage [kg]

In addition, dry matter losses were also evaluated by comparing the total dry weight of the semi-piles, measured at the beginning and at the end of the storage period. To this purpose, all the chips constituting each semi-pile were weighed by taking the incoming transports to a certified weighbridge.

At the end of the storage trial, when the semi-piles were dismantled, all the chips from each semi-pile were loaded on trucks and taken again to the same weighbridge. The weights were corrected for moisture content to determine the dry weight of each semi-pile before and after storage. The results obtained from this operation were compared with those calculated by weighing the sample bags for validation.

Bulk density was determined before and after storage on both types of materials, according to UNI EN 15103. A steel cylinder of known internal volume was filled with chips and then weighed. Chips were dropped into the cylinder from a height of about 20 cm, to simulate the compacting effect of a conveyor. The ratio between the net weight of the cylinder and its internal volume represented chips density, expressed in kg/m³. Bulk density measurements were repeated 24 times at the start of the trial and 36 times (6 replicates for each semi-pile) at the end.

Particle size distribution was determined on 20 samples (10 per treatment) weighing 1 kg each, by using a mechanical sieve according to UNI EN 14961 [32].

Variations in energy content were calculated with the following equation (equation 2) (Thörnqvist and Jirjis, 1990):

$$\Delta \text{En. \%} = \left\{ \left[\left(1 - \frac{\text{Dry matter losses}}{100} \right) * \text{final LHV} \right] - \text{initial LHV} \right\} \frac{100}{\text{initial LHV}} \quad [2]$$

Final LHV = Lower Heating Value after storage

Initial LHV = Lower Heating Value before storage

Data were analyzed with the Statview advanced statistics software (SAS 1999), in order to check the statistical significance of the eventual differences between treatments. After checking the data for normality, the software was used for performing typical analyses of variance (Anova), especially suited to the experiment just described. Anova tables were drawn, in order to see how the sum of squares was divided between main effects,

interactions and residuals. Before statistical analysis, nitrogen content data were arcsine transformed in order to normalize their distribution.

2.3 Results

Weather conditions

During the storage period the mean daily temperature ranged between 9 and 30 °C, while the accumulated precipitations amounted to 259 mm. Rainfall events were mainly concentrated in the first two months of storage, followed by a drought period of about three months (Figure 9).

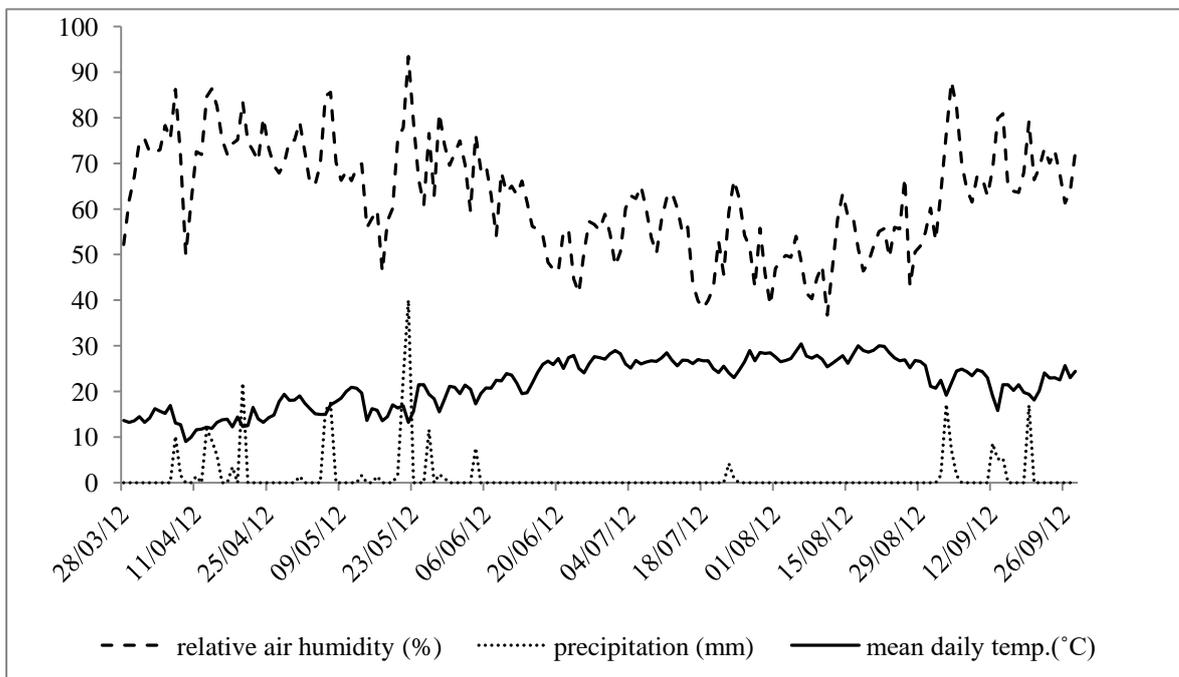


Figure 9 - Climatic characterization of the storage site during the storage period.

Temperature inside the semi-piles

Internal heat development was monitored for the entire period of storage in the six regions of each semi-pile. Figures 6 and 7 show the average internal temperature trends recorded during the storage period in the semi-piles of crown and stem chips, respectively, and it was built by averaging the temperatures recorded for that same level in the three semi-piles. Temperature trends were very similar in the semi-piles of the same material. In contrast, temperature patterns were very much different between the two treatments

(Figure 10 and 11). In particular, the three semi-piles built with crown chips showed a rapid peak near 70 °C in the first week of storage. Then temperature decreased over time and stabilized around the fourth month of storage. Heat development in these semi-piles did not differ much between pile layers.

The temperature inside the stem chip piles did not rise as fast as in crown chip piles, nor did it reach similar levels. In fact, temperatures started from a level lower than the one observed for the crown chips (Figure 11) and the maximum temperature did not exceed 55°C. Furthermore, there was a clear difference between pile layers, with the lower layers (T01, T03, T05) remaining very close to ambient temperatures throughout the whole storage period, and the upper layers (T07, T09, T011) heating up during the initial months in storage.

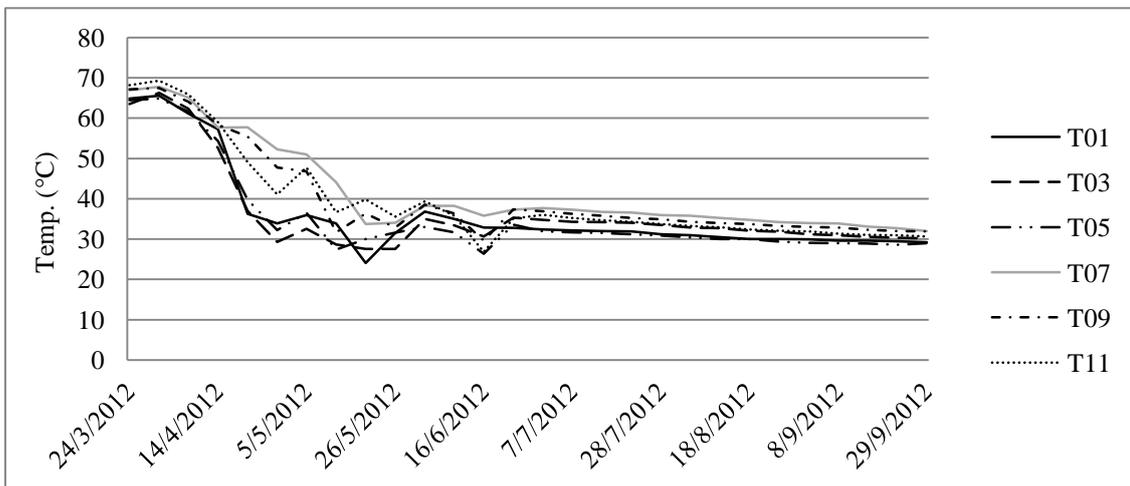


Figure 10 - Temperature changes during storage at the different points of crown wood piles. Each line represents the temperature recorded over time in each measurement point. Each value is the average of the three semi-piles.

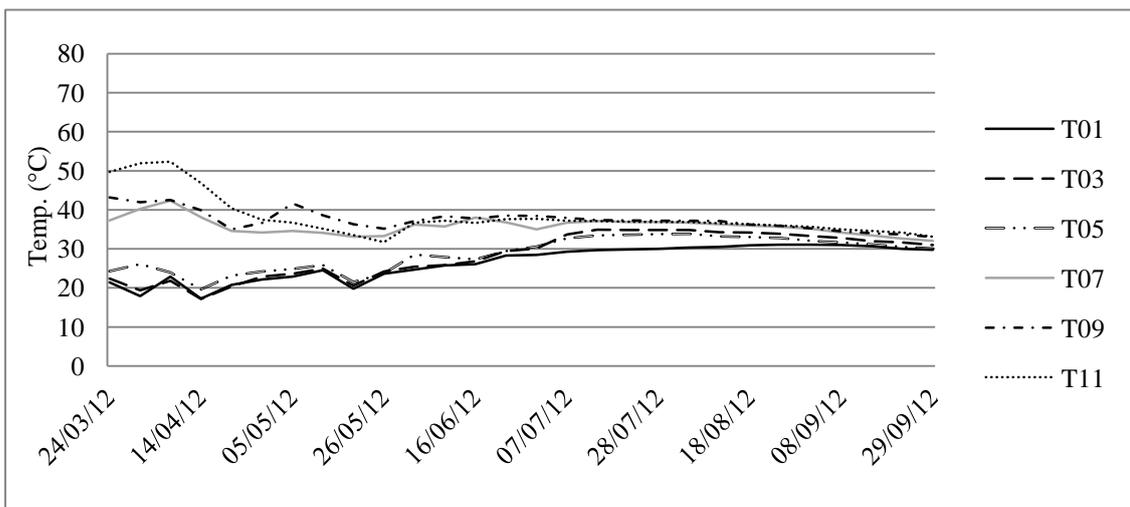


Figure 11 - Temperature changes during storage in the different measurement points of stem wood piles. Each line represents the temperature recorded over time in each measurement point of the pile layers.

Particle size distribution, moisture content, woodchip density and dry matter losses

Particle size distribution did not differ significantly between chips from stem or crown biomass, which implies that pile permeability and air circulation was similar. The most represented particle length was between 8 and 16 mm. Chips within this class represented over 50% of the total weight of the semi-piles, for both material (Figure 12 and 13).

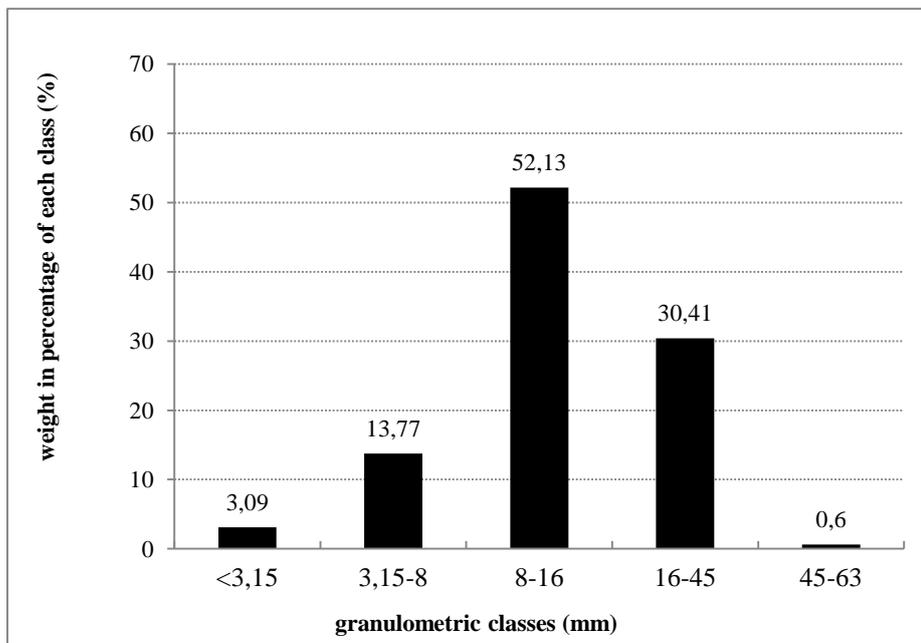


Figure 12 - Particle size distribution of crown woodchips.

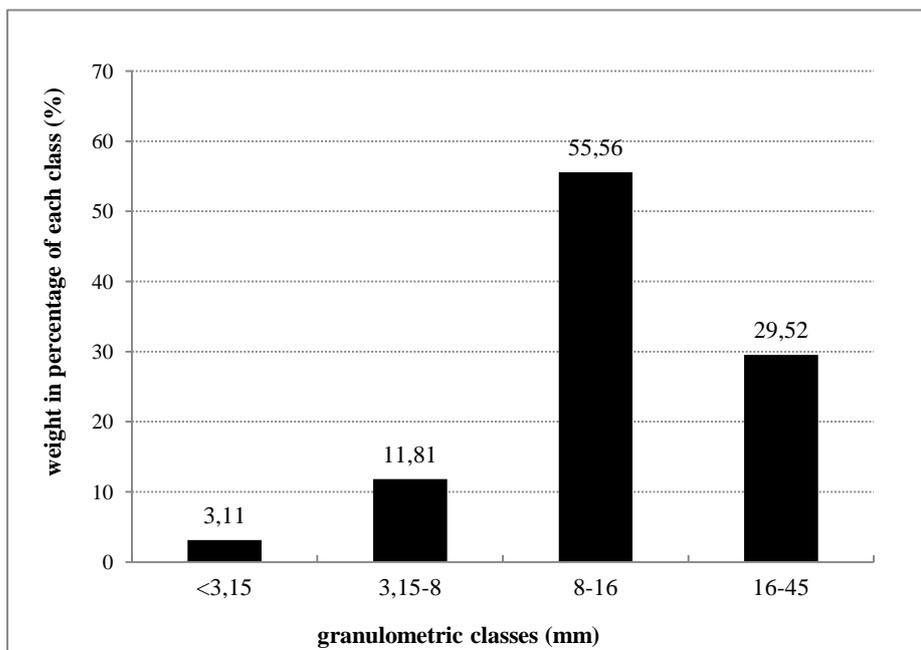


Figure 13 - Particle size distribution of stem woodchips.

Concerning the moisture content (MC), all the tested samples showed a clear reduction in moisture content after six months of storage (Table 4). Despite the higher initial mc of the crown chips, the average MC after storage was lower than that of the stem wood chips.

Analysis of variance confirmed that differences between the two assortments, the two periods (beginning and end of storage) and their interaction were significant at the 1% level. No significant differences in MC were found between different layers at the end of the storage period.

Table 4 - Mean moisture content (\pm Standard.deviation) of the two wood chips assortments at the beginning (T_0) and after six months (T_6) of storage in the three layers.

Source	Moisture content (%)			
	T_0	T_6		
		L1	L2	L3
Stem	47,7 \pm 2,7	35,9 \pm 5,8	33,6 \pm 4,9	31,6 \pm 6,6
Crown	54,2 \pm 1,5	28,3 \pm 3,5	30,3 \pm 1,4	33,7 \pm 2,3

The results of bulk density measurements showed that crown chips had a higher mean value than stem wood chips at the beginning of the trial. However, stem wood had a higher bulk density after storage (table 5). In both cases, bulk density increased after storage, by 25% for stem chips and by 18% for crown chips. Analysis of variance indicated that the differences between the two treatments were not significant, but the differences between the two periods and the interaction of period with treatment were significant at the 1% level. Differences between bulk densities values measured in different layers of the piles were not statistically significant (data are not shown).

