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Ramie fibers in a comparison between chemical and microbiological retting proposed for application in biocomposites

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ABSTRACT

Due to light weight, renewability, sustainability and generally moderate costs, natural fibers are addressed for the production of composites for application in packaging, automotive and other industries. Several approaches are under investigation to improve compatibility with polymer matrices and improve mechanical performances of composites with natural fibers. The retting process is the major limitation to efficient and high-quality natural fiber production. The conventional retting is normally done chemically by treatment of decorticated fibers with hot alkaline solutions. Such a process requires high energy input and produces hazardous wastes. Microbiological and enzymatic methods represent a reliable replacement, however their application on ramie (*Boehmeria nivea* (L.) Gaud.) has not yet been optimized and tuned for use on a large scale. Consequently, the aim of this work was to evaluate the role of microbiological retting on the morphological, chemical and physical–mechanical properties of the derived ramie fibers for application in biocomposites. The decorticated ramie fibers, obtained by mature crop stands grown at the experimental station of the Department of Agriculture, Food and Environment (DAFE) of the University of Pisa, were subjected to a water based microbiological degumming performed with the use of two selected strains of *Clostridium felsineum* L. at 30 °C for 7 days. The results obtained with this method were compared with those recorded adopting the conventional chemical process with NaOH water solution at 100 °C for 2 h. The morphological, chemical (hemicellulose, cellulose, lignin and ash) and physico-mechanical (tensile strength, elastic modulus and elongation at break) properties of retted ramie fibers were investigated. The fibers produced were evaluated for the production of composites by using polyhydroxyalkanoates (PHAs) as polymeric matrix, as targeted in the EC running project OLI-PHA.

Significant differences were observed between the two types of degumming in terms of yield and quality of the fibers. Even if the highest fiber yields were recorded with chemical retting, the performances of fibers modified by microbiological treatments were comparable with those of the composite prepared with fibers modified by chemical treatment. Scanning electron microscopy analysis revealed a good removal of non-cellulosic gummy material from the surface of ramie fibers. According to the mechanical properties, the ramie fibers obtained by both degumming processes, were suitable for use in PHAs composites.

1. Introduction

Development of synthetic polymers, used to produce plastics such as polyethylene, polypropylenes, polyesters and polyamides (including nylon), has brought about environmental concerns for over the past two or three decades (Nkwachukwu et al., 2013). In fact, most of these polymers are not biodegradable, and the wastes produced are solid, visible, and usually quite persistent. In addition, plastic wastes can also impose negative externalities such as greenhouse gas emissions or ecological damage, posing risks to human health and the environment (Nkwachukwu et al., 2013). These concerns include also composite materials, where plastic is the continuous matrix and fibers are used as filler or as reinforcing phase. Consequently, scientific efforts toward the design,

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synthesis, and production of sustainable or green materials have expanded tremendously in the last two decades (Miller, 2013). Currently, the demand of fibers is met mainly by the production of man-made fibers (Kozlowsky et al., 2008). The most dominant reinforcing fibers for polymers are glass, aramid, and carbon fibers, and their applications are found in construction, automotive, aerospace, leisure and sporting industries (Terzopoulou et al., 2015). These fibers have environmental problems both during the production and disposal (Wambua et al., 2003). Due to the above, natural resources are being exploited substantially as an alternative to synthetic ones, thanks to the renewability of the raw materials and due to non-renewable resource savings. A good alternative is represented by natural fibers, and the agricultural production of plant fibers is an interesting opportunity for many Mediterranean countries (Alexopoulou and Shouwei, 2014). Natural fibers have many remarkable advantages over synthetic fibrer such as light weight, low cost and biodegradability (Terzopoulou et al., 2015). Nowadays, various types of natural fibers, including flax (*Linum usitatissimum* L.), hemp (*Cannabis sativa* L.), jute (*Corchorus capsularis* L., *Corchorus olitorius* L.), wood, rice husk, sugarcane (*Saccharum* spp.), bamboo (*Bambusa* spp.), kenaf (*Hibiscus cannabinus* L.), ramie (*Boehmeria nivea* (L.) Gaud.), sisal (*Agave sisalana*), coconut coir (*Cocos nucifera* L.), kapok (*Ceiba pentandra* L.), paper mulberry (*Broussonetia papyrifera* L.), banana pseudo-stem fiber (*Musa sapientum* L.), pineapple leaf fiber (*Ananas comosus* (L.) Merr.) and papyrus (*Cyperus papyrus* L.) (Taj et al., 2007; Saxena et al., 2011) have been investigated for use in environmental-eco-friendly composites in order to substitute the conventional non-degradable plastics.

Among those fibers ramie, a member of the Urticaceae, has several interesting features. In fact, this herbaceous perennial plant, native to China, but widespread in Asia (Kirby, 1963; Pignatti and Anzalone, 1982), presents good prospects for introduction in Mediterranean area, according to long-term agronomic field evaluation (Oggiano et al., 1997; De Mastro, 1999; Di Bene et al., 2011; Angelini and Tavarini, 2013). Furthermore, the ramie's bast fibers are considered of excellent quality and are the longest and most durable bast fiber known (Xu et al., 2001; Nishino et al., 2004; Liu et al., 2005; Lu et al., 2006). The ramie fibers are commonly used for the production of textiles due to the characteristics of comfort of the finished product (Shihong et al., 1994; Cengiz and Babalik, 2009). Even in technical applications, such as the production of composite materials, ramie has excellent performance, as demonstrated by many studies (Angelini et al., 2000; Chen et al., 2005; Levita et al., 2009; Zhou et al., 2014).

