*Manuscript

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

Luciana G. Angelini[1,2*], Mattia Scalabrelli[1], Silvia Tavarini[1], Patrizia Cinelli[2,3] Irene Anguillesi[2,3] and Andrea Lazzeri [2,3]

[1]*Department of Agriculture, Food and Environment (DAFE), University of Pisa, Via del Borghetto 80, 56124 Pisa, Italy*
[2] *INSTM, National Interuniversity Consortium of Materials Science and Technology, Via G. Giusti 9, 50121, Firenze, Italy*
[3] *Department of Civil and Industrial Engineering, Via Diotisalvi 2, 56122, Pisa, Italy, University of Pisa, Italy*

* Corresponding author. Tel.: +39 050 2218901

E-mail address: luciana.angelini@unipi.it

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 hours. 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.

Keywords: *Boehmeria nivea* (L.) Gaud., *Clostridium felsineum* L., Fiber characteristics, Pectinolytic bacterial strains, Polyhydroxyalkanoates biocomposites.

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, 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 (Kozlowski 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. 280604 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, 8x4 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 *Clostridium 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 *Clostridium 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 (500 mL culture broth of 4000 mL demineralised water). The ratio between the weight of the plant sample and water was 1:50. The retting parameters were 30°C for 7 days.

The chemical retting (CHEM) was performed according to Bredemann method (Bredemann, 1942) used for hemp and Dasgupta and Sen (1971) and Dasgupta et al. (1976), carried out on decorticated ramie fiber, first boiled in aqueous alkaline solution and then washed in water.

Samples of bast, gathered in bundles, were placed in glass containers airtight, filled with a 2% NaOH solution until complete coverage of the bundles. The containers were placed in boiling water for 2 h.

After completion of the retting, the retted raw fiber samples were washed properly in cold running water and dried. The raw fibers were then combed through the use of special combs of different dimensions (distance between the teeth) in order to separate the long fibers (called also line fibers) for quality assessment.

2.2 Determination of morphological, chemical and physic-mechanical properties of retted ramie fibers.

The surface of the long fibers was characterized by scanning electron microscopic (SEM), performed with a FEI QUANTA 200. The diameter of the fibers was not uniform, therefore suitable samples were selected with the aid of a microscope (200×magnification); the diameter of each fiber was measured at different places by an ocular micrometer and the average value was used. The number of samples analysed was 360 for each retting method.

Fiber’s chemical characterization was performed according to Van Soest’s method (Van Soest, 1963) using ANKOM Fiber Analyzer, model A200. This method consists of the gravimetric determination of residues previously treated with acid and neutral detergent solutions. Hemicellulose and cellulose contents were calculated from acid detergent fiber (ADF), neutral detergent fiber (NDF) and acid detergent lignin (ADL) measurements, as (NDF–ADF) and (ADF–ADL) for hemicellulose and cellulose, respectively.

The ash content was determined in a muffle furnace at 525 ± 25°C for 60 minutes according to the method TAPPI (Technical Association of the Pulp and Paper Industry) T 211 om -12. Experiments were performed on three replicates.

The tensile properties of selected filaments were determined with an Instron 5500 universal testing machine (load cell 10 N) with a cross-head speed of 1 mm/min at room temperature (20 ± 2°C) and 70 ± 5 % relative humidity. Since the diameter of ramie fibers was not circular and uniform, selection of suitable samples was made with the help of a low magnification microscope. The diameter for each fiber was taken at different places with the help of a precision gauge meter and the average value was used. Data were acquired and processed with Merlin calculation software V.4.42 of Instron Corporation

Tensile strength, elastic modulus and elongation at break, have been calculated for each batch of long fiber. The strength was evaluated by using different length, in the range 10-50 mm, with 45 filaments for each gauge length to give to the data a statistical meaning. The elastic modulus was measured by the slope of the stress-strain curve, taking the distance between grips as the gauge length.

2.3 Production of composites

The composites were prepared by processing Polyhydroxyalkanoate (PHA) PHI 002 from Natureplast, Ifs, France,it is a polyhydroxybutyrate valerate, with 1.25 density, 10-20 g/10 min melt flow rate, processed with 10% by weight of polyethyleneglycol 400 (Aldrich) as plasticizer, and 10% by weight of ramie fibers modified by respectively chemical retting, and microbiological retting. Processing was performed in a MiniLab II Haake Rheomex CTW 5 conical twin-screw extruder at 180 °C with a screw speed of 90 rpm. After extrusion, the molten materials were transferred through a preheated cylinder to a Haake MiniJet II mini injection molder to obtain Haake type 3 specimen (557-2290) dog-bone tensile bars used for measurements and analysis. The dog-bone shape is able to avoid the fracture outside the gauge section The injection mould temperature was 176 °C for the cylinder and 35 °C for the mould, the pressure was 30 MPa and the time was 5 seconds. The specimens were stored at 50% humidity. Tensile tests were performed at room temperature, at a crosshead speed of 10 mm/min, by means of an Instron 5500 universal testing machine (Canton MA, USA) equipped with a 1 kN load cell, in order to ensure an appropriate measuring range, interfaced with a computer and data were acquired with Merlin calculation software V.4.42

2.4 Statistical analysis

The results for the yields of retted raw fibers and long fiber, as well the data concerning the chemical composition, were subjected to the analysis of variance (ANOVA) using the statistical software CO-STAT Cohort V6.201 (2002). Means were separated on the basis of Least Significance Difference (LSD) test only when the ANOVA *F*-test per treatment was significant at the 0.05 or 0.01 probability level (Gomez and Gomez, 1984). Linear regression analyses were performed using GraphPad PRISM V4.0 (2003). Fiber's strength has been analysed in terms of Weibull's statistics.

3. Results and discussion

The manual decortication of the stems of *B. nivea* provided the bast for retting. The percentage of bast on stem dry weight varied between 27.8 and 32.2% of the stem dry weight. These data are in agreement with those reported by Angelini and Tavarini (2013). The yields of water based chemical and microbiological retted fibers are reported in Table 1, expressed as a percentage of the bast dry weight. The retting method significantly affected the fiber yield, with the highest values recorded for both raw fibers, that are fibers with no treatment, and long fibers which are those obtained by chemical retting. Between the two microbial strains, the strain NCIMB 9539 has provided higher yield in raw fiber but not in the long fiber, which was greater with the use of NCIMB 10690 strain. In a previous study carried out on Spanish broom vermenes, NCIMB 10690 strain provided higher fiber yield than NCIMB 9539 (Angelini et al., 2013).