Table 5 - Bulk density expressed in kg m^{-3} (\pm St. Dev.) at the beginning (T_0) and after six months (T_6) of storage on both wood chips assortments.

Time	Source	
	Stem	Crown
T_0	327.2 \pm 11.7	341.2 \pm 7.6
T_6	408.0 \pm 19.6	402.3 \pm 20.9

Dry matter losses ranged between 6 and 27% (dry weight basis), depending on treatment and measurement method (net bag samples or whole pile), and they were consistently higher for stem wood chips. Both measurement methods returned similar figures and

trends, validating each other (table 6). Analysis of variance indicated that the difference between treatments was significant at the 1% level.

Table 6 - Dry matter losses (% , dry weight basis) determined by net-bag sampling method and by the total weight of the semi-pile before and after storage.

Source	Net bag				Semi-piles weighing			
	Layer			Mean	Semi-piles			Mean
	1	2	3		A	B	C	
Crown	4	7	9	6.6	11	10	9	10.0
Stem	24	21	20	21.6	26	26	28	26.6

Fuel characteristics

Table 7 shows the main quality parameters for the two chip types at the beginning of the trial, whereas table 8 shows the same parameters at each layer of the piles at the end of storage period. The good results obtained after storage are marked by the heating values of the two chip kinds which increased in both cases.

Table 7 - Chemical analyses and heating values at the beginning (T_0) and after six months (T_6) of storage in the studied wood chip assortments.

Parameter	Unit	Source			
		Crown		Stem	
		T_0^*	T_6^{**}	T_0^*	T_6^{**}
Ash	% s.s.	$2,91 \pm 0,70$	$3,09 \pm 0,54$	$3,02 \pm 0,30$	$3,20 \pm 0,73$
Nitrogen	%	0,32	0.20	0,21	0.15
Carbon	%	47,70	46.88	47,20	47.81
Hydrogen	%	5,90	6.07	5,96	6.16
Sulfur	%	0,03	0.03	0,03	0.03
HEATING VALUE	-				
Higher Heating Value	MJ/kg d.w.	$16,28 \pm 0,29$	$17,18 \pm 0,25$	$15,61 \pm 0,64$	$17,69 \pm 0,34$
Lower Heating Value	MJ/kg d.w.	$15,02 \pm 0,31$	$15,87 \pm 0,26$	$14,34 \pm 0,57$	$16,35 \pm 0,33$

Concerning the analysis of the chemical elements (C, H, N, S), the only significant difference between the two chip types at the beginning of the trial is in Nitrogen content, which was 0.32% in the crown wood chips and 0.21% in the stem wood chips. After six months, Nitrogen content had decreased significantly, and it was 0.21% in the crown wood chips and 0.15% in the stem wood chips. Nitrogen content differences between tree part and sampling time (initial vs. T_6) were statistically significant at the 5% level, according to

the Scheffe's post-hoc test. After six months in storage, a slight increase in ash content for both chip types was also observed.

Table 8 - Chemical analyses and heating values of stem and crown wood chips after six months of storage. The values are reported for each semi-pile layer.

Source	Layer	Parameter						
		Ash (%)	N (%)	C (%)	H (%)	S (%)	HHV (MJ/kg)	LHV (MJ/kg)
STEM	1	3,70 ± 0,60	0,20	47,2	6,0	0,030	17,08 ± 0,20	15,74 ± 0,22
	2	2,50 ± 0,23	0,10	47,5	6,1	0,020	17,27 ± 0,27	15,98 ± 0,25
	3	3,00 ± 0,40	0,16	47,9	6,3	0,030	17,32 ± 0,33	16,04 ± 0,30
CROWN	1	3,20 ± 0,30	0,21	47,7	6,2	0,030	17,83 ± 0,28	16,49 ± 0,28
	2	3,00 ± 0,80	0,20	45,2	5,9	0,030	17,61 ± 0,42	16,27 ± 0,41
	3	2,70 ± 0,10	0,20	47,8	6,2	0,030	17,43 ± 0,13	16,10 ± 0,08

After six months of storage, changes in energy content calculated according to equation 2, using dry matter losses obtained by the two evaluation methods, were minimal in crown wood chips. Losses in the energy content of stem wood chips, however, were substantial irrespective of calculation method (Figure 14).

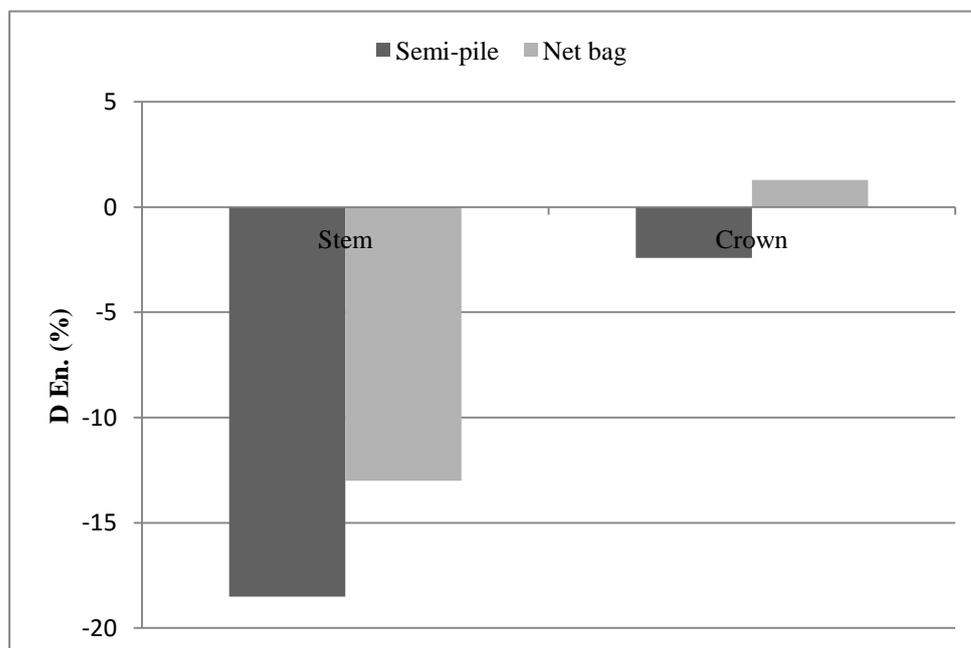


Figure 14 - Changes in energy content (%) in stem and crown wood chips of poplar, Dry matter losses were calculated by weighing the whole semi-pile or by using the net bags weighing method.

2.4 Discussion

Temperature level inside a stored chip pile can be considered as an indicator of storage performance, since aerobic degradation reactions are exothermic and generate a marked temperature rise. Different species of microorganisms have varying capacity to degrade cellulose, lignin and other wood components present in a chip pile. Most mould fungi, for example, utilize mainly available soluble nutrients but unable to degrade more complicated substrates such as lignin. The presence of such fungi normally leads to small dry matter loss. On the other hand many species of rot fungi can consume lignin and cause high substance losses.

The immediate rise in temperature in the crown chip pile could be due to the fast establishment of moulds on the nitrogen-rich biomass. As such substrate diminishes, temperature starts to slowly decline to more moderate levels allowing other species to grow. Stem wood normally contain lower concentrations of nitrogen and soluble nutrient are less available than the crown part of the tree. This is most probably the reason behind the moderate rise in temperature in the stem pile.

In all cases, the piles temperature was close to the ambient temperature after about 150 days of storage. Similar observation was recently reported by Manzone et al.. Different heat development in the piles may explain their different moisture content. In general, the chips were dried by the end of the trial, indicating the occurrence of natural drying. Stem chip piles had relatively higher moisture content than crown piles. The high heat development in the crown chip piles facilitated better drying of the material.

The dry matter losses were clearly higher in the stem wood piles compared with crown wood. The moderate temperature and the higher moisture content in the former provided favorable conditions for the growth of rot fungi species which are known to lead to high substance loss. Just as high dry matter losses was measured after storing *Salix* chips in a three meters high pile during May- September (Jirjis and Lehtikangas, 1998). The study further reported the presence of an intensive growth of white rot fungi in the pile.

The values of dry matter loss obtained through the weighing of net bags were systematically lower than those obtained by weighing the whole semi-piles. This may be related to semi-pile geometry, and in general with the difficulty of integrating the behavior of a whole semi-pile through 24 samples only, however aptly located. Differences in bulk

density at the beginning of the trial are explained by different moisture content in the two treatments, because particle size distribution turned out to be the same for both treatments. Different moisture content may explain the switchover in bulk density values at the end of the trial, when stem chip samples were denser than crown wood samples.

The chemical analyses made at the beginning and at the end of the storage period did not detect any differences between treatments and periods, except for nitrogen content. Changes in energy content was closely related to moisture content and dry matter losses, and it was marginally higher for crown chips at the end of the storage period. In fact, crown chips did incur negligible energy loss after the six-month storage period, whereas stem chips lost between 13 and 18% of their energy content. Indeed, these results are somehow unexpected.

Studies on the crown part of the woody species often show substantial deterioration in quality while stem wood has generally better storage properties. However, all indicators concurred to the same results, so we can categorically exclude any errors in the measurements. It is possible that poplar chips behave differently, partly because poplar wood is quite vulnerable to microbial attack in general which would explain the poor performance of stem chips. Another possible explanation for this discrepancy is that studies made on the crown of soft wood species almost always contained needle fraction. The crowns tested in this study were chipped in winter, without any leaves.

2.5 Conclusions

Crowns generally represent a residue to be disposed of rather than used as a commercial commodity. Easier problem-free storage may increase their attraction as a renewable fuel for the biomass energy market. The building of uncovered heap on plastic sheets is the cheapest and most suitable solution for a low-value commodity such as wood chips.

From the practical viewpoint, the results of the study indicate that it is advisable to store poplar stem wood as logs and chipping it just before use, whereas poplar crown wood can be chipped immediately and stored as chips. Due to the lower dry matter losses and the retaining of higher energy content, chips obtained from the crown can be stored for a longer period compared with stem chips without a remarkable decline of feedstock quality. This has a practical implication and particularly convenient, because it is easy to

manufacture and store logs from stem wood, while it would be much more complicated to store uncomminuted crowns, which are difficult to handle and take up much space.

Stem and crown chips can possibly support the growth of different biota, adapted to the specific substrate and to the climate of central Italy, leading to a different decay rate during storage and influencing the fuel quality after the storage period.

Future studies will be needed in order to model the exact storage dynamics of different chip types, under different conditions. Ideally, such tests should be conducted under a controlled environment, in order to analyze all main factors affecting chips decay.

Chapter 3: Open-air drying of cut and windrowed short-rotation poplar Stems

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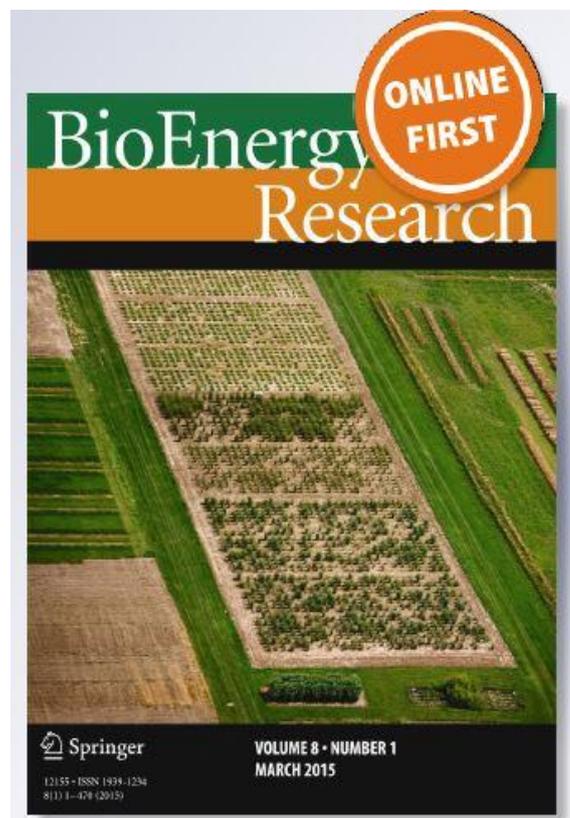
Open-Air Drying of Cut and Windrowed Short-Rotation Poplar Stems

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Abstract Two-pass harvesting of short-rotation forestry plantations offers the opportunity to accumulate large biomass stores without occupying costly industrial areas, while letting the biomass dry before comminution. This study aimed at developing a simple model for predicting moisture content reduction of short-rotation forestry poplar stems felled and windrowed in the field. In a controlled experiment, cut stem windrows were built and left in the field for up to 6 months (from early December to early June). Thus stored, poplar stems incurred a reduction of moisture content between 10 and 20 percent points. Drying rate varied with the period of storage, and it was faster for later felling dates. Precipitation accounted for 20 to 40 % of the drying rate. No dry matter losses due to microbial activity were recorded during the whole storage period, lasting up to 6 months. The models developed with this study are simple and robust, and allow precision management of collection operations in order to guarantee a constant flow of biomass to user plants.

Keywords Biomass · Wood · Chips · Storage · Harvesting



3.1 Introduction

Dedicated tree plantations can help meeting the increasingly large demand for wood fiber, experienced at a global level (Sims et al., 2006). Large amounts of wood biomass can be obtained from tree farms, established on ex-arable land with fast-growing hardwoods and harvested every 2 to 10 years, according to site conditions and product strategy (Spinelli et al., 2012). Over the years, improved management techniques have been developed, which offer a good energy balance (Manzone et al., 2009) and acceptable economic results (Spinelli et al., 2011).

Although not exclusive, coppice management is very popular, because it allows reducing regeneration cost while simplifying operation management (Heller et al., 2003). Pioneering studies have explored the technical possibilities of year-round coppicing (Sims and Venturi, 2004), which remains a theoretical option and is generally excluded from common practice. As a rule, coppicing is performed during the dormant season only, in winter time.

That restricts the production window to 5 months at most, and places a significant strain on production planning when trying to meet a demand for wood biomass that is steady and sustained all along the year (Nord-Larsen and Talbot, 2004). Diachronic supply and demand create a need for a product buffer to secure supply at all times (Jirjis, 1995).

Biomass storage strategies change with product type and local conditions (Jirjis, 2005), but they can be described based on the site where the product is stored and on the form under which it is stored. Biomass can be stored at the user plant, at wood terminals or near the plantations (Kanzian et al., 2009), with the last option being generally considered as the least expensive. Furthermore, biomass can be stored before or after comminution.

Storage before comminution allows minimizing wood decay, but results in higher handling costs compared with storage after comminution (Afzal et al., 2010). Comminuted wood is much easier to handle, but very difficult to store: unless they are dried, fresh wood chips are subject to rapid decay, leading to high dry-matter losses (Barontini et al., 2014), reduction of energy content (Pecenka et al., 2014), increased emissions (Wihersaari, 2005), and risk of self-ignition (Thörnqvist, 1987).

Storing uncomminuted stems on the field seems an ideal solution, because it combines the most durable product form with the cheapest storage site, while minimizing handling cost. The new harvesting system developed by the Italian Council for Research in Agriculture

(CRA) allows efficient twopass harvesting, through the full mechanization of all process steps (Pari et al., 2013). Comminution and extraction (pass 2) are completely separate from cutting and windrowing (pass 1), which allows collecting the product whenever ready, thus turning the field into a low-cost diffused storage site. This strategy accrues the added benefit of open-air drying that is generally faster for thin windrows than for large wood piles at a landing (Nurmi, 1999). That also solves any landing space problems, often encountered at short-rotation forestry (SRF) sites (Eisenbies, 2014).