Retting is the process necessary for the separation of fibers from non-cellulosic tissues in phloem. In ramie, the elementary fibers are bound by gums and pectins (Jarman et al., 1978; Batra, 1981). Currently, the separation of the fiber from the stem occurs mainly through the use of chemical methods (Bhattacharya and Shah, 2007). Microbiological and enzymatic methods, although reported in the literature, have not yet been optimized and tuned for use on a large scale (Pandey, 2007). This is due to a lack of knowledge relating to the optimization of the different parameters involved, and the effects of these parameters on the chemical and physical-mechanical properties of the fibers. Therefore, our specific objectives were to evaluate the morphological, chemical and physical-mechanical properties of ramie fibers subjected to two methods of retting. The first method is a chemical retting with NaOH water solution, the second one is water based microbiological method performed with the use of *Clostridium felsineum* L. The fibers produced, were evaluated for the production of composites by using polyhydroxyalkanoates (PHAs) as polymeric matrix, as targeted in the EC running project OLI-PHA (European Community's Seventh Framework Programme (FP7/2007-2013) under NMP grant agreement n. 280,604 Oli-PHA "A novel and efficient method for the production of polyhydroxyalkanoate (PHA) polymer-based packaging from olive oil waste water"). The properties of composites prepared with fibers treated with NaOH and with microbiological methods were compared.

2. Materials and methods

2.1. Plant material and sampling

A long-term field trial was set up from 1996 to 2013 at the Experimental Centre of Department of Agriculture, Food and Environment (DAFE) of the University of Pisa (San Piero a Grado, Pisa countryside, Italy 43°40′N latitude; 10°19′E longitude; 10 m elevation). The crop was cultivated on an alluvial deep loam soil, Typic Xerofluvent (Soil Survey Staff, 2006). It was representative of the lower Arno River plain, with a good fertility and water retention capacity, and a fairly high water table (0.12 m deep in driest conditions) as reported in Angelini and Tavarini (2013). The crop was planted in April 1996 with a density of 55,000 plants/ha (0.5 m between rows and 0.4 m intra row) on experimental plots (plot size 32 m^2, 8 × 4 m) with four replications. The plants were maintained under identical fertilizer regimes. Mineral fertilizer was applied at pre-planting at the rates of 50/100/100 kg ha^{-1} of N (as urea), P (as triple superphosphate) and K (as potassium sulphate), respectively. A further amount of nitrogen (50 kg N ha^{-1}, as ammonium nitrate) was supplied in late-spring of the same year. From the second growing season onward, plots received 100/65/165 kg ha^{-1} of N/P/K at the end of winter and further 50 kg N ha^{-1} were supplied after the first harvest. Starting from the second year, the plants were harvested twice a season (approx in June and October). Plots were kept weed free by hand hoeing. No crop diseases were detected during the experimental period and there was no need of irrigation treatment.

The plants were harvested in June 2013 on a minimal area of 10 m^2 in the inner part of each plot by cutting 10–15 cm above ground level and weighed to determine fresh weight. The fresh stems were manually decorticated, in order to remove the outer bark/epidermis and the bast from the inner woody core of the stem. The stem was cut into three equal parts, and the bark and the adhering fiber were separated from the third median part of the stem. The weight ratio of bast to stem was measured. The sub-samples were placed in a forced-draft oven at 75 °C for 72 h to determine the dry matter percentage. The bark obtained from the central part of the stem was utilized for subsequent analysis.

2.2. Degumming

The microbiological retting of the bast – obtained from pre-decorticated ramie stems – has been realized into tanks, testing two bacterial strains of *C. felsineum*, NCIMB 10690 (MIC 10690) and NCIMB 9539 (MIC 9539), previously selected for their high pectinolytic activity. Isolation and characterization of NCIMB 10690 has been already reported (Tamburini et al., 2001). The pure culture, on yeast extract-pectate agar medium (YP-agar) were kindly supplied by Prof Giorgio Mastromei from University of Florence, Italy. For retting experiment each organism was grown separately in a liquid growth medium composed by 5 g L^{-1} yeast extract, 5 g L^{-1} peptone, 10 g L^{-1} tryptone, 20 g L^{-1} glucose. Each liquid substrate was inoculated with the specific strain of *C. felsineum*. Then the liquid was transferred into a 17 L capacity jar in which were laid n. 5 sachets of AnaeroGen AG35 to create anaerobiosis. The jar was placed at 37 °C for 96 h to achieve a reasonable development of microorganisms. Each retting treatment was carried out with 100 g dry weight of bast samples, replicated four times, placed in 9 L plastic tanks. Each replicate was put into separated bags (prepared with a meshed net of polymer), inside the plastic tank. The dilution obtained was 1:8