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Tracking the dynamics of hemp dew retting under controlled environmental conditions

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ABSTRACT

The use of natural fibers such as hemp (*Cannabis sativa* L.) as substitutes for nonrenewable fibers increases the life cycle performance of composite materials. The management of retting in fields as a natural pretreatment prior to fiber extraction remains challenging due to a lack of knowledge about the relative importance of environmental and biotic factors, which continually interact under field conditions. Here, we studied the dynamics of hemp retting under controlled air temperature and humidity conditions and with simulated rain. We tracked the color and infrared spectral absorbance of the stem surface, the chemical composition and microbial enzyme activities of the bast tissues, and the stem architecture over 42 days at 15 °C. Color changes on the stem surfaces were the first indicators of retting progress, with a significant decrease in the L*, a* and b* values (CIELAB) from day 14 onward. These findings were closely correlated with the surface colonization progress as revealed by scanning electron microscopy, the changes in enzymatic activities and the decohesion of the bast tissues. Additional investigations are needed to study other environmental scenarios to provide an accurate assessment of the retting process over time.

1. Introduction

Improving the life cycle performance of products has become a priority for consumers, and, consequently, for industry (Sauvageon et al., 2017). The use of natural fibers such as hemp (*Cannabis sativa* L.) as a substitute for synthetic or mineral fibers in applications such as composites is part of this trend, and it is broadly under study for industrial applications in Europe. However, natural fibers are still used only in small quantities because, among other reasons, of quality issues related to the heterogeneous properties involved in any biomass production (Fernandez-Tendero et al., 2017; George et al., 2016) (Fernandez-Tendero et al., 2017; George et al., 2016). To facilitate defibration and preserve the fiber quality, a pretreatment step known as retting is performed, and it involves the selective degradation of the parenchyma cells surrounding the fiber bundles located in the bast tissues of the stem, leading to their decohesion from the rest of the stem (Müssig, 2010). This interbundle decohesion can continue with intrabundle decohesion involving the selective degradation of the thin primary cell wall and middle lamella around the thick cellulosic secondary cell wall of the cells composing fiber bundles (Liu et al., 2017b). The fiber extraction from the plant is thus easier, making it possible to preserve the fiber integrity and homogenize the fiber properties by reducing the amount of energy needed for the decortication step (Liu et al., 2015a). In the case of hemp, traditional methods of retting, such as dew (or field) retting and water, have been used for centuries and are still the dominant procedures.

Dew retting consists of leaving stems in the field, after the harvest, at the interface between the soil and the atmosphere, facilitating the natural and partial degradation of the bast tissues. Degradation of the pectin-rich middle lamella allows separation of the bast fibres from the stem with partial dissociation of the bundles (Ribeiro et al., 2015). This process occurs under local environmental conditions and is regulated by abiotic and biotic factors such as local weather conditions and soil microbial colonization (Liu et al., 2015a). The process of dew retting is not yet fully characterized and is highly climate-dependent, which makes it difficult to obtain a predefined quality for industrial applications (Bacci et al., 2010; Jankauskienė et al., 2015). Most of the literature published on hemp field retting focuses on comparisons with other retting processes involving only a single field experiment (Jankauskienė et al., 2015; Liu et al., 2017a). With this approach, it is difficult to characterize the factors that drive the retting process and to compare results between experiments. Limited research has been

Abbreviations: 4-MUB, 4-methylumbelliferone; AHC, ascending hierarchical classification; ANOVA, one-way analysis of variance; ATR-FTIR, attenuated total reflection-Fourier transform infrared; DNS 3, 5-dinitrosalicylic acid; EMC, equilibrium moisture content; PCA, principal component analysis; SEM, scanning electron microscopy

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conducted under controlled laboratory conditions, which would allow for the environmental parameters to be set and to be decoupled from one another and thereby avoid confounding effects, e.g., the harvest date, which influences both the hemp composition and the climatic conditions at retting time.

In this study, we developed a new experimental approach performing hemp dew retting under controlled conditions (in relation to the air temperature, relative humidity, simulated rains and lighting cycle). This approach was derived from plant litter decomposition studies (Iqbal et al., 2015; Lee et al., 2014) that were aimed at acquiring a better understanding of the relationships between the properties of plant residue mulches, environmental conditions and decomposition. The objectives of this work were i) to verify the capacity of the controlled experimental approach to reproduce the hemp dew retting process and ii) to dynamically link the changes in the macroscopic properties of stems with chemical properties and observations of biological processes.

2. Materials and methods

The experimental approach implies miniaturization and simplifications to ensure homogeneity, standardization and reproducibility for further experiments (i.e., in terms of the soil granulometry, rain, hygrometry, hemp stem segment length, litter layer shape and height).

2.1. Plant material and soil sampling

Hemp (*Cannabis sativa* L. cv. Fedora 17) was grown in the Champagne area (eastern France) and harvested in September 2015 at the physiological stage of seed maturity. The stems were cut a few centimeters above the ground, dried in a forced-air oven at 40 °C for one week and then stored at room temperature. The median parts (between the fourth node from the apex and the third node from the stem base) of the hemp stems were selected to reduce the heterogeneity of the initial chemical composition of the stems, which differs along the stems, as revealed by a previous study (Liu et al., 2015b), and they were hand-cut into 10 cm-long sections.