However, in-field storage cannot extend indefinitely. Windrows must be removed before coppice regeneration is as tall as to hinder machine traffic and suffer from it. In temperate climates, that sets the limit to early June and results in a potential duration of storage ranging between 12 and 30 weeks, depending on the time of cutting. Clearing all fields by early June requires a careful scheduling of the second pass, which could be further refined by integrating moisture content considerations, in order to maximize the benefits accrued from open-air drying.

However, it may be impractical to monitor wood moisture content on all fields. A more convenient solution may consist in developing a simple model to forecast moisture content variation in felled and windrowed stems. Moisture content is likely to decrease with time, but its variation will be affected by precipitation and air temperature as well (Erber et al., 2012; Filbakk et al., 2011a; Filbakk et al., 2011b) thus reducing the accuracy of a model that is just based on the time elapsed after cutting.

On the other hand, an accurate model integrating weather conditions can only be used if one can actually monitor such conditions. Otherwise, one will have to rely on rough estimates for weather conditions, which will reduce the accuracy of the model itself.

Therefore, it may be more convenient to develop a regional set of models that account for both the time elapsed after felling, and the time of felling within the cutting season. For the same storage duration, a felling date closer to the end of the harvest season will imply storage at higher air temperatures (nearer to the Spring), which may allow reaching a lower moisture content compared with an earlier felling date. On the other hand, late cutting may encounter with variations of tree moisture content, due to fluctuations in the vegetative state of the plants as Spring approaches (Giorndano, 1986), which may further complicate the situation.

The goal of this study was to develop such set of models for SRF poplar grown in Central Italy. In particular, the study aimed at determining: (1) if the initial wood moisture content at cut changed along the cutting season; (2) what was the rate of drying of cut poplar stems at different periods along the harvest season; (3) what was the impact of precipitation on drying rate, which would allow a rough correction of the eventual prediction, in case of a seasonal precipitation above or below the expected values, and (4) if trees windrowed in the field also incurred dry matter losses as a result of microbial activity and not just a reduction of moisture content.

The study focused on poplar (*Populus* spp.), because this is the most common species used for the establishment of SRF plantations in Central, Southern, and Eastern Europe (Bergante et al., 2010), as well as North America (Alig et al., 2000). The experiment was conducted in Italy, because the Mediterranean climate may prove especially favourable to air-drying, which has justified investment in the new two-pass harvesting system developed by CRA.

3.2 Materials and methods

The research was conducted at the CRA experimental farm in Monterotondo, near Rome, Central Italy. One of the experimental SRF poplar plots available at the farm was cut for the occasion, using a chainsaw.

The stand had been established with the AF2 clone 7 years earlier and was being cut for the first time (table 9). The stand grew on a slightly inclined field, which drained into a small ditch at its lower edge. The field had not been weeded after the third year from establishment, but weeds had been suppressed by tree cover and there was very little grass on the floor at the time of cut.

After cut, weed growth was limited by the presence of the windrows and remained quite scarce. Cut trees were laid in windrows, aligned with the row axis, as if they had been cut with the prototype felling machine developed at CRA and specifically designed to fell trees and lay them down in windrows at the center of the inter-rows.

Four parallel windrows were produced, i.e., one for each of the following felling dates: early December (02 Dec 2012), mid January (18 Jan 2013), early February (04 Feb 2013)

and mid March (18 Mar 2013). These dates spanned over the whole felling season, at regular intervals.

Table 9 - Characteristics of the poplar stand.

Region	Lazio	
Province	Roma	
Placename	Name	Monterotondo
Coordinates	Longitude	12°E 37'26.23"
	Latitude	42°N 05'56.86"
Elevation	m a.s.l.	23
Stand surface	ha	0.5
Species	<i>Populus x Euroamericana</i>	
Clone	code	AF2
Plantation age	years	7
Stand density	stems ha ⁻¹	7140
Inter-row distance	m	2.8
Distance within the rows	m	0.5
Stem diameter at 10 cm	mm	100 (±17)
Stem height	m	10.5 (±1.8)

Each date corresponded to an individual windrow, from which five trees were randomly collected every 15 days for all the duration of the experiment, which lasted until early June (10 June 2013). These trees were sampled for moisture content, at the CRA laboratory.

Due to variable length of the storage periods, a different number of sample trees and a different number of sampling events were obtained for each felling date: 65 trees and 13 sampling events for the early December cut; 50 trees and ten sampling events for the mid-January cut; 45 trees and nine sampling events for the early February cut; 30 trees, and six sampling events for the mid-March cut date (Table 8).

Additional 66 trees were collected from the same windrows and placed inside a shed, so that moisture content could be determined for the same cutting dates and storage period in the case where the trees received no precipitation. Due to space constraints, this sample was reduced to only three trees per sampling event and treatment, and the sampling events were spaced at 30-day intervals, instead of 15-day intervals.

Nevertheless, the monthly sampling dates corresponded with those of the open-air windrows, so as to match the same samples over the same dates for the open-air and the covered treatments. This part of the experiment was designed to isolate the effect of precipitation on stem drying. Again, a different number of trees and a different number of sampling events were obtained for each felling date, as a result of the different duration of the storage periods (Table 10).

Finally, dry matter losses were determined on five trees per felling date and cover treatment (i.e., open-air windrow vs. shed), for a total of 40 trees. Each tree was weighed at felling and then again at the end of the storage period, on 10 June 2013.

Initial moisture content was assumed as the mean moisture content for that felling date, as obtained from the five sample trees collected at the time of felling and immediately analyzed for moisture content. In contrast, final moisture content was destructively sampled from each of the original trees, after the final weighing. These trees were not weighed at intermediate dates in order to avoid any disturbances, which may have affected the results of the analysis.

In all cases, moisture content was determined with the gravimetric method, according to European standard EN 14774-2, which is still the most reliable and accurate (Samuelsson et al., 2006). Moisture content was determined on 5 roundels per sample tree, which were cut at equal distances along the tree, starting from 10 cm above the base cut and ending 50 cm below the tip of the tree.

A weather station was installed near the experimental plot, so as to obtain daily records of air temperature, precipitation, and relative humidity.

The statistical significance of differences between treatments was tested with non parametric statistics, because the distribution of data violated the normality assumption. In particular, the statistical significance of any differences between the mean moisture content figures recorded for the different treatments was checked with the Kruskal–Wallis test, whereas any differences between the open-air and covered treatments for each felling date were tested with the Mann–Whitney U test (SAS institute Inc, 1999).

Table 10 – Sampling periods.

Moisture content				
Felling date*	Open-air windrows		Shed	
	Sample trees	Sampling events**	Sample trees	Sampling events***
Early December	65	13	18	6
Mid January	50	10	15	5
Early February	45	9	12	4
Mid March	30	6	9	3
All	190		54	
Dry matter losses				
Felling date*	Open-air windrows		Shed	
	Sample trees	Sampling events****	Sample trees	Sampling events****
Early December	5	2	5	2
Mid January	5	2	5	2
Early February	5	2	5	2
Mid March	5	2	5	2
All	20		20	

Notes: * the experiment was concluded on June 10th;
 ** one sampling event (5 trees) every 15 days;
 *** one sampling event (3 trees) every 30 days;
 **** one sampling event (5 trees) at the beginning of the time of felling and another at the end of the experiment (June 10th), using the same 5 sample trees.

Regression analysis was used to test the significance of any relationships between moisture content and potentially influencing variables, such as time in storage. Indicator (dummy) variables were used to test the effects of discrete factors, such as felling date or cover (Olsen et al., 1998). For all analyses, the significance level was set at 5 % (i.e., $\alpha=0.05$).

3.3 Results

Moisture content at the time of cutting varied between 52.9 and 58.2 % (n=20). The overall mean was 56.6 %, with no significant differences between the means obtained for the different felling dates. Figure 15 shows the weather data recorded by the on-site weather station over the whole duration of the experiment. Starting at the end of February, air temperature showed a steady increase while springtime approached.

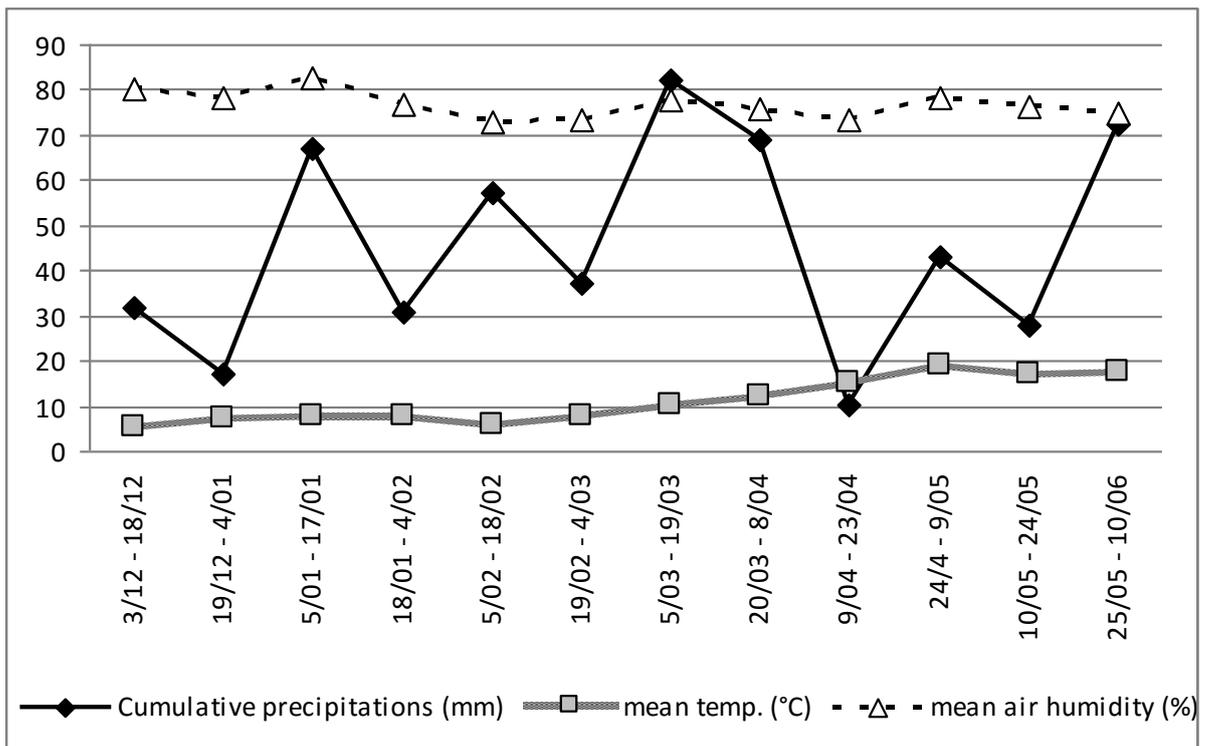


Figure 15 - Meteorological data recorded during the entire duration of the experiment.

In contrast, precipitation was sustained all along, with a temporary lull in April. In fact, the precipitation recorded for April and May 2013 was lower than the average for the previous 7-year period, whereas the precipitations recorded for the other months were generally higher than the 7-year average figures (Figure 16).

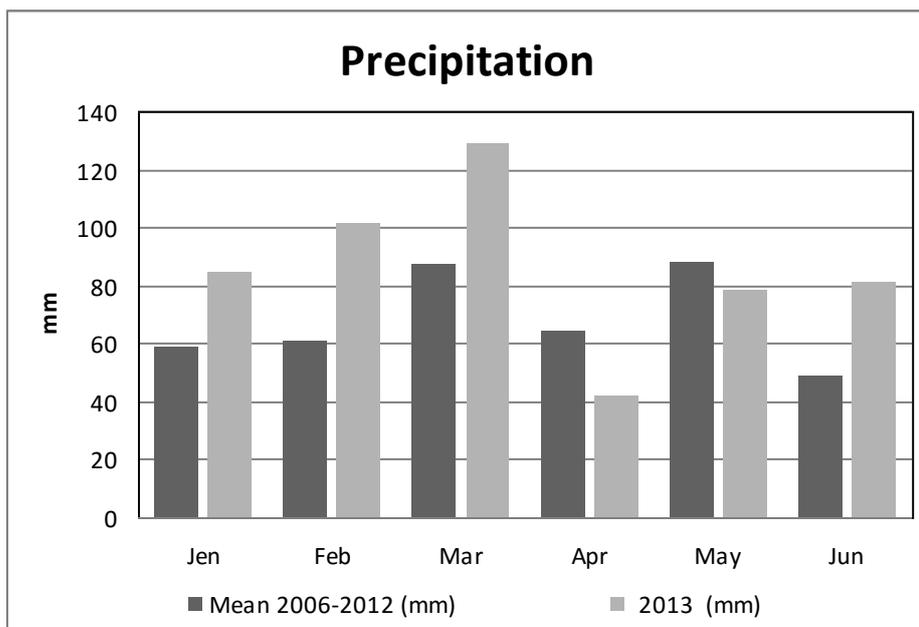


Figure 16 - Mean precipitation between 2006 and 2012 and mean precipitation in 2013.

Figure 17 shows the moisture content trends recorded over the whole duration of the experiment for the open-air windrows, felled at different dates. Open-air storage in windrows allowed reducing moisture content by 10÷15 percent points within the first 3 months. After 6 months (trees felled in early December), moisture content was down to 36 %, or 20 percent points lower than the initial value at the time of felling.

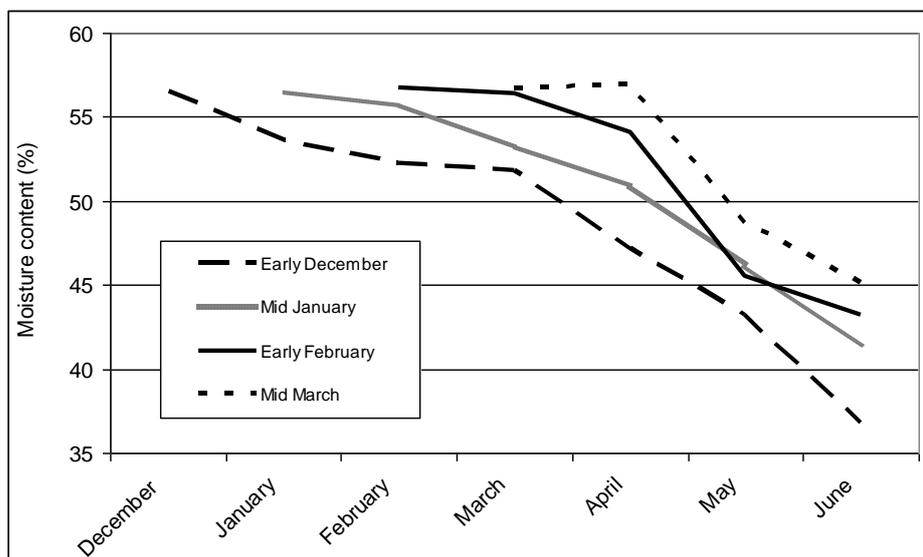


Figure 17 - Moisture content trends according to the treatment.