The diameter of combed fibers was measured with the aid of an optical microscope (200x magnification) (Fig. 1). Fibers from microbial retting had an average diameter greater than those obtained by chemical maceration (32 µm). No significant differences were found between the fibers obtained with the two strains of *C. felsineum* (47 µm and 51 µm, for strain 10690 and 9539, respectively).

To investigate the morphology changes of ramie fibers treated with different retting methods, SEM pictures were examined and compared with those of untreated fibers (Fig 2a). The SEM analysis showed the surface of the ramie retted fibers, composed of a single cell elongated in accordance to Ilvessalo-Pfaffli (1995). These appeared smooth and uniform, with the presence of some material arranged irregularly over the entire length (Fig 2b-d). The main components of these encrustations are lignin and pectin that have the function of intercellular glue (Fan et al., 2010) and that were removed, in a more or less efficient way, by retting. The diameter range is between 30.8 and 51.5 µm, similarly to that reported by Pandey (2007). The diameters of ramie fibers are highly variable due to different factors but the influence of the retting method is not clear. According with Boruah et al. (2002), fibers present a reduction in diameter with the intensity of the treatment. The fibers present a spontaneous wrap on itself, known as crimp.

In Table 2 the composition in cellulose, hemicelluloses, lignin and ash of fibers has been reported.

From the results obtained it is possible to observe how the type of maceration significantly affected the chemical composition. Fibers from chemical retting had the highest percentage content in cellulose. The most effective capacity to remove hemicellulose was achieved by chemical method, even if a wide variability was observed. The microbial retting showed no difference in the reduction of hemicellulose between the two strains, in accordance with the results obtained on Spanish broom (Angelini et al., 2013). The chemical method was more effective than the microbial one in lignin removal, furthermore no statistical differences had been found between the two strains. Microbiological retting is generally less efficient then the chemical method in hemicellulose and lignin removal as demonstrated in other crop such as kenaf (Ramaswamy et al., 1994). The removal of the pectic material is important since allows the separation of the fiber bundles from the surrounding cells of the stem. In addition, Yu and Yu (2010), evaluating the influence of different retting methods on kenaf fiber properties, concluded that the chemical retting was the most effective methods yielding the least gum, while microbe retting induced higher residual gum content. Mooney et al. (2001) and Kawahara et al. (2005) reported that the chemical retting was generally more efficient and produced clean and consistent long and smooth surface bast fibers within a short time. Our results seem to confirm these findings, being the chemical retting more effective in the removing the non-cellulosic materials, consisting mainly of pectin and hemicellulose, attached to fibers. On the other hand, Kapoor et al. (2001) carried out a degumming of bast fibers using a combination of chemical (2% NaOH) and enzymatic treatment, showing a degumming efficiency in terms of gum removal up to 37 and 56% from ramie and sunn hemp bast fibers, respectively.

Fibers obtained by microbiological retting with NCIMB 10690 strain showed the lower ash content, while, on the other hand, the chemical retting showed the higher content. The lower the ash content, the better the quality of the fiber (Pandey, 2007).

Ramie fibers present stress-strain diagrams almost linear until fracture. Irregularities may occur for a failure of some of the individual fibrils, of which filaments are made up, prior to the final cumulative rupture, or for an internal rearrangement of fiber subunits under the action of the tensile stress.

The mean elastic module did not varied among the retting treatments and averaged 6.08, 5.02 and 7.54 GPa for the MIC 10690, MIC 9539 and CHEM retting, respectively. Within each treatment, great variability in the data was observed. The brittle behaviour of ramie fibers allowed their strength to be analyzed in terms of Weibull's statistics. As a natural product, vegetable fibers present variability in the tensile data (Fidelis et al., 2013). Broad distributions in tensile strength of fibers is usually attributed to flaws or defects that can naturally exist or be introduced during handling or processing or, finally, resulting from surface ageing. It is widely accepted that these defects are the main cause of premature failure of the fiber under tensile load (Curtin, 1994). The Weibull's distribution function (Weibull, 1951) is a statistical model that can represent the random variability of the natural fibers. In the two parameter models, the cumulative probability of failure $P_n(\sigma)$, i.e. the fraction of filaments having tensile strength not exceeding σ, is given by:

$$P_n(\sigma) = 1 - e^{-l(\sigma/\gamma)\alpha}$$

where α and γ are the parameters that characterize the fiber and experimentally-derived, σ is the stress at break and *l* is the gauge length.

The previous equation can be written as:

$$f(P_n, l) = \ln[\ln(1 - P_n(\sigma))^{-1}] - \ln l = \alpha \ln \sigma - \alpha \ln \gamma$$

So that a plot of $f(P_n(\sigma), l)$ versus $\ln(\sigma)$ is linear; α and γ are thus obtained by the slope and the intercept, respectively. For each gauge length, a plot was created in order to obtain the slope and the intercept. Once that α and γ are known, the mean fiber strength (σ_m) at a given gauge length can be calculated by the following equation:

$$\log \sigma_m(l) = \alpha^{-1} \log(l) + \log(\gamma) + \log[\Gamma(1+\alpha)\alpha^{-1}]$$

with Γ as the complete Gamma function. A plot of $\log \sigma_m$ versus log (l) is again expected to be linear. The mean tensile strength at the gauge lengths required in the fragmentation analysis is experimentally inaccessible and it is evaluated by extrapolation of such plots. The plots of log (mean stress) versus log (gauge length) of the different retted ramie fibers are reported in Figure 3.

The solid line represents the regression line. In each case, it was observed that the fibers strength increased with a decrease of the gauge length. The strength of microbial retted fibers was in the range of 58-25 MPa for the 10690 strain, and 56-23 MPa for the 9539 strain. The chemical retted fibers had strength between 164-88 MPa. These values are lower than data reported from different authors (Mwaikambo, 2006; Angelini et al., 2000). Anyway, elastic modulus and strength data were large enough for present fibers to be proposed as reinforcing means of PHA matrices.

