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The short-term degradation of cellulosic pulp in lake water and peat soil: A multi-analytical study from the micro to the molecular level

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ABSTRACT

Cellulosic pulp was analysed after two and five years of natural ageing in two different burial environments - lake water and peat soil - in order to investigate the chemical and structural changes undergone by the material. This research is part of a monitoring program developed in the archaeological site of Biskupin, whose final aim is to estimate the best re-burial conditions for an *in situ* conservation of archaeological wood. We applied SEM, FT-IR, XRD and Py-GC/MS with thermally assisted silylation using hexamethyldisilazane (HMDS) to obtain broad information on cellulosic pulp degradation.

SEM provided information on the structural changes undergone by the fibres and on the microbial activity. FT-IR highlighted a depletion of the carbohydrates in the most degraded samples. Py(HMDS)-GC/MS confirmed these observations and provided detailed molecular information on cellulose alteration. The crystallinity index (CI) of cellulose was estimated by XRD, showing that the degradation began in amorphous regions. The correlation between the CI and the relative abundance of anhydrosugars detected by Py(HMDS)-GC/MS demonstrated that pyrolytic reactions are also influenced by the degree of crystallinity of cellulose.

A mechanism of cellulose degradation in these natural environments was formulated: the degradation of cellulose in these natural environments proceeds through a depolymerisation step, initially involving the amorphous regions of cellulose, until the size of oligomers becomes sufficiently small to allow metabolisation by microorganisms or solubilisation by water. Secondly, a loss of carbohydrates was detected after five years of ageing and the loss reached 25% and 55% for the dark areas of samples aged in peat soil and lake water, respectively. Peat soil was found to be more suitable than lake water in terms of preservation of carbohydrates.

1. Introduction

The archaeological site of Biskupin (Poland) preserves the remains of a fortified settlement dating back to the Bronze and early Iron Ages (around the mid 8th century BC) which is almost completely made up of oak and pine wood. In recent years increasing attention has focused on *in situ* conservation strategies for archaeological wood, which entails leaving the wood remains in the environment in which they were found, either in the ground or water. This approach has been adopted for archaeological wood remains found in Biskupin (Babiński et al, 2007; Tamburini et al, 2015; Waliszewska et al, 2007).

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The preservation state of some of the archaeological woods found in the site has been evaluated from both chemical and mechanical points of view and the changes were in some cases related to the burial conditions (Babiński et al, 2014; Tamburini et al, 2015; Waliszewska et al, 2007). Nevertheless, the risks of the re-burial of these archaeological woods and the dynamics and extent of possible degradation processes in different burial conditions are not assessed, and need to be evaluated. To achieve this aim, a monitoring program was set up, whose final aim is to estimate the best re-burial conditions for an *in situ* conservation of archaeological wood in Biskupin site.

Cellulosic pulp and sound oak and pine woods were buried in peat soil and in lake water in Biskupin, using the same burial conditions as archaeological wood. The materials were periodically removed from the burials and analysed. This paper presents the results obtained by the analysis of cellulosic pulp during the monitoring program, aimed at obtaining a reliable and correct evaluation of the state of degradation of cellulosic pulp after two and five years of natural ageing in lake water and peat soil in Biskupin site. We decided to start from the analysis of cellulose, as this is the most sensitive wood component and these results will be useful to better understand the changes occurring in the wood samples (still under investigation) under these specific burial conditions, and finally to identify the best re-burial conditions for the *in situ* conservation of archaeological wood.

Cellulose is the world's most abundant naturally occurring organic substance and the major building block in the cell wall structure of higher plants. The structural basic unit consists of glucose residues connected by β-(1,4)-links, thus the polymer is linear. Around 40–50% of wood is made up of cellulose and wood cellulose has an average degree of polymerisation (DP) of 10000–15000 (Moldoveanu, 2010; Rowell et al, 2005). The tendency of cellulose molecules to form intra- and intermolecular hydrogen bonds leads to the formation of microfibrils, which are arranged in stacked layers forming crystalline regions. Wood-derived cellulose can be up to 65% crystalline. The remaining portion is referred to as amorphous cellulose. The amorphous zones are more reactive than the more highly ordered crystalline areas (Coffey et al, 2006; Rowell et al, 2005).