The relationship between moisture content, duration of storage and felling date was estimated through regression analysis after cutting to 90 the number of days in storage, in order to have the same maximum duration of storage for all felling dates. Moisture content was significantly related to days in storage as well as to felling date (Table 11). A later felling date resulted in a faster drying rate.

Table 11 - Regression equation for estimating moist. content (y) as a function of days in storage and felling date (x).

$MC = a + b D + c D F1 + d D F2 + e D F3$			
n = 129			
$R^2 = 0.713$			
F = 80.452			
Parameter	Coefficient	t-Value	p-Value
a	57.351	177.399	<0.0001
b	-0.156	-16.428	<0.0001
c	0.084	8.356	<0.0001
d	0.070	6.836	<0.0001
e	0.057	5.373	<0.0001
Where: MC = Moisture content (%); D = Days elapsed from felling; F1 = Indicator variable for felling date 1 (it is 1 if felling date is early December; 0 if otherwise); F2 = Indicator variable for felling date 2 (it is 1 if felling date is mid-January; 0 if otherwise); F3 = Indicator variable for felling date 3 (it is 1 if felling date is early February; 0 if otherwise);			

Table 12 shows the cumulated precipitation over the first 3 months in storage for the four felling dates, as well as the mean air temperature and relative humidity calculated over the same periods. The largest difference was between air temperatures means, indicating that stems cut at a later date were normally subjected to higher temperatures. In contrast, the total precipitation received in the first 3 months of storage was quite similar for all felling dates.

Table 12 - Cumulate precipitation, mean air temperature, and mean relative humidity over a period of three months from felling for the four treatments.

Treatment	Period	Cumulated precipitation	Mean Air temperature	Mean Relative humidity
Felling date	From - to	mm	C°	%
Early December	Dec 03 - Mar 03	241	6.9	77.5
Mid January	Jan 18 – Apr 18	276	9.9	75.1
Early February	Feb 04 - May 04	264	11.6	74.7
Mid March	Mar 18 –Jun 18	232	16.0	75.8

Table 13 shows the regression equations used to estimate the relationship between moisture content, days in storage, and cover treatment, separately for each felling date.

Table 13 - Regression equation for estimating moisture content (y) as a function of days in storage and cover treatments (x) for each felling date.

MC = a + b D + c DC				
Felling date	Early Dec	Mid Jan	Early Feb	Mid Mar
n =	56	46	38	31
R ² =	0.770	0.783	0.782	0.820
F =	93.041	82.300	67.407	59.501
Parameters				
a	57.318	56.301	57.907	56.968
b	-0.095	-0.092	-0.109	-0.142
c	-0.022	-0.027	-0.080	-0.015*

Where: MC = Moisture content (%); D = Days elapsed from felling; C = Indicator variable for cover treatment (it is 1 if the stems are covered; 0 if they are not); * Parameter not significant for $\alpha=0.05$.

The effect of a cover was always significant, except for the latest felling date. However, the strength of this effect depended on the felling date. Protection from rainfall increased drying rate by about 20 % if the stems were cut within mid January and by about 40 % if the cut occurred in early February, that is, if storage was prolonged until early June, of

course. Finally, Table 14 shows the mean dry matter content of the sample stems stored all along the experiment and tested for dry matter losses. In no case, the difference between initial weight and final weight was statistically significant, which denied the occurrence of dry matter losses caused by microbial activity, regardless of storage period, cut date, and cover treatment.

Table 14 - Dry matter losses at the beginning and end of the storage period, for the four felling dates.

Felling date	Dry kg beginning	Dry kg end	p-Value
Open-air windrows			
Early December	11.85 (± 1.36)	12.09 (± 1.42)	0.793
Mid January	11.35 (± 5.01)	11.14 (± 4.30)	0.943
Early February	10.15 (± 1.50)	9.80 (± 1.38)	0.709
Mid March	9.24 (± 2.08)	9.33 (± 1.81)	0.939
Covered			
Early December	8.02 (± 1.81)	8.64 (± 2.06)	0.718
Mid January	17.71 (± 0.99)	16.98 (± 2.00)	0.599
Early February	12.83 (± 1.10)	12.64 (± 1.68)	0.874
Mid March	10.74 (± 2.75)	10.31 (± 3.07)	0.867

3.4 Discussion

High initial moisture content is a characteristic of SRF poplar, as reported in previous studies (Spinelli et al., 2009). However, none of these studies ever explored the issue of moisture content variation along the felling period, which is relevant to moisture content management in biomass fuel. Plant moisture content does change with the season, but our study indicates that it does not change significantly within the same season, at least for SRF poplar in winter. That definitely relieves moisture content management by removing one complicating variable from the eventual modeling. In fact, the model obtained from this study is relatively unsophisticated, because it does not account directly for weather trends, but only for storage duration and felling period.

Simplification may represent one of the main advantages of this model, because it makes the model robust and convenient to use. Classic models based on weather data are

expensive to build and somewhat difficult to use, because they require exact knowledge of weather data on the part of the user. In contrast, this study is designed for maximum efficiency, which is achieved by developing a viable, user-friendly model at minimum cost. Of course, one may not expect from this model the same level of accuracy as in classic weather-based models, but it is unlikely that such high accuracy is reflected in industrial operation scheduling. Operation managers may favor simple tools, capable of returning viable predictions for a minimum effort.

When talking about model development, it is important to stress that the cover treatment in this study was only meant to isolate the effect of precipitation, not to suggest a new practice. Covering the windrows with film or tarpaulins would be very expensive and would probably drive supply chain costs beyond acceptable levels.

What is more, covering the windrows may not accrue any drying benefits, after all. A cover placed directly over the felled stems is likely to hinder air circulation and intercept evaporation at the same time, thus slowing moisture content release, rather than accelerating it (Manzone et al., 2013). In the experiment, stems were protected from precipitation by placing them in an open shed, where the cover was well above the stems, and air circulation was allowed. Such procedure was just meant to replicate windrowing in the open field, minus precipitation.

Covered storage showed that precipitation had but a secondary effect on the air drying of windrowed SRF poplar stems, except for the case when felling was performed in February. In all other cases, precipitation accounted for 20% of the drying rate, at maximum. This result must be interpreted in the light of the unusually high precipitation recorded for the winter season 2012–2013, which made this model especially robust against any variations of weather conditions, as may result from the expected climate changes (Klaus et al., 2001; Schelhaas et al., 2010).

Such simple model may help drawing harvesting schedules that can reach the most favorable balance of moisture content reduction, duration of storage time, and machine utilization (Gautam et al., 2012). Precision supply chain management may accrue substantial benefits in term of increased fuel quality, decreased storage cost and losses, higher product value, and better transportation efficiency through energy densification of hauled loads (Acuna et al., 2012; Petterson and Nordfjell, 2007). These benefits may offset

the lower cost of singlepass harvesting as compared with two-pass harvesting, and bridge the cost gap between the two alternative supply chains (Berhonagary et al., 2013).

Compared to single-pass harvesting, in-field storage, and delayed comminution offer two main advantages: (1) avoiding the occupation of costly storage space at the plant or at a terminal and (2) preventing dry matter losses caused by microbial activity (Schweier and Becker, 2012). In contrast, prolonged open-air storage of fresh poplar chips results in substantial dry matter losses, which may vary between 10 % and 25 % over a period of 6 months (Barontini et al., 2014).

If the storage period extends to a whole year, then dry matter losses can be even higher. However, one must bear in mind that the results of this study cover a maximum storage period of 6 months. It is uncertain whether windrowed stems may store as well as they did, if the storage period was extended to 1 year, in the unlikely case where the constraints of coppice regeneration were overcome and one could occupy the field for that long.

Recent studies suggest that uncomminuted poplar stems may suffer severe decay when left piled for as long as a year, which is compatible with the generally low durability of poplar wood. Furthermore, readers must recall that this study was conducted only with one specific clone. Therefore, results are verified for that clone only and may vary with other clones. However, the model developed in this study is simple enough that it might reflect the general behavior of SRF poplar, although less accurately with clones other than AF2.

Although significant, the moisture content reduction obtained through in-field storage and delayed chipping is not large enough to guarantee safe storage of the chips eventually produced, which would still contain about 40% moisture. For this reason, these chips must be used or actively dried shortly after comminution, in order to prevent microbial decay.

3.5 Conclusions

This study shows that open-air storage in windrows is a viable solution for SRF poplar management. This technique allows accumulating large biomass stores without occupying costly industrial areas. Thus stored, poplar stems incur a substantial reduction of moisture content, without suffering any dry matter losses. Windrows can be left in the field for up to 6 months and are collected with new automatic machines specifically designed for the purpose. The models developed with this study allow precision management of collection operations, so as to maximize all the potential benefits of the new supply chain.

Chapter 4: Alternative storage systems of *Arundo donax* L. L. and characterization of the stored biomass

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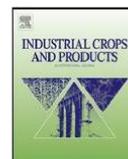
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Alternative storage systems of *Arundo donax* L. and characterization of the stored biomass



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Arundo donax L. has received much attention from researchers as biomass crop because of its vigorous growth, high productivity and low agronomic inputs requirements. Once harvested, the chopped biomass of *A. donax* L. may be stored for a variable period according to energy demand or logistic reasons. As consequence of storage, the chopped biomass may undergo processes of degradation that can compromise the energy yield of the product and the economic convenience of the cultivation.

In the view of preserving the *A. donax* L. biomass for producing different kind of energy, such as bio-ethanol, biogas, and fuel for combustion, the aim of this study was to investigate, in small-scale, different storage systems assessing the dynamics of storage and their effects on energy losses and fuel quality. The work has included different studies, such as (i) the monitoring of heat development, moisture content and dry matter losses, (ii) the analysis of polymeric compounds and combustion performances. Results indicate that according to the storage method adopted, the conservation of the product can be significantly influenced, with clear impacts on its energy potential. Polymeric composition changed after storage according to the method used, while energetic characteristics were not remarkably affected.

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4.1 Introduction

The widespread commercialization of biofuels and biochemicals deriving from lignocellulosic biomass is strictly linked to the feedstock availability and production costs. Among perennial grasses, *Arundo donax* L. (*Poaceae* family) presents several attractive characteristics as dedicated biomass crop. This species is a hydrophyte that grows spontaneously and abundantly in southern Europe and in many subtropical temperate regions of the world (Pilu et al., 2013, 2012).

Since the 80's, in Europe, several studies regarding the *Arundo donax* L. biomass potential for energy production have been carried out (Cosentino et al., 2006; Nassi di Nasso et al., 2007; Angelini et al., 2009; Lewandowski et al., 2003). *Arundo donax* L. has received much attention from researchers for its vigorous growth, high productivity, low agronomic inputs (fertilizers, pesticides), high tolerance to environmental stress, and suitability to be cultivated in unproductive soils or marginal lands. As perennial biomass crop, *Arundo donax* L. would have also the advantage to distribute its planting costs throughout the entire productive cycle (one annual harvest for 10-15 years), thus giving a more favourable energy output/input ratio respect annual grasses (Bell, 1997).

According to the climatic conditions and geographical location, the potential dry matter yields are between 29 and 46 t/ha/year (Angelini et al., 2009; Lewandowski et al. 2003). These values combine well with the energy features of the biomass. In fact, *Arundo donax* L. feedstock can be used as fuel for combustion in biomass power plants, but also for producing bio-ethanol and biogas in transforming plants and bio-digesters.

In the case of combustion, the use of *Arundo donax* L. is limited to specific power plants capable to tolerate determined fuel characteristics such as high ash content and low ash fusion point respect traditional sources. In fact, the biomass of *Arundo donax* L. presents about the 3 - 8 % of ash content and an Initial Deformation Temperature of 930 C° (Coulson et al., 2004); these features may determine troubles in traditional combustion plants such as clogging and slugging.

The bioethanol production from *Arundo donax* L. has been proposed by several years; recent works have been finalized to improve the destructuration of the lignocellulosic fraction and facilitate the sugar release (De Bari et al., 2013; Scordia et al., 2012).

Bura et al. (2012) estimated the potential bio-ethanol production as 1/kg of raw biomass and achieved lower yield for *Arundo donax* L. compared to hybrids of poplar. However, by virtue of the higher biomass yield/ha/year of *Arundo donax* L. respect that of poplar, the ethanol production could be approximately twice than poplar hybrids per area of land cultivated.

The use of *Arundo donax* L. biomass for biogas production is becoming attractive (Di Girolamo et al., 2013) and hypothesized as a valid alternative to maize, due to the concerns about input requirements, land use and price of food and feed commodities (Ragaglini et al., 2014). However, the few works available are still contrasting. Whereas Toscano et al. (2013) observed low yields of biogas and biohydrogen, Schievano et al. (2012) demonstrated that the biogas production from *Arundo donax* L. in the long period is more convenient (cost in euro/Sm³ of CH₄ or cost in euro/Kwh) than that of other crops commonly used for this purpose.

Interestingly, Ragaglini et al. (2014) emphasized the role of harvest time and frequency of cutting on morphological features (stem vs. leaves proportion) and chemical traits (nitrogen concentration, C/N ratio, Non-Structural Carbohydrates and cell wall components) as factors strongly affecting the biogas production.

Bearing in mind the multi-purposing of *Arundo donax* L. biomass, currently it is deemed feasible the harvesting all year long in order to assure the continuous supply of the feedstock to transformation plants. However, in order to satisfy the constant demand for energy, suitable storage methods aimed at preserving the biomass characteristics as a function of the final use should be identified. For instance, in Southern Europe during summer the moisture content of the plants may exceed 60%, indicating the necessity of dehydration processes or storage to stabilize the biomass for energy conversion (Angelini et al., 2005, Sanzone et al., 2010).

Till now, few experiences on storage of *Arundo donax* L. biomass have been carried out. The infield drying seems to be the most economic and rapid method in order to obtain proper material for combustion.

To evaluate the changes in fuel quality and dry matter losses that occur during time, Curt et al. (2013) stored chopped *Arundo donax* L. in field windrows for twenty days achieving moisture content lower than 15%. Successively, the dry material was stored outdoor in

bales for eight months without noticing relevant changes in physical, chemical and energetic properties of the biomass. In another study *Arundo donax* L. was stored in bundles varying the initial period of storage from December to March and monitoring the trend of moisture content during time. In this case, the initial moisture content of the plant was always around 50%, while acceptable levels of moisture for energy conversion through combustion were reached in all cases in late spring (Sanzone et al., 2010).

However, specific and comprehensive studies focused on *Arundo donax* L. storage are still lacking. In the view of preserving the *Arundo donax* L. biomass for producing different kind of energy, such as bio-ethanol, biogas, and fuel for combustion, the aim of this study was to investigate, in small-scale, different storage systems assessing their effects on energy losses and fuel quality.

4.2 Materials and methods

The Arundo donax L. feedstock

The plants of *Arundo donax* L. *donax* utilized for the storage trials were harvested in June 2013 from a five-years old cultivation at the experimental farm “Ca Rossa” located at Anzola dell’Emilia in central Italy (Lat. 44° 31’ N, Long. 11° 21’ E; 135 m a.s.l.). The mechanical harvest was carried out with a self-propelled harvester presenting a modified chopping system manufactured by Spapperi Ltd for improving the process of comminution. Soon after the harvest, the green chopped *Arundo donax* L. was transferred to “Unità di ricerca per l’Ingegneria agraria” (CRA-ING) at Monterotondo (Lat. 42° 10’ N, Long. 12° 62’ E; 300 m a.s.l.) near Rome, where the storage site was set up.