The slopes of lines in Figure 3 showed that fibers from chemical retting were more reliable then the fibers from microbial retting, which presented the same slope in both involved strains. In each case, the slope was comparable with those from man-made fibers, like E-glass and carbon. This was somehow stunning since one would expect natural fibers to exhibit a much wider variability and a more pronounced effect of filament length on fracture stress, instead all fibers appeared to be very similar in this respect.

As for the strength, gauge length influences also the elongation at break (ε_B). In Figure 4 the behaviour of fibers is reported. The behaviour was almost linear so that $\sigma_B \approx \varepsilon_B$. Since there is no influence of gauge length on elastic module (fluctuation of elastic modulus values was independent of gauge length), it follows that the gauge length dependence of strength has to be paralleled by that of elongation.

Breaking elongation is directly correlated with the presence of lignin in fibers (Zhang and Yan, 2013). Long fibers from chemical retting, which presented a lower level of lignin, had a higher elongation then microbiological one.

Ramie fibers can be proposed for application in bio-composites production where the presence of fibers can have both benefit of increasing tensile strength, and elastic modulus of the materials, as well as lowering cost, since most biodegradable polymers are pretty expensive (Marsyahyo, 2011; Choi 2012; Kumar et al., 2012). For example, polyhydroxyalkanoates (PHAs) are interesting biodegradable polymers proposed for production of composites since compostable, but also degradable in soil and marine environment. The cost of PHAs ranges between 5-10 Euro (Bugnicourt, 2014); furthermore, the addition of natural fibers in materials produced with these polymers lowers the cost and promotes the disintegration in compost test, that is an important factor for materials with consistent thickness (> 2mm), such as rigid packaging (Seggiani 2015). Composites based on a plasticised polymeric matrix of PHA and PEG400 (90/10), with 10% by weight of ramie fibers were easily prepared by extrusion and injection moulding in the mini lab extruder and minijet. The materials were homogeneous and fibers were well distributed in the polymeric matrix. Mechanical properties of the samples prepared with fibers respectively modified by microbiological and chemical treatment are reported in Table 3. The results of mechanical properties are compared with those of the plasticised polymeric matrix based on PHA (90%) and PEG400 (10%), as well as with a similar composite prepared with 10% by weight of good quality commercial fibers of micro cellulose (CMC, that were Arbocell BE 600-30, natural cellulose fibers with average fibers length 40 μm, and average fibers thickness 20 μm.

The higher strength and modulus observed in composites versus the polymeric matrix attest for good dispersion and reinforcement of the matrix. This is expected for fibers treated by alkali since, in general, alkali-treatment onto the natural fibers is an effective treatment in terms of improving its hydrophilicity by breaking the extensive hydrogen bond network in the fiber structure and creating many free reactive hydroxyl groups (Goda et al., 2006). The performance of ramie fibers are comparable with those of commercial micro cellulose, and most interesting. The performances of composites prepared with fibers modified by microbiological treatments were comparable with those prepared with fibers modified by chemical treatment. Foulk et al. (2011) found that flax enzyme retting via the pectinase PL-BRI was capable to produce consistent high-strength renewable fibers for use in novel resins developed for natural fiber agricultural feedstock composites.

Our findings assess the potential value of microbiological treatment versus the chemical one, allowing achieving composites with similar mechanical properties, eventually even better properties, using fibers modified with a treatment that is more sustainable and based on a green chemistry approach.

5 Conclusions

The present work contributes to the development of natural fiber supply in pursuit of greater sustainability. This investigation has shed some light on the influence of the retting process on ramie fibers in terms of morphological, chemical and physical-mechanical characteristics, with the aim to evaluate the microbial retting as an alternative to the chemical one. Chemical retting method has serious environmental implications, with high energy consumption and heavy release of caustic residue in waters. Significant differences were observed between the two types of retting in terms of yield and quality of the fibers. In particular, from the viewpoint of the hemicelluloses removal, cellulose content and fibers tenacity, the chemical retting appeared to be the most effective one. On the contrary, microbiological retting gave fibers characterised by lower tenacity and lower quality. The microbiological retting was conducted without varying process parameters, such as time and temperature that can influence the results. Therefore, there are possibilities for optimization, and further studies are needed in order to increase the process yield and quality characteristics of the fibers obtained by this method. Nevertheless, the performances of the two kind of fibers (by chemical and microbiological retting), when used in composites production, based on biodegradable polymeric matrices, such as polyhydroxyalkanoates, were comparable.

Acknowledgements

The authors wish to thanks Dr Lara Foschi for her technical assistance in plant sampling and laboratory chemical retting.

References.

Alexopoulou, E., Shouwei, T., 2014. The importance of the fiber crops for Europe and China, in: Miller, T., E. Alexopoulou, and M.T. Berti, Eds. 2014. International Conference in Industrial Crops and 26th Annual Meeting of the Association for the Advancement of Industrial Crops (AAIC). September 13-19, 2014, Athens, Greece, Program and Abstracts p. 96.

Angelini, L.G., Lazzeri, A., Levita, G., Fontanelli, D., Bozzi, C., 2000. Ramie (*Boehmeria nivea* (L.) Gaud.) and Spanish Broom (*Spartium junceum* L.) fibres for composite materials: agronomical aspects, morphology and mechanical properties. Ind.Crops Prod. 11, 145–161.

Angelini, L.G., Tavarini, S., 2013. Ramie [*Boehmeria nivea* (L.) Gaud.] as a potential new fibre crop for the Mediterranean region: Growth, crop yield and fibre quality in a long-term field experiment in Central Italy. Ind. Crops Prod. 51, 138-144.

Angelini, L.G., Tavarini, S., Foschi L., 2013. Spanish broom (*Spartium junceum* L.) as new fiber for biocomposites: the effect of crop age and microbial retting on fiber quality. Conf. Papers Mater. Sci. (Online), 5pp., Article ID 274359.