A detailed characterisation of cellulose requires a multi-analytical approach, often applied after isolation of cellulose from the matrix. Cellulose can be isolated from wood since it is analytically distinguished from extractives by its insolubility in water and organic solvents, from hemicelluloses by its insolubility in aqueous alkaline solutions, and from lignin by its relative resistance to oxidizing agents and susceptibility to hydrolysis by acids. Generally a delignification step is required in order to separate the polysaccharide components. The non-cellulosic materials are then removed by alkaline extraction (Browning, 1967; TAPPI, 1996-7). In the pulp and paper industry, various wet chemical analytical methods have been developed and standardised to characterise cellulose and the other wood components, in order to exploit the individual components in industrial processes or to produce energy (Bond et al, 2014; Carlson et al, 2011). These methods are referred to as TAPPI methods and they are useful to accurately quantify the components present in lignocellulosic materials. Nevertheless, the high amount of sample needed, the time required for the analysis and the possible artefacts produced during the chemical treatments are the main drawbacks of wet chemical methods. This is why various instrumental techniques are often applied to obtain information on cellulose material at different levels, from microscopic and spectroscopic observations to hyphenated techniques, including a chromatographic separation step.

The morphological characterisation of cellulose material can be performed in terms of shape, size, roughness and compactness of fibres by both scanning and transmission electron microscopes (SEM and TEM) (Chinga-Carrasco, 2010). Information on specific functional groups and their relative amounts is obtained using spectroscopic methods, such as FT-IR (Oh et al, 2005; Schwanninger et al, 2011; Yue et al, 2015) and NMR (Bardet et al, 2009; Newman, 1999; Santoni et al, 2015; Yue et al, 2015). Extremely valuable for establishing the preservation state of degraded cellulose is the determination of the crystallinity index, that can be assessed by XRD, FTIR or NMR (Åkerholm et al, 2004; French and Santiago Cintrón, 2013; Oh et al, 2005; Park et al, 2010; Yue et al, 2015). Information on the molecular weight of cellulose polymers can be obtained by size exclusion chromatography (SEC), gel permeation chromatography (GPC), asymmetric flow field–flow fractionation (A4F) and viscosimetry. A critical aspect is to establish accurate standards for the calibration when a degraded polymer is under study, and inter-laboratories standard deviations of the absolute values obtained are often high (Kačík et al, 2016).

It has to be underlined that when cellulose is analysed after the dissolution, precipitation and derivatisation processes, the obtained results may be affected by alteration of the polymer introduced during the pre-treatment.

In our research on lignocellulosic polymer a central role is played by analytical pyrolysis. Analytical pyrolysis coupled with gas chromatographic separation has been used to study cellulose in detail (Moldoveanu, 1998). Pyrolysis reactions begin around 150 °C and up to 300 °C these reactions mainly involve a reduction in the degree of polymerisation, the formation of free radicals, the elimination of water, the formation of carbonyl, carboxyl and hydroperoxide groups, and the evolution of carbon monoxide and carbon dioxide (Lu et al, 2011; Pouwels et al, 1989; Shafizadeh and Fu, 1973). The most significant weight loss starts around 300 °C (Yang et al, 2007), when energy becomes sufficient for a rapid cleavage of the glycosidic bonds, followed by the evaporation of the products. The primary volatile products derive from chain scission and water elimination reactions, which lead to the formation of anhydrosugars of which levoglucosan is the major product isolated (Moldoveanu, 1998; Pouwels et al, 1989). After this first pyrolytic step, further decompositions take place, resulting in the formation of furans, pyranones, cyclopentenones with different functionalities. When fast pyrolysis is used with a relatively high temperature (500–600 °C), all the mechanisms involved in pyrolysis reactions operate in parallel, and their relative yields strongly depend on pyrolysis conditions, the average DP value, crystallinity, water content and pH of the sample (Azeez et al, 2011; Lu et al, 2011; Moldoveanu, 1998, 2010; Ramirez-Corredores, 2013). Many cellulose pyrolysis products have been identified and reported in the literature (Galletti and Bocchini, 1995; Moldoveanu, 1998, 2010; Pouwels et al, 1989; Ralph and Hatfield, 1991).

When GC is used to separate pyrolysis products, the high abundance of polar hydroxyl functionalities in most of the cellulose pyrolysis products can cause peak broadening with a loss in resolution and adsorption in the Py-GC interface. A derivatisation step is therefore suggested and the most common thermally assisted on-line derivatisation reactions are methylation and silylation reactions (Challinor, 2001; Fabbri and Chiavari, 2001; Fabbri and Helleur, 1999).

The use of TMAH (tetramethylammonium hydroxide), the most