The storage tests began on 13th of June and ended on 13th of July. Climatic data were recorded using a weather cab “SIAP-MICROS DA9000” certified by SIAN (National Informative System for Agriculture). The collected data were recorded during the entire storage period. These included parameters concerning precipitations, external temperature, and air humidity.

The storage’s systems and conditions

Three different storage methods were tested outdoor: material stored in ventilated bins (A), in perforated bins (B), and in piles built on plastic platforms (C). All treatments were replicated twice (on the whole, 6 experimental units) using about 100 kg of chopped

Arundo donax L. for each. The bins used in the test were those commonly utilized for the collection and transport of fruits (100 cm length, 110 cm width, and 55 cm height).

In treatment A, the airflow was generated by two electrical engines manufactured by EuroMotors Italia Mod. 800CFM (figure 18). The engines were provided singularly with two centrifugal fans fixed at the bottom of the bins in two opposite sides. In this regard, four openings in the plastic were created in order to ensure efficient air entrance. Such system allowed pumping in air with an air flow of 1500 m³/h and an air speed of 27 km/h. The air was pumped in at intermittent intervals of 20 minutes in order to avoid overheating of the system.

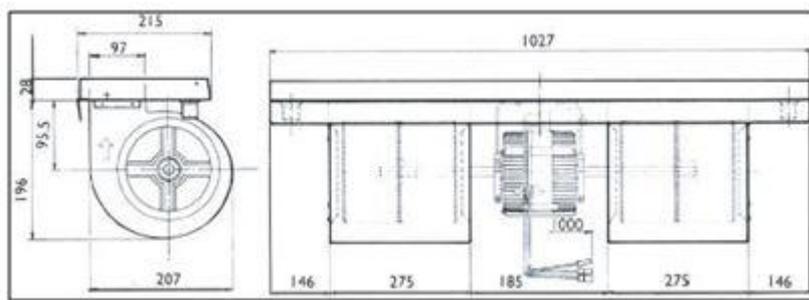


Figure 18 - Scheme of the fan used in the ventilated treatment.

In the treatment B, the perforations were applied by drilling the walls of the bins in order to permit the air circulation. The holes had a diameter of 1 cm and were applied every 9 cm² of the wall surface. Air circulation was also favoured by the presence of perforated pvc tube placed in the middle of each bins.

In treatment C, the open piles (conventional storage system) were built on two plastic platforms laid on two wood pallets of the same dimension. The pallets were utilized in order to isolate the material from the ground and facilitate the lifting and weighing of the biomass (figure 19).



Figure 19: letter A indicates the storage treatment in ventilated bins, letter B indicates the storage treatment in perforated bins, letter C indicates the storage treatment in small pile on plastic platform.

The temperature inside all treatments was continuously monitored during the storage through two thermocouples “pt100” placed respectively at 15 and 30 cm from the bottom of the bin or from the base of the pile. The thermocouples were connected to an electronic control unit which was created *ad-hoc*. The probes transmitted a mean value of temperature every 5 minutes as results of 240 observations (high frequency observation system). The system automatically removed from the final data the errors due to malfunctioning or electronic disturbances, ensuring the reliability of the data during time.

After placing the green material, the setting of the entire systems took about twelve hours, so the temperature recording was delayed of this time frame. The moisture content of the material was measured according to the UNI EN 14774-2 standard. Two samples (300 g each) were drawn randomly at different height from each treatment at four storage time intervals, respectively at the beginning of storage (T_0) after 10 days (T_{10}), after 15 days (T_{15}) and at the end of storage (T_{30}).

The material was sealed in non-breathable bags, duly tagged and dispatched to laboratory where they were oven dried for 24 hours at $103 \pm 2^\circ\text{C}$. The data deriving from moisture measurements were used for the calculation of dry matter loss. To this aim, in correspondence with the moisture determination, the heaps and the bins were weighted positioning them manually on a balance.

Analysis of lignin and cellulose

Two samples of biomass (about 200g each) were taken from each treatment at T_0 and T_{30} in order to study the effects of storage on lignin and cellulose. Immediately after sampling, the product was sealed in plastic bags under vacuum and frozen at -20°C till the lab analyses. The defrosted product was then grounded with lab-scale MF109 miller (IKA, Staufen, Germany) in particles <1 mm. Successively, 1 g of biomass was taken from each sample, washed twice with deionized water, and dried. Cellulose and Klason lignin content of the feedstock were determined according to the method reported by Santi et al. (2012).

In particular 0.5 g of biomass was suspended in 3 ml of 72% (w/w) H_2SO_4 and kept at 30°C for 60 min to be hydrolyzed. Thereafter, the mixture was diluted with deionized water to adjust the acid concentration at 2.5%, w/w and then autoclaved at 121°C . The mixture was then vacuum filtered through a calibrated sintered glass crucible with standard

porosity 2 (PBI, Milan, Italy). The solid fraction of the filtrate was dried at 105 °C for 24 h and weighed, so Klason lignin was calculated by the difference with ash content determined in other tests.

On the other hand, liquid fraction was neutralized at pH 6.5 by adding solid CaCO₃. The neutralized filtrate was then centrifuged (6000 × g, 15 min) and passed through a 2.7 μm MGD glass filter (Sartorius Stedim) prior analysis. Glucose was determined by using the Enzytec D-Glucose Code E1210 (R-Biopharm AG, Darmstadt, Germany). The cellulose content in the starting material was calculated using the following equation as previously reported by Ververis et al. (2007):

$$\text{Cellulose content (\%, w/w)} = [(0.9/0.96) \times C1 \times (V/M) \times \alpha \times 100]$$

where 0.9 is the coefficient that results from the molecular weight ratio of the polymer and the monomer hexose, the saccharification yield was taken as 0.96, C1 is the glucose concentration (g/L), V is the total volume of sugar solution (L), M is the dry weight of the biomass sample (g) and α the dilution of the sample (if any).

Efficiency of combustion: fuel quality

In order to determine the combustion efficiency of this product and to evaluate the effects of storage on fuel quality, the principal reference parameters such as ash content, heating value, elemental analysis, and ash fusion temperatures were determined before and after storage.

Heating values (HHV and LHV on dry basis) were measured with isoperibolic calorimeter (Anton Paar 6400). All operations were carried out respecting the European standard UNI EN 14918: 2010; the measurements were replicated three times for each treatment. The ash content of the biomass was determined burning in a muffle furnace three samples per treatment (about 1.0 g each). The operations were performed as according European standard UNI EN 14775:2010; the measurements were replicated three times for each treatment.

The analyses of carbon, hydrogen and nitrogen were performed before and after storage on 1 sample per treatment by means of an elemental analyser (Costech ECS 4010), as prescribed by the respective European standard UNI EN 15104:2011. As for the elemental analysis, the ash fusion temperature was measured on one sample per each treatment

before and after storage by means of a analyser for the fusibility of ash (Sylab SHVIF 1500) following the standard UNI CEN/TS 15370-1:2006.

Statistical analysis

In order to check differences with statistical significance among treatments, the values of dry matter losses and cellulose/lignin content were analyzed with the software PAST by using one-way Anova and Tukey's tests. Statistically significant differences among treatments were not checked for heat development because this parameter is considered only an indicator of potential storage dynamics. This means that significant differences cannot exclude the occurrence of one phenomenon respect another. Regarding the quality parameters, statistical significant differences among treatments were checked for moisture content with the software PAST by using one-way Anova and Tukey's tests.

4.3 Results

Weather conditions

The experiment started in the middle of June and ended in the middle of July. During the storage period, the mean daily temperatures were between 19.7°C and 27.5°C, while the cumulated precipitations were equal to 39.8 mm concentrated into five raining events. The mean daily relative air humidity ranged between 41% and 66% (figure 20). A comparison made with average values of long-term period showed that precipitations were above the mean, while temperatures were slightly below the mean (<http://www.idrografico.roma.it>).

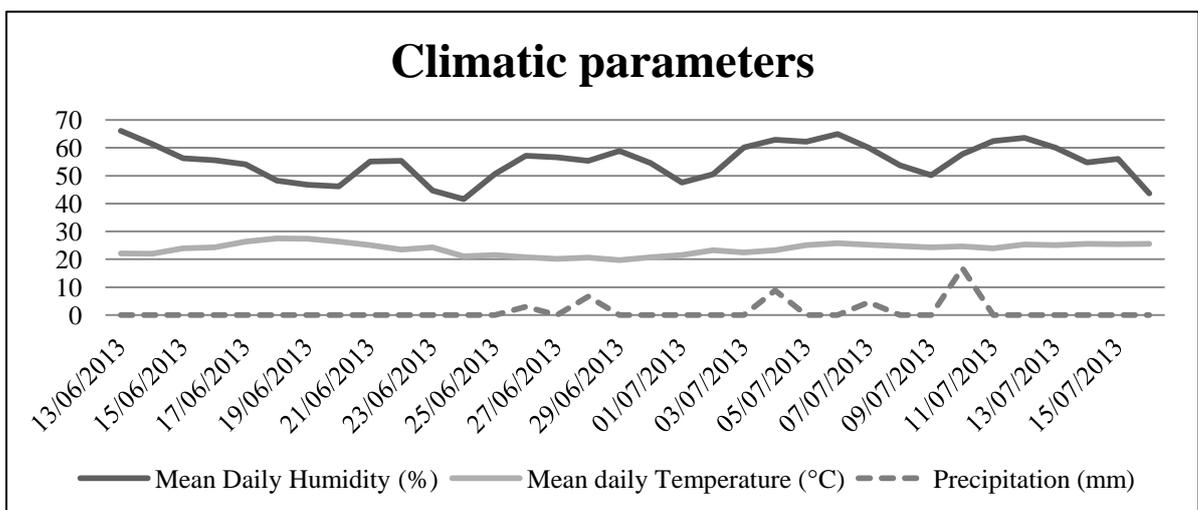


Figure 20 - Climatic parameters recorded during the storage period.

Temperatures during storage

The inner temperature of the biomass is a reliable indicator of storage performance, since degradation reaction are exothermic and generate a marked temperature rise. The most noticeable difference among treatments was observed during the initial 10 days of storage (figure 21).

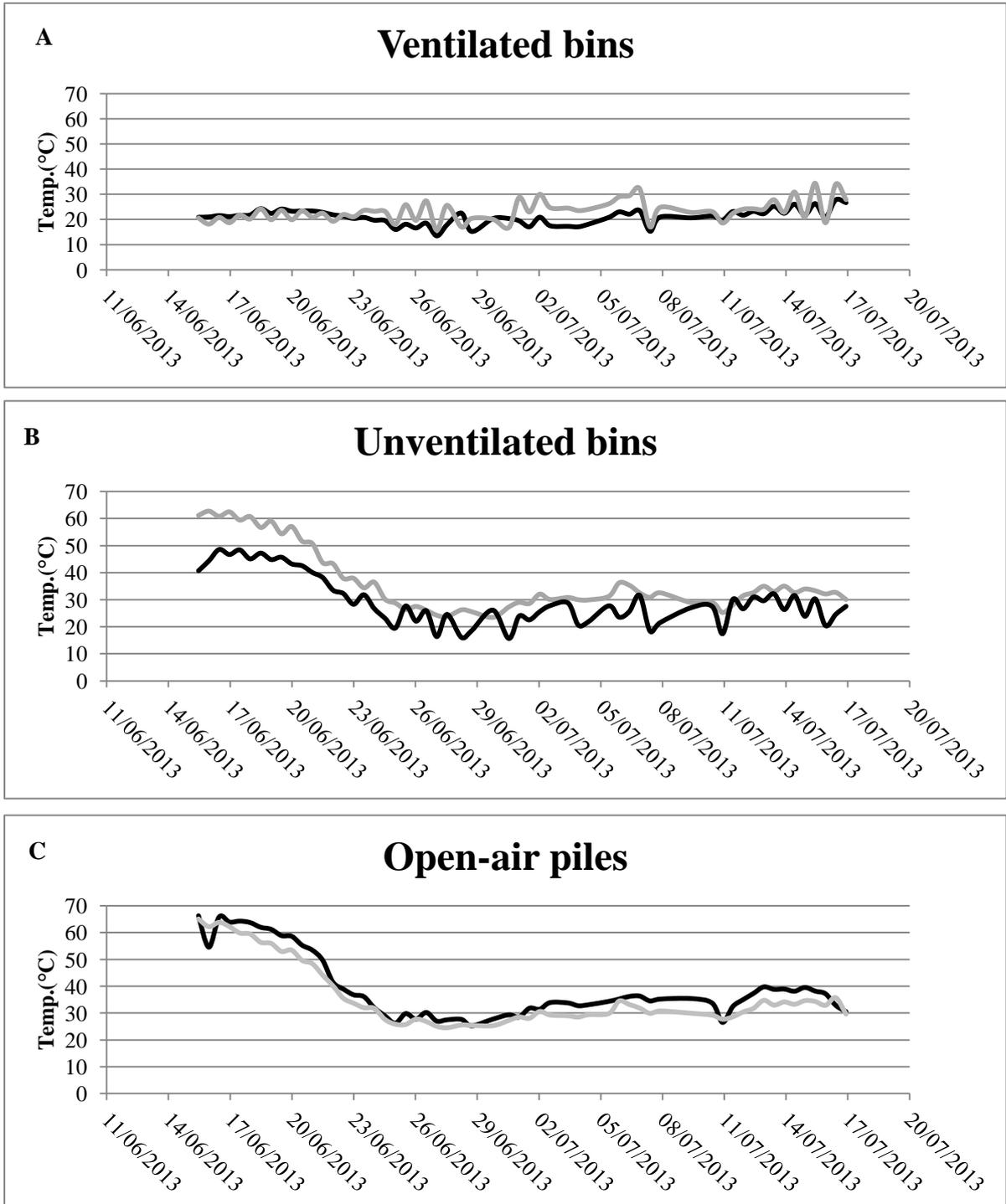


Figure 21 - Time course of the temperature in: A) ventilated bins; B) unventilated bins; C) open-air piles. The grey line correspond to the data recorded with the probes located at 15 cm from the bottom, while the black line represents those recorded at 30 cm.

During this time, the temperature in ventilated bins have always fluctuated between 10°C and 30°C, while in the other two treatments it rose very quickly reaching 60°C and taking about 10 days to decrease and stabilize. The temperatures at the end of the cycle reached 30°C in both outer heaps and bins without ventilation, while in those with engines it was leveled off at slightly lower levels.

Moisture content

The moisture content of the fresh biomass was about 59% (figure 22). After 10 days of storage, the average moisture content of the feedstock put in the ventilated bins decreased about 50 percent points while in unventilated bins and open-air piles it decreased about 7 percent points.

The difference between ventilated bins and the other treatments was highly significant at $p < 0.01$ after Tuckey’s test. At the end of storage, the moisture content in ventilated bins did not changed significantly, while in unventilated bins it decreased until 43%. A countertendency was shown in open-air piles, whereas the biomass at T₃₀ overcome the 60% of moisture content, showing statistically significant differences respect the other treatments.