Batra, S.K., 1981. Other long vegetable fibres, in: Lewin, M., Pearce, E.M. (Eds.), Handbook of Fibre Science and Technology, vol. 4. Marcel Dekker, New York, pp. 727–808.

Bhattacharya, S.D., Shah, S.R., 2007. Degumming of decorticated ramie: effects of alkalis on gummy compositions vis-à-vis their properties. J. Text. Inst. 98 (5), 431-436.

Boruah, R.K., Baruah, P.P., Bordoloi, A.K., Rahman, H., 2002. Chemical and chemi-mechanical process for degumming of ramie and their characterization by XRD, FT-IR and microscopic methods. J. Sci. Ind. Res. 61 (6), 449-453

Bugnicourt E., Cinelli P., Lazzeri A., Alvarez V. 2014, Polyhydroxyalkanoate (PHA): Review of synthesis, characteristics, processing and potential applications in packaging, Polymer Letters, , 8(11), 791-808,

Bredemann, G., 1942. Die Bestimmung des Fasergehaltes bei Massenuntersuchungen von Hanf, Flachs. Fasernesseln und anderen Bastfaserpflanzen. Faserforschung 16, 14–39.

Cengiz, T.G., Babalik, F.C., 2009. The effects of ramie blended car seat covers on thermal comfort during road trials. Int. J. Ind. Erg. 39, 287–294.

Chen, Y., Sun, L., Chiparus, O., Negulescu, I., Yachmenev, V., Warnock, M., 2005. Kenaf/ramie composite for automotive headliner. J. Polym. Environ. 13, 107–114.

Choi H Y, Lee J S, 2012, Effects of Surface Treatment of Ramie Fibers in a Ramie/Poly(lactic acid) Composite, Fibers and Polymers, 13, (2), 217-223.

Curtin, W.A., 1994. Determining fiber strength versus gage length. Polym. Compos. 15 (6), 474.

Dasgupta, P.C., Sen, S.K., 1971. Gum of ramie fibre, Proceedings of 58th Science Congress, p. 184.

Dasgupta, P.C., Sen, K., Sen, S.K., 1976. Degumming of decorticated ramie fibre for textile purposes, Cellul. Chem. Technol. 10, 285-291.

De Mastro, G., 1999. Boehmeria o Ramiè, in: Venturi, G., Amaducci, M.T. (Eds.), Colture da fibra, Edizioni Edagricole, Bologna, pp. 26–32.

Di Bene, C., Tavarini, S., Mazzoncini, M., Angelini, L., 2011. Changes in soil chemicalparameters and organic matter balance after 13 years of ramie [*Boehmeria nivea* (L.) Gaud.] cultivation in the Mediterranean region. Eur. J. Agron. 35, 154–163.

Fan, X.S., Liu, Z.W., Liu, Z.T., Lu, J., 2010. A Novel Chemical Degumming Process for Ramie Bast Fiber. Text. Res. J. 80(19), 2046–2051.

Fidelis, M.E.A., Pereira, T.V.C.; a, da Fonseca Martins Gomes, O., de Andrade Silva, F., Toledo Filho, R.D., 2013. The effect of fiber morphology on the tensile strength of natural fibers. J. Mater. Res. Technol. 2, 149-157.

Foulk, J.A., Rho, D., Alcock, M.M., Ulven, C.A., Huo, S., 2011. Modifications caused by Enzyme-retting and their effect on composite performance. Adv. Mater. Sci. Eng. doi:10.1155/2011/179023.

Goda G Sreekala MS, Gomes A, Kaji T, Oh J, 2006, Improvement of plant based natural fibers for toughening green composites—Effect of load application during mercerization of ramie fibers, Composites: Part A 37, 2213–2220.

,

Gomez, K.A., Gomez, A.A., 1984. Statistical procedures for agricultural Research ed. John Wiley and sons, Inc. London.

Ilvessalo-Pfaffli, M.S., 1995. Fibre Atlas, Identification of Paper Making Fibres, ed. T.E. Timmel, Springer-Verleg.

Jarman, C.G., Canning, A.J., Mykoluk, S., 1978. Cultivation, extraction and processing of Ramie fibre: a review. Trop. Sci. 20 (2), 91–116.

Kapoor, M., Beg, Q.K., Bhushan, B Singh, K., Dadhich, K.S., Hoondal, G.S., 2001. Application of an alkaline and thermostable polygalacturonase from *Bacillus* sp. MG-cp-2 in degumming of ramie (*Boehmeria nivea*) and sunn hemp (*Crotalaria juncea*) bast fibres. Process Biochem. 36, 803-807.

Kawahara, J., Tadokoro, K., Endo, R., Shioya, M., Sugimura, Y., Furusawa, T., 2005. Chemically retted kenaf fiber. Sem'i Gakkaishi 61, 115-117.

Kirby, R.H., 1963. Vegetable Fibres: botany, cultivation, and utilization, ed. Leonard Hill, London.

Kozlowsky, R., Kozlowsky, J., Barriga, J., 2008. The state of art in fibrous plants production and their application. Conference "Future crops for food, feed, fiber and fuel", September 17, 2008, Bologna, Italy.

Kumar, K.A.A., Sreekala, M.S., Arun, S., 2012, Studies on properties of bio-composites from ecoflex/ramie fabric-mechanical and barrier properties. J. Biomater. Nanobiotechnol., 3, 396-404.

Levita, G., Micaelli, F., Belfiore, G., 2009. Proprietà meccaniche di fibre di ramié. Conference “Le filiere delle colture da fibra: aspetti tecnico-scientifici e sperimentazione correlata alla produzione e all’impiego dei loro prodotti in ambito agricolo, industriale ed energetico”, Viterbo, Italy, University of Tuscia, 16 February 2009, Amaro (UD): Cirmont vol. CD ROM, ISBN: 978-88-903361-1-9.

Liu, F., Liu, Q., Liang, X., Huang, H., Zhang, S., 2005. Morphological, anatomical, andphysiological assessment of ramie [*Boehmeria nivea* (L.) Gaud.] tolerance to soil drought. Genet. Res. Crop Evol. 52, 497–506.