The soil was collected from the 0–10 cm soil layer from the same plot as the plant material and stored at 4 °C. The soil is a silty clay loam with 38.8% clay, 59.2% silt and 2% sand. The initial total carbon and nitrogen concentrations were 15.8 and 1.8 g kg^{-1} soil, respectively, and the pH_{H2O} was 6.97. Prior to the experiment, the moist sample soil were sieved (< 4 mm), all the plant residues were removed manually, and the moist soil was kept at 4 °C to prevent microbial activities before sample use.

2.2. Experimental system and retting protocol

The experimental system consisted of twelve 3-L PVC columns (5 destructive sampling dates x 3 replicates per date) containing a 10-cm layer of soil onto which hemp stems were dropped and lined up to form a 3-cm height pile (Fig. 1a). The pile of hemp stems was held in place by a metal frame to maintain its structure and form a hemp litter layer, and in the rest of this article, it will be known as the "litter layer" to distinguish it from the stem segments that are considered individually (Fig. 1a). The columns were set up in a climatic chamber in which the environmental parameters were regulated. The air temperature (15 °C) and relative humidity (60% moisture content) were recorded every 30 min using sensors (174 H, TESTO, Germany) and analyzed with software (Comfort Basic 5.0, TESTO, Germany). A 12-hour daily lighting cycle was provided with a horticultural LED light rail (Rail 36 W, Gemma LED, Sweden) to reproduce the visible spectrum of natural lighting, and regulated with a time switch. Deionized water was applied with rain simulators (Iqbal et al., 2015), allowing for the control of the amount, intensity, and composition of applied water (Fig. 1b). A 6-mm equivalent rain was applied to the columns once per week. These climatic parameters correspond to the mean climatic conditions for hemp retting as performed after harvesting at the seed maturity stage in the area where the hemp and soil were sampled. According to the temperature response function (Brisson et al., 2009), a 42-day duration of retting at 15 °C under our experimental conditions represented (approximately) in the field, for a dew retting duration of 59 days with a mean temperature of 12 °C and 33 days with a mean temperature of 17 °C.

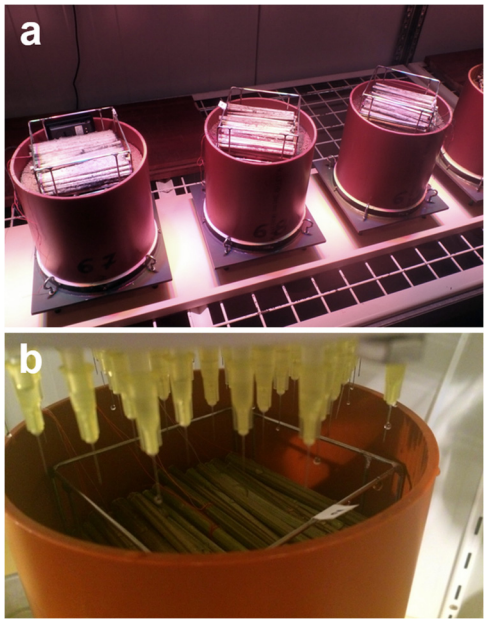

Fig. 1. Retting under controlled conditions, experimental system description: (a) experimental columns inside the climatic chamber under the LED light rail (b) rain simulator: © Bleuze/Inra.

The soil water content was adjusted to 20 g of H_2O 100 g^{-1} dry soil with deionized water to ensure that the retting process would not be limited by the soil moisture. The soil was placed in the columns to form a 10-cm-high soil layer at a bulk density of 1.3 g cm^{-3} by adding the corresponding mass (2426 g of moist soil), and the height was adjusted by applying pressure with a manual press (Sauvadet et al., 2016). Before the start of the experiment, the columns containing soil were maintained for 24 h in the climatic chamber at 15 °C. The hemp stems were premoistened between two cotton pieces humidified with deionized water at 4 °C for 24 h to reach an average moisture content of 78%. The excess water (the same free water as that contained in the pith hole) was removed before retting. The litter layer consisted of 50 g of equivalent dry matter in stems per soil column for an average of 70 stem sections and corresponded to approximately 26 t DM ha^{-1}. The experiment was run for 42 days. The litter layer was turned over on days 7 and 28 to homogenize the retting process vertically.

2.3. Stem sampling

Three randomly selected columns were destructively sampled after 7, 14, 28 and 42 days. This dynamic was selected to monitor the key retting periods in accordance with preliminary experiments that are not reported here. The sampling strategy included three litter layers that were sampled at the start of incubation (day 0). For each sampled litter layer, the analyzed stem samples were taken from the center of the litter layer after removing the stems that were in contact with the soil and those in contact with the atmosphere to minimize the edge effects. The samples were then stored at −20 °C except for 3 stems, which were dried in a forced-air oven at 40 °C for 72 h for dry matter content determination and stem surface analysis. The bast tissues (corresponding to the outer tissues surrounding the xylem core of the stem) (Fig. 2)