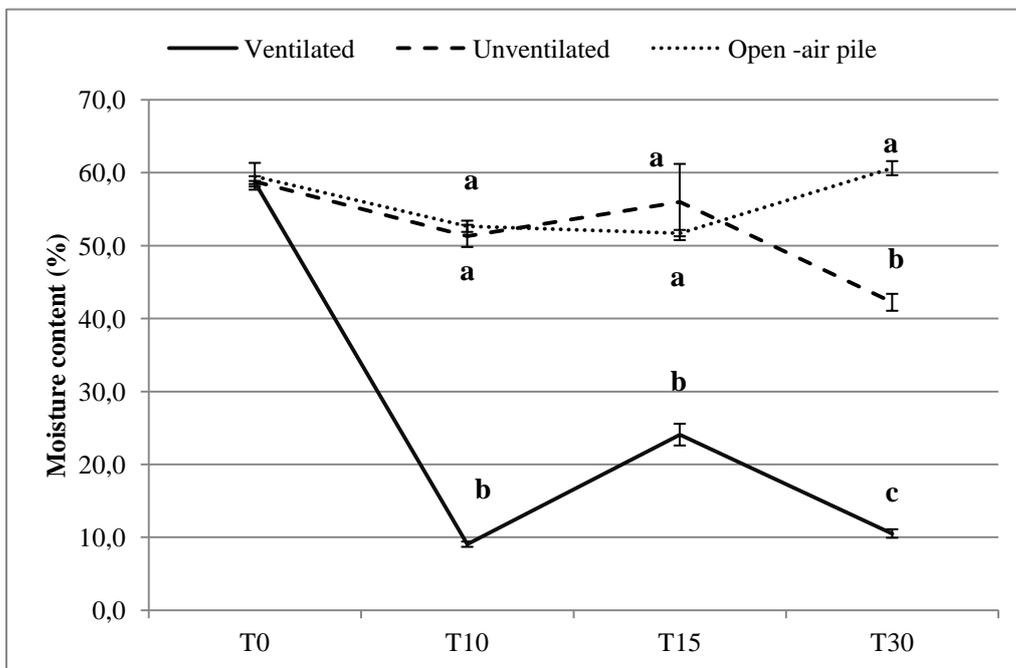


Figure 22 - Time course of chopped *Arundo* moisture content (mean±SD) stored in ventilated bins, bins without ventilation or on open-air. Different letters indicate a significant difference between the treatments at each time-point at the level of $p < 0.01$ after Tuckey’s test. Before the ANOVA analysis the data were transformed as square root of the arcsine.

Dry matter losses

Dry matter losses were consistently higher in open-air piles and unventilated bins than in ventilated one (figure 23). At the end of storage these two treatments have showed losses higher than 39-40% and 27-28%, respect the ventilated bins. Statistical analysis has showed significant differences between treatments at each time-point at the level of $p < 0.01$ after Tuckey's test.

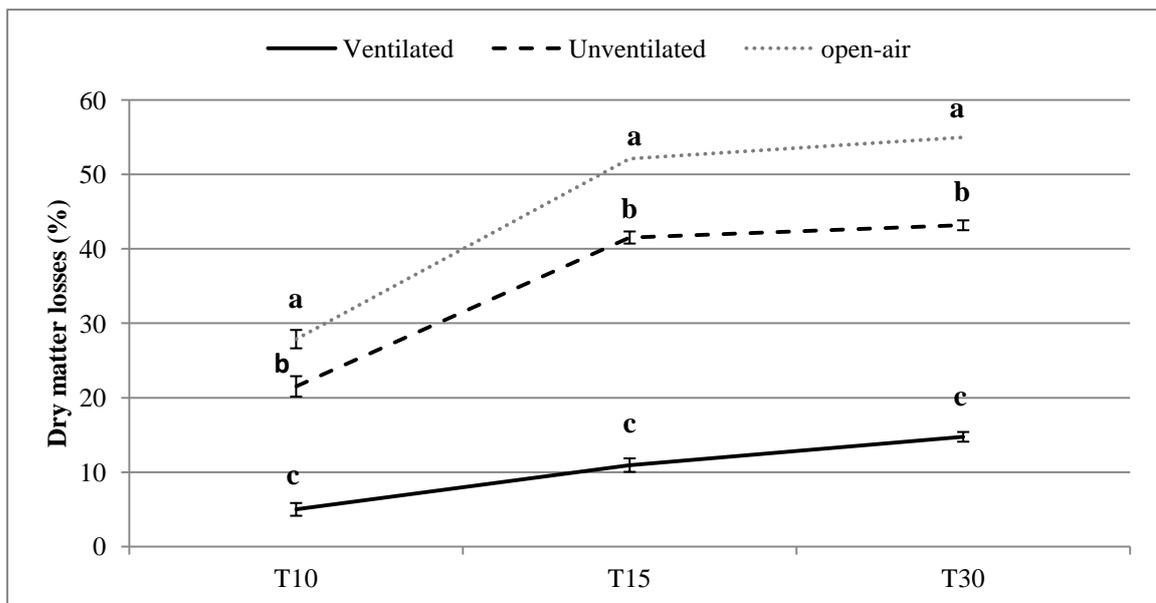


Figure 23 - Time course of dry matter losses (mean \pm SD) of chopped *Arundo* stored in ventilated bins, bins without ventilation or on open-air. Different letters indicate a significant difference between the treatments at each time-point at the level of $p < 0.01$ after Tuckey's test. Before the ANOVA analysis the data were transformed as square root of the arcsine.

Analysis of lignin and cellulose

Effect of different storage systems was evaluated on the principal polymeric component of *Arundo donax* L. biomass; to this aim, variation in lignin and cellulose content was monitored during the storage time. Figure 24 reports the percentage composition in lignin and cellulose at the beginning and at the end of the storage. No relevant variations were observed in lignin content among different storage treatments, while cellulose content decreased more or less according to the storage method used.

In particular, starting from 33%, the fraction of cellulose (dry matter basis) decreased in all the storage system tested, reaching 22% in the ventilated bin, 18% in unventilated bins, and only 12% in the open-air piles. However, the percentage variation of lignin and cellulose content can be misleading, because these values must be proportioned to the dry matter losses occurred during storage.

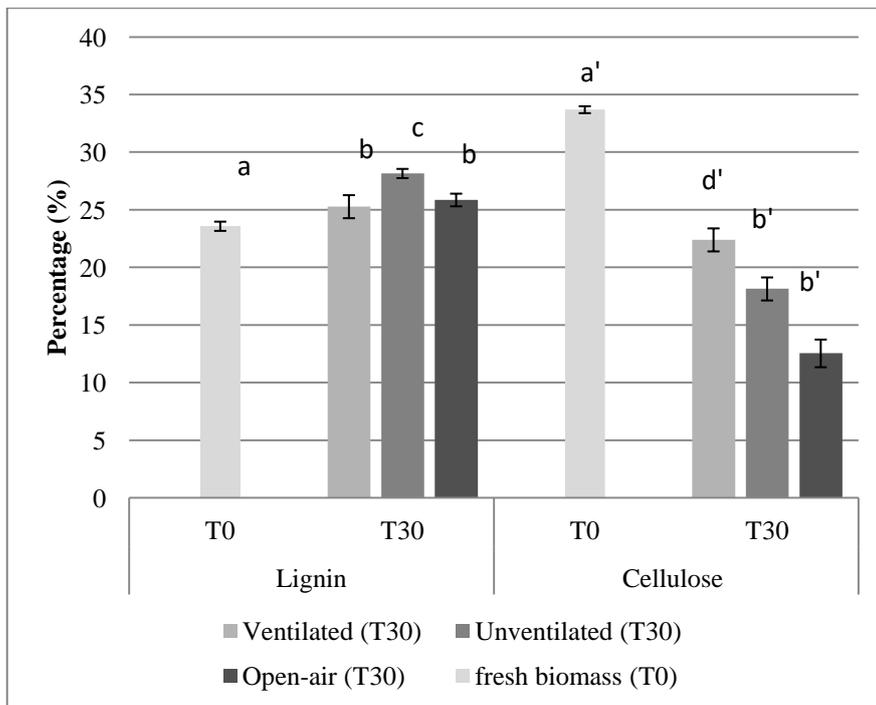


Figure 24 - Lignin and cellulose contents detected before storage on fresh biomass (T0) and after storage on the three treatments (T30). Means \pm SD (two replicates for three samples), same letters above bars indicate the lack of statistically significant differences within each treatment. The apostrophe was used to avoid confusion between the statistical analysis made for lignin and cellulose.

Fuel quality parameters

Table 15 shows respectively the fuel quality parameters before and after storage of the three treatments, while table 16 shows the ash fusion temperatures of the biomass. Despite the moisture content, no remarkable differences were identified among treatments, even before and after storage. On the other hand, the *Arundo donax* L. feedstock demonstrated to possess higher ash content and lower ash fusion temperature than typical wood-chips.

Table 15 - Fuel quality parameters of fresh biomass (T0) or after storage (T30) under different conditions.

Parameter	Ventilated		Unventilated		Open-air	
	T0	T30	T0	T30	T0	T30
MC ¹ (%)	58.65 \pm 0.2	10.5 \pm 0.6	58.8 \pm 0.7	42.2 \pm 1.15	59.5 \pm 1.8	60.6 \pm 0.9
HHV ² (MJ/Kg)	17.85 \pm 0.09	18.06 \pm 0.26	17.89 \pm 0.16	17.97 \pm 0.39	17.95 \pm 0.08	17.96 \pm 0.36
LHV ³ (MJ/Kg)	16.64 \pm 0.13	16.85 \pm 0.22	16.65 \pm 0.14	16.74 \pm 0.38	16.69 \pm 0.08	16.71 \pm 0.34
Ash (%)	7.4 \pm 0.3	7.5 \pm 0.3	7.85 \pm 0.2	7.77 \pm 0.2	7.9 \pm 0.3	7.92 \pm 0.2
C (%)	46.70	46.53	46.53	46.44	46.34	45.42
H (%)	5.38	5.38	5.82	5.76	5.58	5.53
N (%)	1.40	1.42	1.58	1.52	1.58	1.51

Table 16 - *Arundo* biomass - ash fusion temperature.

Ash fusion temperatures (°C)				
start	deformation	sphere point	hemisphere point	flow
550	747	840	876	896

4.4 Discussion

The temperature rise in stored green biomass is a reliable symptom of microbial activity. Indeed, the initial high temperature recorded in treatment B and C is a clear sign that biodegradation processes were rapidly established. The thermocouples started to record twelve hours after the setting of the monitoring system, so this time frame was sufficient to reach 60°C.

The use of ventilation in treatment A was applied with the scope to decrease the moisture content of the biomass. Probably, in this initial part of the test, the ventilation worked also as cooling systems, because the temperatures remained slightly above 20 °C.

Regarding moisture content, the initial value of the fresh biomass was about 58-59%. This value decreased significantly after 10 days in treatment A, while it slightly decreased in treatment B and C. This implies that the ventilation was very effective, even if the air was pumped in at intervals of 20 minutes. The trend observed between T_{10} and T_{15} was probably due to the fact that the material already dry was much more predisposed to suffer rehydration due to precipitations. In the case of unventilated bins and open-air-piles, the moisture content trend was mainly influenced by climatic factors such as precipitations, sun and wind.

Interestingly, open-air treatments were much more exposed to the effect of weather; this resulted at the end of storage in a biomass 20% more humid respect unventilated bins. In addition, it was expected that the initial marked rise in temperature would have favored the drying process, but actually the heat did not affected very much natural drying. In fact, after 10 days of storage without precipitation, the moisture content decreased only a few percentage points in both treatments.

The persistence of moisture in the feedstock of unventilated bins and open-air piles has probably promoted a strong microbial degradation. In fact, the study of dry matter losses confirmed that degradation was much more evident in these treatments. On the other hand, in ventilated bins, the instantaneous evaporation of water lowered in short time the water content to values that did not meet the needs of micro-organisms, thus inhibiting the potential fermentation of biomass.

Similar lab experiences were performed in Sweden on willow chips (de Toro et al., 1994). The tests demonstrated the efficacy of the ventilation systems to reduce moisture content

and dry matter losses. Other trials performed in Italy adopting passive ventilation methods in large scale chip piles gave scarce results (Pari et al., 2008). In fact, in this case the internal systems of tubes created ad-hoc to allow air circulation did not reduce the moisture content and the dry matter losses respect the standard method.

The study of the principal polymeric components showed that lignin content was little affected by storage respect cellulose in all treatments. However, taking into account the dry matter losses, we observed that also the lignin was in part affected by degradation. This fact was probably due to the presence of ligninolytic fungi, especially in treatment B and C.

Considering the potential use of *Arundo donax* L. biomass for bio-ethanol and biogas production, an interesting observation is that the amount of cellulose preserved in unventilated bins was the 10% more respect traditional method (open-air piles). Indeed, without the use of electricity, part of the biomass fraction convertible into energy can be preserved in any case adopting bins and probably other similar solutions.

The use of ventilated bins allowed preserving 40% more cellulose than traditional storage method. This will reflect in energy performance of the biomass much higher during conversion into bio-ethanol or biogas. However, further studies are still needed to verify the convenience of the method in economic terms, i.e. if the costs of ventilation are lower than the cost of energy obtained.

In general, the treatment that allows preserving more lignin and cellulose was the ventilated treatment, while the worse storage performances were exhibited by open-air piles. On the other hand, the study has pointed out that the amount of cellulose degraded may result in any case very high, considering also the relatively short storage period. A possible explanation is that the cellulose, because of its chemical composition, is much degradable if exposed to microorganisms.

Indeed, the chopping of the whole plants determined the breakage of many cells, increasing dramatically the surface exposed to degrading agents present in the air and on the soil. In addition, the material was stored in late spring/early summer in Mediterranean climate, within a range of temperatures that typically favor for microbial growth.

In the case of energy produced from combustion, the study of moisture content and dry matter losses showed that ventilation has remarkably influenced the energy potential of the biomass stored, allowing efficient drying and limiting degradation. The study of fuel

quality revealed very low changes in energy characteristics of the biomass, but at the same time confirmed what is indicated in the available literature (Coulson et al., 2004; De Bari et al., 2013; Dahl and Obernberger, 2004; Corno et al., 2014), i.e. a high presence of ash and the low ash fusion temperature point. It must be highlighted that for heating values and ash content only the standard deviations was indicated; statistical analysis was not applied because variations were in all cases lower than 5%. The same was true also for analysis of C, H, and N.

Finally, the last observation regards the relation between C and lignin/cellulose content. The study showed that the changes occurred in lignin and cellulose composition at T₃₀ did not determine variation in C content. This can be explained because other carbonic components present in the biomass, which were not object of the present study, may have undergone proportional changes, contributing to maintain the final carbon balance.

4.5 Conclusion

The aim of this study was to investigate different storage systems and their effects on energy losses and fuel quality. The work showed that dry matter losses of the biomass can be partially controlled by the conservation system used in the post harvesting phase. Indeed, ventilation and climatic factors have respectively influenced in a positive and in a negative way the storage performance of the biomass.

The study demonstrated also that cellulose is the polymeric component that suffered much more the storage, reducing significantly, according to the system used, the bio-energy potential of the product (bio-ethanol and biogas production). Finally, about combustion, the storage system can significantly influence the energy yield without changing important fuel quality parameters such as heating value and ash content.

Chapter 5: Airborne fungi in biofuel wood chip storage sites

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Airborne fungi in biofuel wood chip storage sites



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ABSTRACT

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ABSTRACT

An experimental biofuel wood chip storage site was studied, as a potential fungal “reservoir,” by means of quantitative and qualitative assessments of airborne fungal spores.