Lu, Y., Weng, L., Cao, X., 2006. Morphological, thermal and mechanical properties of ramie crystallites–reinforced plasticized starch biocomposites. Carbohydr. Polym. 63, 198–204.

Marsyahyo E Astuti S, Ruwana I(2011). Mechanical Improvement of Ramie Woven Reinforced-Starch Based Biocomposite Using Biosizing Method, Advances in Composite Materials - Analysis of Natural and Man-Made Materials, Dr. Pavla Tesinova (Ed.), ISBN: 978-953-307-449-8, InTech, Available from: http://www.intechopen.com/books/advances-in-composite-materials-analysis-of-natural-and-man-madematerials/mechanical-improvement-of-ramie-woven-reinforced-starch-based-biocomposite-using-biosizingmethod

Miller, S.A., 2013. Sustainable Polymers: Opportunities for the Next Decade. ACS Macro Lett. 2, 550−554; dx.doi.org/10.1021/mz400207g

Mooney, C., Stolle-Smits, T., Schols, H., de Jong, E., 2001. Analysis of retted and non retted flax fibers by chemical and enzymatic means. J. Biotech. 89, 205-216.

Mwaikambo, L.Y., 2006. Review of the history, properties and application of plant fibres. Afr. J. Environ. Sci. Technol. 7 (2), 120-133.

Nkwachukwu. O.I., Chima, C.H., Ikenna, A.O., Albert. L., 2013. Focus on potential environmental issues on plastic world towards a sustainable plastic recycling in developing countries. International Journal of Industrial Chemistry, 4, 34.

Nishino, T., Matsuda, I., Hirao, K., 2004. All-cellulose composite. Macromolecules 37, 7683–7687.

Oggiano, N., Angelini, L.G., Cappelletto, P., 1997. Pulping and paper properties of some fibre crops. Ind. Crops Prod. 7, 59–67.

Pandey S.N., 2007. Ramie fibre: part I. Chemical composition and chemical properties. A critical review of recent developments. Text. Progr. 39(1), 1-66.

Pignatti, S., Anzalone, B., 1982. Flora d'italia Vol. 1 ed. Edagricole, Bologna.

Ramaswamy, G.N., Ruff, C.G., Boyd, C.R., 1994. Effect of bacterial and chemical retting on kenaf fiber quality. Text. Res. J. 64 (5), 305-308.

Saxena, M., Pappu, A., Sharma, A., Haque, R., Wankhede, S., 2011. Composite Materials from Natural Resources: Recent Trends and Future Potentials, in: Advances in Composite Materials - Analysis of Natural and Man-Made Materials, ed. Dr. Pavla Tesinova, Publisher InTech; ISBN 978-953-307-449-8

Seggiani M Cinelli P, Verstichel S, Puccini M., Vitolo S, Anguillesi I, Lazzeri A. Development of Fibres-Reinforced Biodegradable Composites, 2015, Chemical Engineering Transaction, 43, in press,

Shihong, L., Benlian, Z., Qiyun, Z., Xianrong, B., 1994. A new kind of super-hybrid composite material for civil use-ramie fibre/Al. Composites 25,225–228.

Tamburini, E., Daly, S., Steiner, U., Vandini, C., Mastromei, G., 2001. *Clostridium felsineum* and *Clostridium acetobutylicum* are two distinct species that are phylogenetically closely related. Int. J. Syst. Evol. Microbiol. 51, 963-966.

Taj, S., Munawar, M.A., Khan, S.U., 2007. Review: Natural fiber-reinforced polymer composites. Proc. Pakistan Acad. Sci. 44 (2), 129–144.

Terzopoulou, Z.N., Papageorgiou, G.Z., Papadopoulou, E., Athanassiadou, E., Alexopoulou, E., Bikiaris, D.N., 2015. Green composites prepared from aliphatic polyesters and bast fibers. Ind. Crops Prod. 68, 60-79.

Van Soest, P.J., 1963. Use of detergents in the analysis of fibrous feeds, II. A rapid method for the determination of fiber and lignin. J. Assoc. Off. Anal. Chem. 46, 829–835.

Wambua, P., Ivens, J., Verpoest, I., 2003. Natural fibers: can they replace glass in fiber reinforced plastics? Compos. Sci. Technol. 63, 1259-1264.

Weibull , W., 1951. A statistical distribution function of wide applicability. J. Appl. Mech. 18, 293-297.

Xu, R., Xu, W., Fan, Y., Luo, L., 2001. Mechanical properties of ramie/LLDPE laminate. Fuhe Cailiao Xuebao/Acta Mat. Compos. Sin. 18, 23–28.

Yu, H., Yu, C., 2010. Influence of various retting methods on properties of kenaf fiber. J. Text. I. 101, 452-456.

Zhang, Q., Yan., S., 2013. Degumming of ramie bast fibers by Ca2+-activated composite enzyme. J. Text. Inst. 104(1), 78-83.

Zhou, N., Geng, X., Ye, M., Yao, L., Shan, Z., Qiu, Y., 2014. Mechanical and sound adsorption properties of cellular poly (lacticacid) matrix composites reinforced with 3D ramie fabrics woven withco-wrapped yarns. Ind. Crops Prod. 56, 1–8.

FIGURE CAPTIONS

Figure 1. Diameter (mean values ± standard deviation) of combed fibers from different retting processes.

Figure 2. Ramie fibers before degumming (A); fibers after degumming with *C. felsineum* 10690 (B); fibers after degumming with *C. felsineum* 9539 (C); fibers after retting with NaOH (D).

Figure 3. Comparison of the influence of gauge length on strength of different retted ramie fibers.

Figure 4. Influence of gauge length on breaking elongation of retted ramie fibers.

Table

Table 1. Percentages of raw and long fibers to bast dry weight obtained by microbiological (MIC) (*Clostridium felsineum* 10690 and 9539) and chemical (CHEM) retting.

Retting	Raw fibers (% dw)	Long fibers (% dw)
MIC 10690	24.3 ± 0.7 c	13.2 ± 1.9 b
MIC 9539	26.7 ± 0.8 b	8.8 ± 0.8 c
CHEM	42.9 ± 0.2 a	22.0 ± 1.3 a
Significance	***	***

For each column, mean values followed by the same letters are not significantly different at 0.05 probability level (LSD test).

***P<0.001

Table 2. Lignin, hemicellulose, cellulose and ash (mean values ± standard deviation) percentages of the fibres from different retting treatments.

Treatments	Cellulose (% dw)	Hemicellulose (% dw)	Lignin (% dw)	Ash (% dw)
MIC10690	84.67 ± 0.40 b	5.93 ± 0.54 a	1.84 ± 0.48 a	1.71 ± 0.08 c
MIC9539	81.29 ± 0.94 c	5.74 ± 0.14 a	2.36 ± 0.43 a	3.07 ± 0.14 b
CHEM	87.49 ± 0.88 a	1.50 ± 0.68 b	0.61 ± 0.19 b	4.16 ± 0.70 a
Mean	84.48	4.39	1.60	2.98
Significance	*	**	*	***
Not retted bast fibres[†]	61.85-73.21	5.27-7.58	4.6-9.06	7.51-9.34

For each column, mean values followed by the same letters are not significantly different at 0.05 probability level (LSD test). ∗*P*<0.05; ∗∗*P*<0.01; ∗∗∗*P*<0.001.

[†] Angelini and Tavarini, 2013.

Table 3. Mechanical properties of composite based on PHA and fibers treated with MIC10690, MIC9539, and NaOH (CHEM).

Fiber Treatments	Elongation at Break (%)	Tensile Strength at Break (MPa)	Young's Modulus (GPa)
MIC10690	3.5	28.8	2.6
MIC9539	2.8	26.8	3.0
CHEM	2.2	25.1	2.9
Significance	*n.s.*	*n.s.*	*n.s*

n.s. not significant (*P*>0.05).