Fungal load in the bio-aerosol, determined through active and passive methods, declined with the distance from wood piles. Occupational exposure was comparatively evaluated when two specific operational tasks, manual and mechanized handling, were performed. Under the conditions tested, the manual operators were more exposed to the airborne fungal spores (4864 ± 580 CFU m^{-3} air). The collected spores were identified as belonging to species of the genera *Alternaria*, *Aspergillus*, *Penicillium*, *Fusarium*, *Cladosporium*, *Pleospora*, *Cochliobolus*, *Epicoccum*, *Absidia*, and *Trichoderma*. Most prevalent were the genera *Alternaria* and *Cladosporium*, with the highest percentages of occurrence (30 and 12%, respectively). To the best of our knowledge, this is the first work reporting the identification through molecular methods of airborne fungi released during the handling of wood chip biofuel biomass.

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5.1 Introduction

The demand of renewable energy sources, i.e. biomass, is steadily increasing worldwide to reduce the need of fossil energy sources. The use of biomass such as energy crops, woody species, forestry and agricultural residues, for energy supply is presently estimated between approximately 2 and 10 % of the global net primary production (Smeets et al. 2007; IEA 2012). Fast-growing wood species such as pine, poplar and others are preferred for biomass-based heat and/or electricity production. However, depending on geographic location and season, the availability of wood do not always meet the demand for energy production and there could be periods of the year in which the requests for energy may not be satisfied (Noll et al. 2010; Noll and Jirjis 2012).

In heating plants, the large scale storage of comminuted wood occurs in almost all cases outdoors in piles. Therefore, outdoor storage of wood in large-scale (thousands of tons of materials) becomes an increasingly important issue.

During the storage time, however, microorganisms especially white, brown and soft rot fungi and also, to a minor extent, bacteria carry out the degradation of wood polymers (De Boer et al. 2005), so consequently, similarly to composting and recycling plant, large scale storage biomass areas (sites) may represents ‘reservoir’ for fungal spores.

Moving large quantities of wood materials leads to the release in air of high concentration of spores that may be inhaled and ingested by workers and deposited onto their skin and eyes.

Since airborne mould spores are ubiquitous, and their diameter falls in the range 2–10 μm , their penetration in the lower airways of the human respiratory tract is easy (O’Gormann 2011). *Conidia* inhalation may affect human health in many ways, causing severe diseases, like invasive pulmonary mould infection termed aspergillosis, or contributing to allergic sinusitis and allergic broncho-pulmonary disease (Cornet et al. 2002).

The allergenic and pathogenic potential of a range of fungi is well established, for example, spores of *Alternaria*, *Aspergillus*, *Penicillium* and *Cladosporium* spp. are responsible for causing an array of respiratory conditions, from allergic rhinitis to asthma (Kurup et al. 2000). Exposure to fungal spores occurs mostly indoors nevertheless, outdoor air is an important source of both aeroallergens and pathogens (Curtis et al. 2006;

O’Gormann et al. 2008). Some epidemiological studies have focused on health risks from indoor exposure to toxigenic fungi and mycotoxins, while less studies considered the assessment of these hazards in outdoor environments. In outdoor air, the concentration of spores may vary considerably, depending on climate, time of day, season, and site (Koch et al. 2000; Ren et al. 2001). Fluctuations in the outdoor concentration are mostly driven by natural parameters, such as seasonal variations of temperature and humidity, geographical position, occurrence of accumulated rotten material, or dispersed soil (Herbarth et al 2003).

There have been several studies examining concentrations of outdoor airborne fungal spores in urban areas, (Guinea et al. 2006; Shelton et al. 2002; O’Gormann et al. 2008), and many others on workers’ personal exposure to bioaerosols associated with specific tasks (Schlosser et al. 2009; Grisoli et al. 2009; O’Gormann 2011; Sakawari et al. 2013).

In biofuel plants it is known the risk associated with handling wood and wood chip (Madesen 2006; Madsen et al. 2009), however no regulatory occupational exposure limit (OEL) is set for airborne biological agents, despite of this operational and health and safety (H&S) managers need recommendations for bioaerosol-related health risk management (Schlosser et al. 2009).

The objective of this study was to investigate the airborne fungi collected in proximity of biomass chip piles storage site in outdoor environment and to evaluate whether there is a risk to worker health from the inhalation of bioaerosol. The study was carried out using a culture-based method for bio-aerosol sampling, in order to evaluate exposure risks for worker during movement activities. To this aim, the bio-aerosol sampling was quantitative and quantitative evaluated for viable airborne fungi; besides the standard enumeration of as CFU/m³, molecular methods have been used to identify the isolates and to assess their possible harmfulness.

5.1 Materials and methods

Sampling site and movement activities

The study was carried in the experimental storage plan of Research Center on Agriculture (CRA-ING Monterotondo Rome, Italy). The storage site had a total area of 6000 m² and where piles of poplar chips were stored for six months. Every pile had a mean volume of 117 m³ and a weight of about 40 tons.

The chips were handled using a cabin cruiser excavator equipped with a forest gripper. The biomass was then loaded on truck and transported in another area of the research center in order to reproduce the operations traditionally performed in heating plants.

Meteorological measurements

The environmental conditions were monitored during the entire storage time from March 2012 to September 2012 by an automatic recording device (SIAP-MICROS DA 9000 Davis, CA) located in the same place, which provided temperature and humidity, wind, rainfall data at 30 min intervals (figure 25).

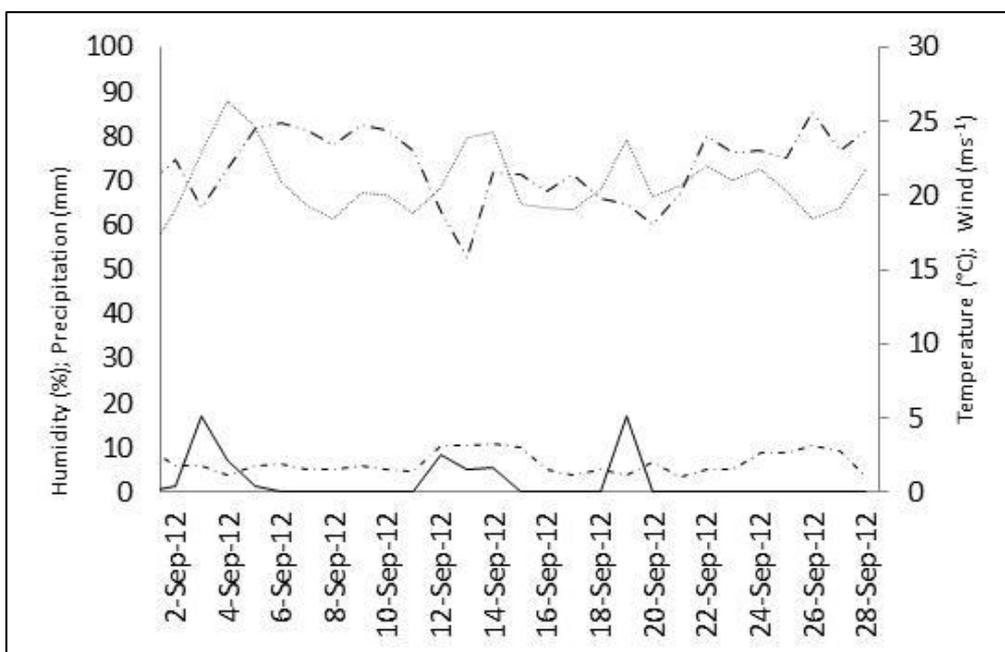


Figure 25 - Meteorological parameters measurements registered during the experiment.

Environmental sampling procedures

The sampling was made for fungal spores and dust quantification during the handling activities of six different wood piles. Dust quantification was performed using sampling devices “BRAVO m2” (TECORA, 94134 Fontenay sous Bois Cedex, France) having a flow rate of 15 L/min. Each device was equipped with Fluoropore Membrane Filter (FHLP04700) (Millipore, MA, USA) with a pore size of 45 μm .

Fungal spore counts were carried out during ordinary work days by means of an active sampler and settling plates (passive sampling) as schematized in figure 26.

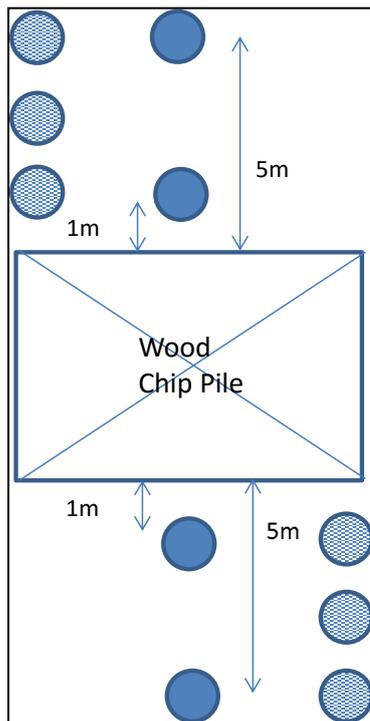


Figure 26 - Schematic disposition of environmental sampling stations: filled circle active air sampling: squared circles passive air sampling.

Active method

Quantitative data were collected in duplicate using a stationary air sampling device “BRAVO m2” (TECORA, 94134 Fontenay sous Bois Cedex, France) that aspirates a defined air volume. Calibration, setting of pumps (range 4-8 L/min) and time of sampling (15-30 min) were defined after a pre-inspection of the site.

The sampler was disinfected with 70% rubbing alcohol, and alcohol was dried before loading the filter in cellulose acetate with a diameter of 47mm and a pore size of 0.22 μ m. The filter was then placed in a Petri dish with agar culture medium, so that the microorganisms collected from the air could give rise to colonies. Following incubation for several days at 30°C, fungal colonies were counted. Results are expressed as numbers of colony-forming units for cubic metre of air sampled (CFU/m³).

Passive method

Using the gravity settling culture method, Petri dishes with culture medium were exposed to air on a tripod at a height of 1.5 m above ground for a distinct period of time (from 20 min to 1h) to provide the optimum condition. The plates were then incubated at 30°C for

the detection and enumeration of fungal colonies. Counts of fungal colonies were expressed as CFU per Petri plate per h of exposition. Colonies with different morphology were isolated in pure culture and identified. The stock cultures of the various isolates were preserved at the University of Tuscia Microbiology Laboratory (DIBAF).

Personnel sampling procedure

In order to determine worker exposure during handling of the piles, different types of personal sampling were carried out, i.e. epidermal and operator breathing sampling (dust and spores).

Epidermal sampling

Sterile swabs were rubbed on the skin and in particular in areas close to the eyes of the operators before and after handling procedures and subsequently used to inoculate Petri plates containing agar growth medium.

Breathing sampling

In order to assess their level of exposure to fungal spores, personnel with different tasks was monitored for time intervals through a mobile air personal sampler ("EGO PLUS TT - PF 11221, Zambelli s.r.l., Italy), having a flow rate of 4,3 L/min and equipped with a filter in cellulose acetate with a diameter of 37mm and a pore size of 0.22 μm .

For personnel dust exposure the same device was equipped with Fluoropore Membrane Filter (FHLP03700, Millipore) with a pore size of 45 μm .

Growth media

Rose Bengal Agar (RBA) supplemented with chloramphenicol (which inhibits the growth of bacteria) was used as culture medium for the germination of spores. This medium is particularly suitable for total counts because of the inhibitory effect of rose-bengal on fast-growing mucoraceous moulds.

Fungal identification

Genomic DNA (gDNA) of isolated strains was extracted according to the method of Dellaporta et al. (1983). Amplification of the internal transcribed spacer (ITS) region of rRNA gene was performed by using ITS1 (TCCGTAGGTGAACCTGCGG) and ITS4

(TCCTCCGCTTATTGATATGC). The optimal amplification conditions were those previously reported by Crognale et al. (2012).

The purified PCR products were used in sequencing reactions with the same set of primers, using a BigDye Terminator cycle sequencing ready reaction kit, version 3.0 (Applied Biosystems, Foster City, CA). Sequencing was performed on an ABI 3730 DNA sequencer (Applied Biosystems). Sequences were deposited in Gen-Bank (<http://www.ncbi.nlm.nih.gov>) and compared with those of all known fungal species available from the same database.

Statistical analysis

The likelihood of statistically significant differences between the concentrations of fungi measured in each sampling was assessed by one-way analysis of variance (ANOVA) and multiple pair-wise comparisons were performed by the HSD-Tukey test. A one-side p-value of less than 0.05 was considered significant in the test.

5.2 Results

The results obtained from active air sampling are reported in table 17. The total amount of fungal spores measured in the storage area during movement operations was over the value of 1000 CFU m₃ air.

Table 17 - Active and passive environmental air sampling at different distance from the wood chip piles. Results from passive sampling are obtained after 1 h of exposition.

Distance from pile (m)	Active sampling				Passive sampling	
	w/o movement activities		With movement activities		w/o movement activities	With movement activities
	Particulate (mg/m ³)	Fungal spores (CFU/m ³)	Particulate (mg/m ³)	Fungal spores (CFU/m ³)	Fungal spores (CFU/plate)	Fungal spores (CFU/plate)
1	<0.1 ^a	291 ± 26 ^{aA}	0.17 ± 0.04 ^a	1689 ± 412 ^{bB}	28 ± 6 ^{aA}	207 ± 12 ^{bB}
3	----	---		---	32 ± 3 ^{aA}	212 ± 15 ^{bB}
5	<0.1 ^a	227 ± 38 ^{aA}		1510 ± 467 ^{abB}	26 ± 2 ^{aA}	219 ± 22 ^{bB}
300	<0.1 ^a	310 ± 56 ^{aA}	0.019 ^a	614 ± 85 ^{aB}	23 ± 4 ^{aA}	55 ± 9 ^{ab}

*Data are the mean ± SD. Multiple pair-wise comparisons were performed by the HSD-Tukey test (P ≤ 0.05): same lowercase and uppercase letters denote the absence of statistically significant differences between column and row means within of the same sampling method, respectively.

No significant differences in total amount of fungal spores were found within the first 5 m from the piles, while at a distance of 300 m the level of fungal spore concentration declined to 614 CFU m⁻³, due to atmospheric dispersion and dilution.

Results listed in Table 16 indicate high levels of exposure to fungal spores and mycelial fragments (4864 ± 580 CFU m⁻³) for the manual operators working on the pile, while that of the machinist was significantly lower (894 ± 82 CFU m⁻³).

Since most of the mold-correlated symptoms are allergic reactions, with inflammation of the eye or skin and congestion (Edmondson et al., 2009), in this study the workers' exposure was evaluated not only in terms of respirable spore concentration, but also in terms of epidermal exposure of the skin close to the eyes. After 1 h of manual work, a mean value of 7 CFU cm⁻² was recorded (table 18).

Table 18 - Personnel exposure to particulate and airborne fungi as a function of task. Epidermal sampling are referred to 1 h of exposure.

Personal task	Breathing sampling				Epidermal sampling	
	w/o movements activities		with movement activities		w/o movements activities	with movement activities
	Total dust (mg/m ³)	Fungal spores CFU/m ³	Total dust (mg/m ³)	Fungal spores CFU/m ³	Fungal spores CFU/plate	Fungal spores CFU/plate
Manual operator	< 0.1 ^{aA}	20 ± 3 ^{aA}	0.35 ± 0.03 ^{bB}	4864 ± 580 ^{dB}	2 ± 0.1 ^{aA}	7 ± 1.5 ^{bB}
Inside vehicle cabin	-	25 ± 8 ^{aA}	0.12 ± 0.02 ^{aB}	894 ± 82 ^{aB}	-----	4 ± 1 ^{aB}

*Data are the mean ±SD. Multiple pair-wise comparisons were performed by the HSD-Tukey test (P ≤ 0.05): same lowercase and uppercase letters denote the absence of statistically significant differences between column and row means within of the same sampling method, respectively.