Figure 1

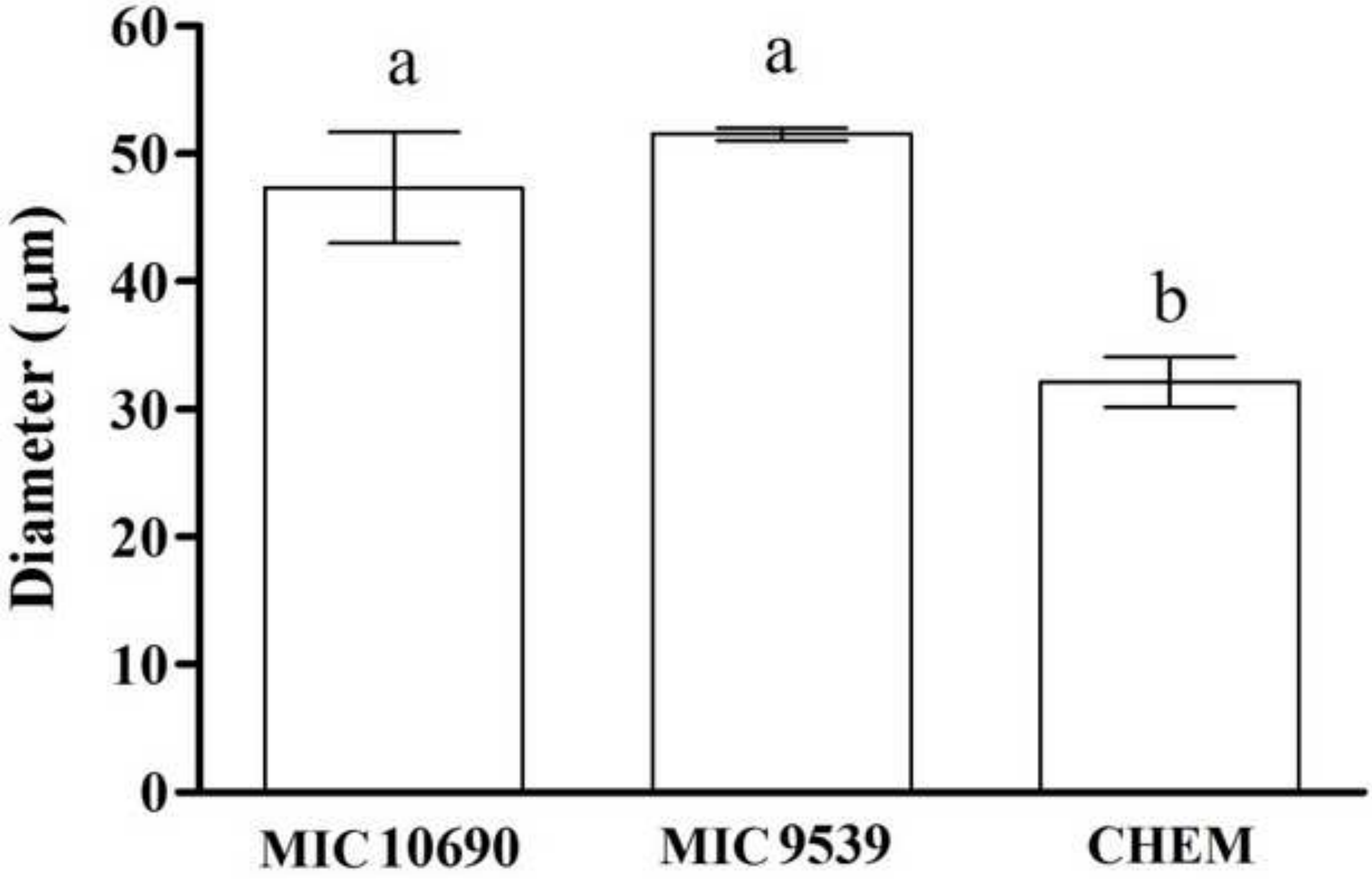


Figure 2

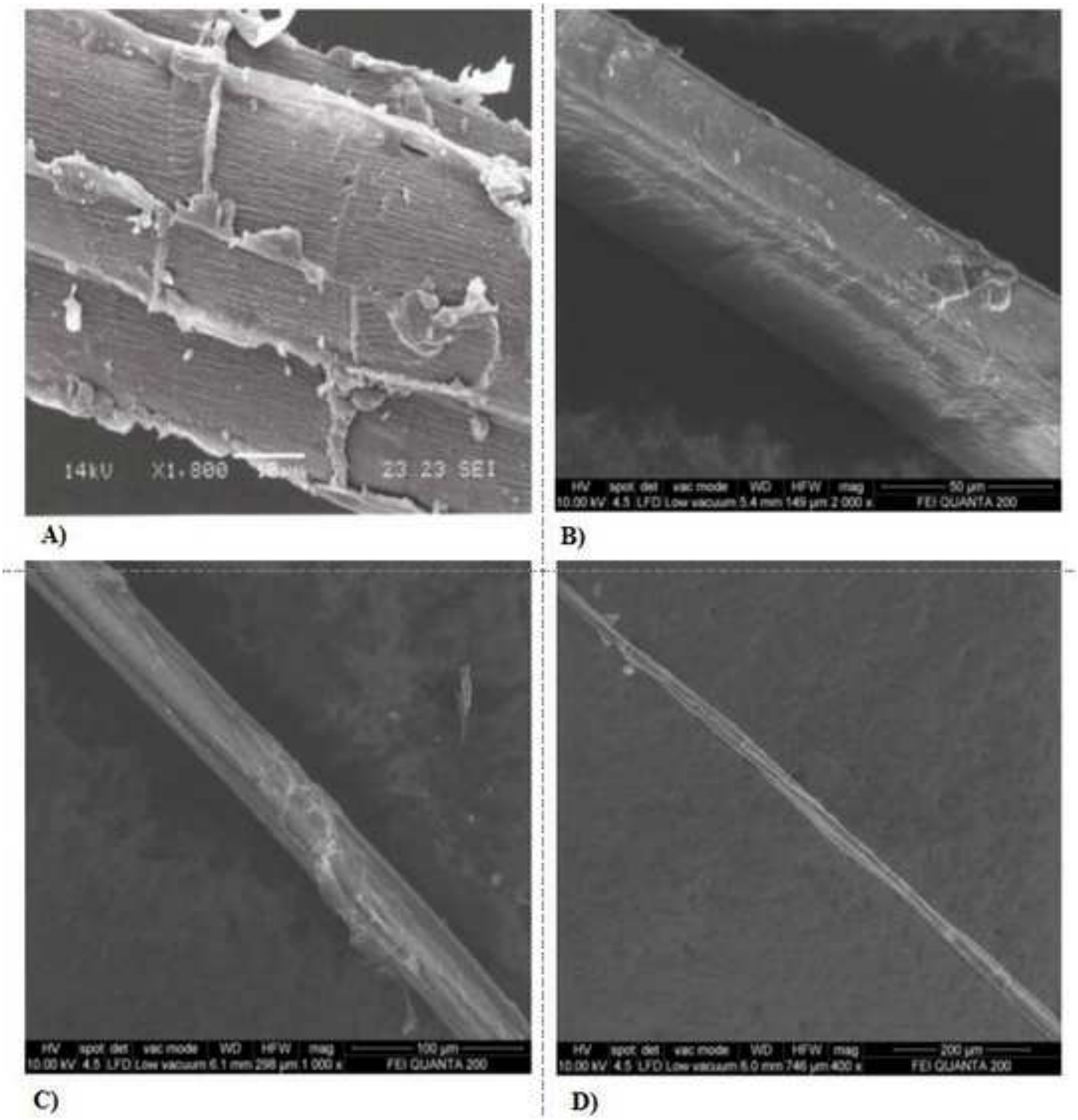
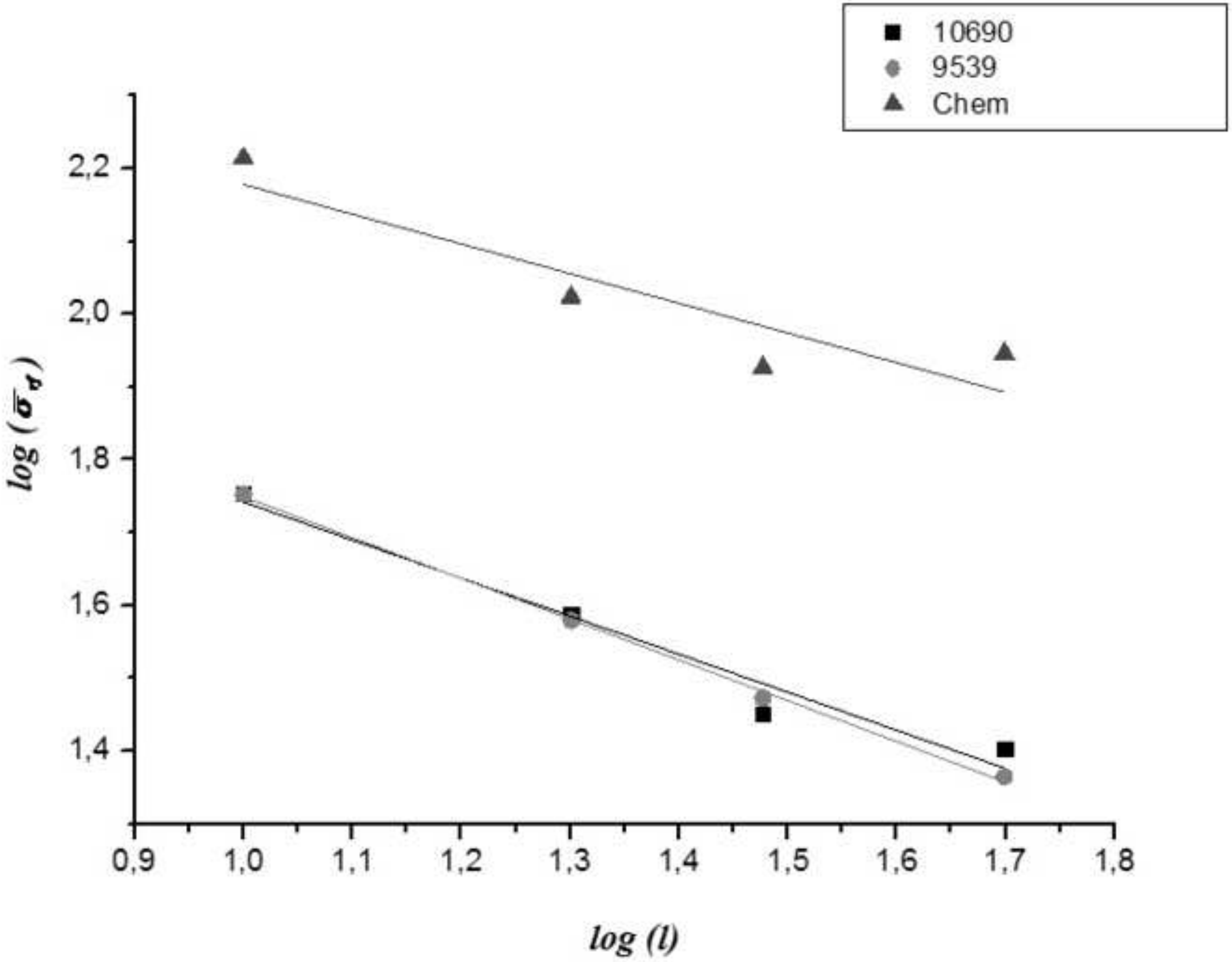
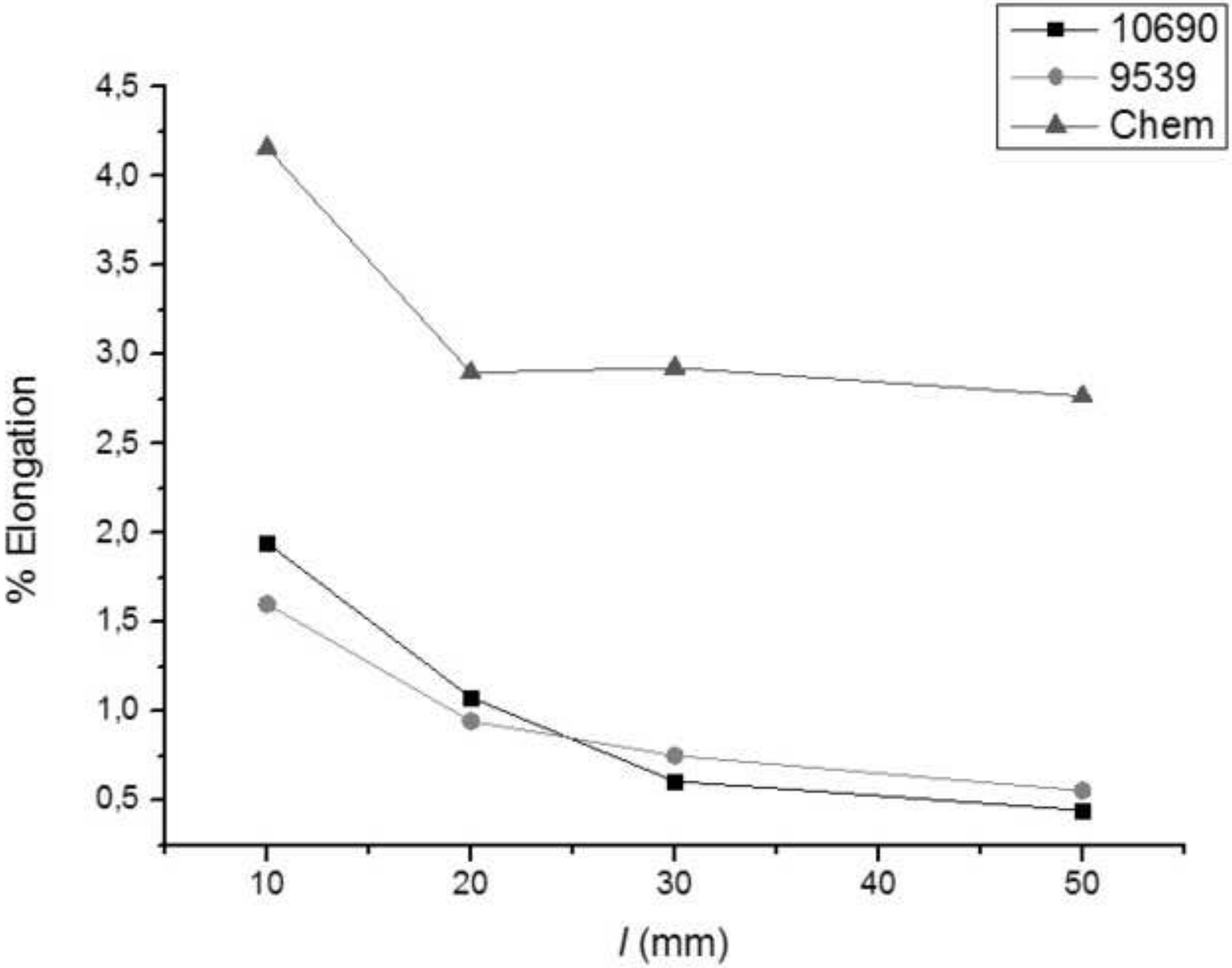

14kU X1,800 10μm 23 23 SEI
A)
HV spot det vac mode WD HFW mag 50 μm
10.00 kV 4.5 LFD Low vacuum 5.4 mm 149 μm 2 000 x FEI QUANTA 200
B)
HV spot det vac mode WD HFW mag 100 μm
10.00 kV 4.5 LFD Low vacuum 6.1 mm 298 μm 1 000 x FEI QUANTA 200
C)
HV spot det vac mode WD HFW mag 200 μm
10.00 kV 4.5 LFD Low vacuum 6.0 mm 746 μm 400 x FEI QUANTA 200
D)

Figure 3



■ 10690
● 9539
▲ Chem
$\log(\overline{\sigma}_d)$
2,2
2,0
1,8
1,6
1,4
0,9
1,0
1,1
1,2
1,3
1,4
1,5
1,6
1,7
1,8
log (l)

Figure 4



10690
9539
Chem
% Elongation
4,5
4,0
3,5
3,0
2,5
2,0
1,5
1,0
0,5
10
20
30
40
50
l (mm)