More than 300 fungal isolates were collected by means of the gravity settled method. Based on colony and hyphal morphology and characteristics of spores, the isolates were classified into 14 different morphological taxa and one of each was chosen for the identification phase, the results of which are reported in table 19.

Table 19 - Taxonomic assignment of the fungal spores and mycelial fragments collected from bio-aerosol (environmental and personal sampling) and from workers' skin in wood chip storage plan.

Microorganisms	Homology (%)	Deposited accession number	Source of isolation
<i>Alternaria sp.</i>	100	KF482451	Skin/Bioaerosol
<i>Arthrinium arundinis</i>	99.8	KF482448	Skin/environment
<i>Aspergillus fumigatus</i>	100%	KF482453	Bioaerosol
<i>Aspergillus tubingensis</i>	100%	KF482450	Skin/bioaerosol
<i>Cladosporium sp.</i>	100%	KF466173	Bioaerosol
<i>Cochliobolus specifiers</i>	100%	KF482452	Skin / Bioaerosol
<i>Epicoccum nigrum</i>	100%	KF466170	Bioaerosol
<i>Fusarium chlamydosporum</i>	100%	KF466171	Bioaerosol
<i>Lewia infectoria (Alternaria)</i>	100%	KF466174	Skin/Bioaerosol
<i>Lichtheimia corymbifera (Absidia)</i>	100%	KF466172	Bioaerosol
<i>Penicillium sp.</i>	99.8%	KF494298	Bioaerosol
<i>Penicillium citrinum</i>	100%	KF466169	Bioaerosol
<i>Pleospora herbarum</i>	100%	KF482449	Skin/Bioaerosol
<i>Trichoderma longibrachiatum</i>	100%	KF466168	Bioaerosol

As expected, all the isolates were sporogenic fungi, mostly phytopathogens, i.e., *Pleospora herbarum*, *Cochliobolus* and *Epicoccum nigrum*, and some were allergenic and potential human pathogens (i.e., *Aspergillus fumigatus*). *A. fumigatus* was detected in the bioaerosol but not on the workers' skin surface, while many other allergenic fungi were detected in air and in epidermal samples.

Figure 27 shows the occurrence of the prevalent airborne fungi collected from environmental air sampling. *Absidia sp.*, *P. herbarum*, *Arthrinium aurundinis*, and *Trichoderma sp.* are not included in the graph, since their presence was not revealed in all the samples analyzed.

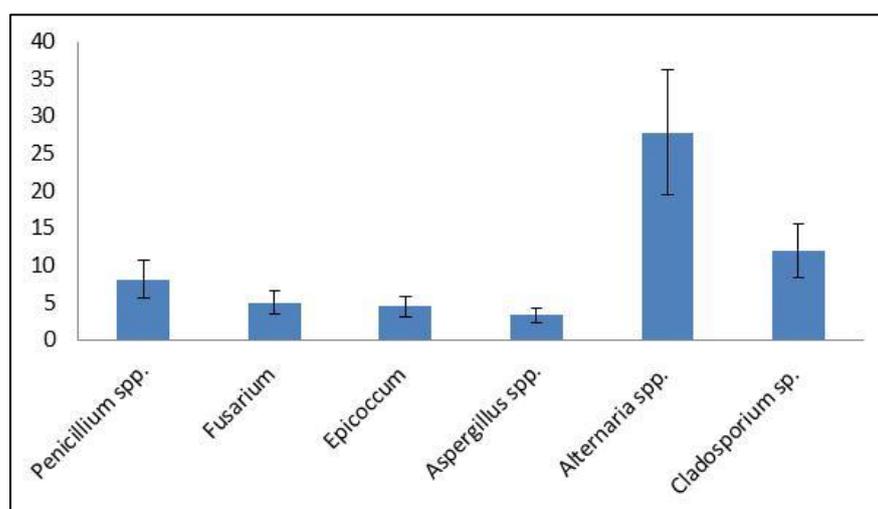


Figure 27: Taxonomic assignment of the fungal spores and mycelial fragments collected from bio-aerosol (environmental and personal sampling) and from workers' skin in wood chip storage plan.

The most prevalent genus was *Alternaria*, species of which (*A. infectoria* and *Alternaria* sp.) were detected in almost all the samples and reached the highest percentage of occurrence (ca. 30%). Conidia of *Cladosporium* sp. were the second most commonly isolated spore type, with an average occurrence of 12%. Members of the genera *Fusarium*, *Epicoccum*, *Aspergillus*, and *Penicillium* were also collected, with occurrences ranging between 5 and 10%.

Biofuels represent sustainable energy resources of growing economic importance, but at the same time they may pose significant health problems (Sebastian et al., 2006). The outdoor environment, where wood chips are usually stored in piles for long periods before use as biofuels, is a natural habitat for fungal microorganisms. Biological risks can arise from potentially pathogenic microorganisms living as saprophytes or as parasites grown on wood before storage, as well as from microbial forms that can develop during the degradation of woody polymers.

Recent work focusing on the microbial community of energy chip piles (Noll et al., 2010; Suchomel et al., 2012) found that microorganisms growing during the storage period may be potential human pathogens and toxic or allergenic species (Suchomel et al., 2012). Moreover, Sebastian et al. (2006) found that the microbiological dustiness, mainly due to spores and mycelia fragments, measured as ergosterol content and total counted fungi, was at a maximum during the summer.

On this basis and considering the geographic and climatic characteristics of the biomass storage plant location monitored in this study, sampling and fungal spore enumeration were carried out in late summer. During the movement activities, the level of airborne fungal spores (1689 _ 412 CFU m₃) exceeded the suggested limit of 1000 CFU m₃ for composting and sewage treatment plants (Oppliger et al., 2005; Grisoli et al., 2009). Similar fungal spore concentrations (2377 CFU/m₃), indicating a significant level of fungal air pollution, were also found by Madsen et al. (2009).

Since the air spore concentration declined to less than 1000 CFU m₃ at distance of 300 m from the wood pile, this distance may be considered sufficient as a buffer zone to limit exposure to potential risks. Interestingly, our results are in agreement with the recommendation of the UK Environment Agency of a 250- mset-back distance from a composting facility site boundary to the nearest dwelling to remove the potential for increased exposure to fungal conidia (Environment Agency, 2009).

The effect of distance on airborne microbial pollution was also studied by Grisoli et al. (2009), who investigated an open composting facility and a wastewater treatment plant, and found that the contamination levels were highest within a distance of 40 m. It must be noted that in the present study the highest spore concentrations were registered during working activities; this means that the movement of a wood pile represents the major cause of fungal dispersion, promoting the detachment of spores from hyphae and their release into the air. On the other hand, in static storage conditions, aerosolization by wind did not lead to a significant dispersion of airborne spores.

The exposure of personnel to bio-aerosols in biomass storage sites appeared strictly dependent on the work task. Workers handling the wood were exposed to a level of spores that could be considered critical. This is particularly true considering that the cultural methods usually reveal lower fungal concentrations than those based on spore trap. Many spores, in fact, may be non-viable, dormant, or unable to grow on the media used. The machine operator was less exposed since the vehicle cabin offered protection against the diffusion of fungal spores (Bünger et al., 2007; Schlosser et al., 2009, 2012).

The fungi collected from environmental and personal samplings were identified by molecular methods, after a cultivation step on RBA. This medium was particularly suitable for fungal count and isolation, inhibiting bacterial growth and limiting the colony size of faster-growing moulds (e.g., *Mucor* sp). Usually, this approach provides proper identification of the fungal airborne species if compared with the use of spore traps, which allow the easy identification of spores of *Alternaria* and *Epicoccum*, but cannot distinguish between *Aspergillus* and *Penicillium* spores (Eduard, 1996; Fernández et al., 2012; Skjøth et al., 2012) or identify or count mycelial fragments.

The identified airborne fungi reflected a microbial community typical of wood storage ecosystems and were mostly represented by the so called “opportunistic molds” able to cause allergic reactions in healthy people and infections in immune-compromised subjects (Friedman et al., 1991; Washburn, 1996). The substrate homogeneity typical of wood storage plants may be the cause of the lower fungal diversity as compared to that found by Grisoli et al. (2009) in compost facilities; the total amount of airborne fungi, however, was of the same order of magnitude.

Among the different fungi isolated, *A. fumigatus*, the major airborne fungal pathogen (Rizzetto et al., 2013), has been identified. This species is typically found in self-heating

organic biomasses, where it has an essential role in the degradation of plant polymeric material (Tekaiia and Latgé, 2005), and can seriously affect the immune system of workers in compost facilities and landfills (Bünger et al., 2007; Lis et al., 2010). Indeed *A. fumigatus* is reported to be responsible for about 90% of all invasive aspergillosis cases (Denning, 1998).

On the studied wood storage site, *Alternaria* sp. were most frequently isolated, surpassing the allergenic threshold level for evoking allergic symptoms (Gravesen, 1979), of 100 *Alternaria* conidia m⁻³, proposed by Bagni et al. (1977). Species of this genus have been found growing on different natural habitats, such as cereal crops, rotten wood, compost, and various forest plants (Skjøth et al., 2012), having as a favorite habitat warm dry regions.

Usually, their spore concentration is higher in rural than nearby urban areas (O’Gorman and Fuller, 2008; Oliveira et al., 2009). It must be stressed that the percentage of occurrence of airborne fungi might not strictly reflect the relative abundance of the fungal community degrading the chip pile. This is obviously related to aerodynamic phenomena affecting conidial dispersion depending on spore morphological characteristics (e.g., diameter and surface roughness) (Jones and Harrison, 2004).

5.4 Discussion and Conclusions

Biofuel wood storage sites can definitely be regarded as fungal spore “reservoirs”; however, they do not represent a potential risk for nearby populations. In fact, the air contamination level decreases significantly with increasing distance from the pile, and reaches values below the suggested limit of 1000 CFU m⁻³ at ca. 300 m from the sites.

Significantly high levels of fungal pollution, particularly due to allergenic species such as *Alternaria* sp. and *Cladosporium* sp., can be present near the wood storage plant during the movement of the wood chip piles and may potentially be harmful for employees handling the wood manually. Although among the isolated fungi only *A. fumigatus* may be of pathogenic concern for the workers’ health, the results of this study suggest that the employment of allergic or sensitive workers in wood storage plants should be discouraged, while the use of respiratory and eye protection devices should be recommended to avoid possible allergic reactions. Our findings also point out the need for standard security procedures at wood chip storage sites.

6. Main findings of the research program

Storage is often considered as a simple way to keep large amount of material in a limited space. However, in case of lingo-cellulosic biomass, this process entails different problems and several doubts. The research activities performed in this Ph.D. program showed the different aspects related to the storage of lingo-cellulosic biomass in Mediterranean climate, the factor affecting biomass degradation and the risks related to the conservation of this organic entity. From a scientific point of view, the layout of each study was kept original as much as possible, taking in some cases example from previous studies performed in Europe, but modifying the design and the species tested.

In Chapter two it was analyzed the storage behavior of poplar wood chips produced from different plant parts (stems and crowns). The most interesting finding in this research is embedded in the storage performance of crown wood chips. In fact, the small dry matter losses suffered by crown wood were positively balanced by the fuel quality changes occurred during storage (increase of heating value after storage). In this regard, we verified that the final energy balance of crown wood was close to zero, meaning that six months open-air storage did not affect the potential energetic production of the biomass. It was unexpected because studies on the crown part of the woody species often show substantial deterioration in quality while stem wood has generally better storage properties. However, the measurements were performed using two different methods (sample bags and direct weighing) with similar final results, so errors in the measurements can be excluded.

Beside the scientific interest, this information can be extremely useful for industries and power plants working with poplar, because the crowns till now are often considered a bulky residue to be disposed, difficult to handle and that take up much space. On the other hand, as the present research suggests, the poplar crown wood can be chipped immediately after cut and stored outdoor for long periods without a remarkable decline of the feedstock quality.

Contrary to crowns, the energy balance of stem wood chip was significantly negative, meaning that chips deriving from stem wood suffered significant energy loss during storage. This problem was faced in chapter three, as one of the objectives of the research presented was testing the conservation capacity of poplar stems stored in windrows as logs in the field. In this regard, the interesting finding of this research is undoubtedly the fact that poplar stems stored as whole trunks can be left in windrow, along the inter-row of the

cut plantation, during winter without suffering dry matter losses and achieving acceptable level of moisture content. The mechanical solutions designed by CREA-ING, consisting of new automatic machines, will allow the collection and chipping of the material directly from the windrows in early spring. This technique allows accumulating large biomass stores without incurring in dry matter losses and without occupying costly industrial areas. However, the focus of the Ph.D. program was not limited to the study of the storage dynamics biomass deriving from woody species. In fact, lingo-cellulosic biomass for energy production includes material deriving also from perennial grasses and agro-residues such as pruning, shells, and husks.

In this regard, in chapter four the focus was addressed to the species *Arundo donax* L.. This perennial grass in the last year has received much attention from researchers and bio-energy industrial producers for different reasons, but mainly because it can be cultivated without requiring significant agricultural inputs and in marginal lands, i.e. without subtracting agricultural land to food crops. In this case, the experimentation was kept in small scale, as different storage configurations (ventilated bins, non-ventilated bins, and open-air piles) were tested and replicated for result validation.

The work showed that dry matter losses of the biomass can be partially controlled by the conservation system used in the post harvesting phase. Indeed, ventilation and climatic factors have respectively influenced in a positive and in a negative way the storage performance of the biomass. The most interesting finding in this research was exhibited by the results achieved in ventilated bins, because degradation in this type of configuration was significantly controlled respect the other two layouts. In this case, the very rapid dehydration of the biomass has been key to control microbial activity, as identified with the moisture content and dry matter losses analysis. Actually, after 10 days of ventilation the product presented acceptable levels of moisture content for energy conversion through combustion. At that time the amount of dry matter losses were about the 6%, which is value comparable with wood chips.

However, the study demonstrated also that cellulose was the polymeric component that suffered much more the storage, reducing significantly, according to the system used, the bio-energy potential of the product for bioethanol production respect the potential of the starting material. This is another indication for industries working in the bioethanol generation. Indeed, these producers should be aware that an optimized production of this

fuel can be reached just developing mechanisms that will stop degradation immediately after cut.

The last goal of the Ph.D. was the analysis of the risk to which workers are exposed every time they handle lingo-cellulosic biomass. Specific qualitative and quantitative studies related to the biological risks in a storage site of poplar wood chips were performed. Literature review did not show any similar study performed at national level.

Interesting finding of this research was represented by significant high levels of fungal pollution identified in the storage site. In particular, the fungal identification revealed the presence of allergenic species such as *Alternaria* spp. and *Cladosporium* sp. and pathogenic species such as *A. fumigates*. These fungal entities suggest that the employment of allergic or sensitive workers in wood storage plants should be discouraged, while the use of respiratory and eye protection devices should be recommended for “non-sensitive” workers until a distance of 300 m from the storage site.

Hopefully, the information obtained will be helpful for researchers to give new perspective for the resolution of the problems related to storage, but also for wood and energy industries to understand the opportunities of unexploited products as well as the mechanisms that determine energy losses. In addition, the expectation is that the information provided will furnish new insights for the responsible institutions to enrich human safety regulation in the biomass storage areas.

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